PREFERENTIAL POTENTIATION OF WEAKER INPUTS TO PRIMARY VISUAL CORTEX BY ACTIVATION OF THE BASAL FOREBRAIN IN URETHANE ANESTHETIZED RATS.

by

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Abstract

The ability of the brain to store information and adapt to changes in the sensory environment stem from the capability of neurons to change their communication with other neurons ("synaptic plasticity"). However, the ability of synapses to change (e.g., strengthen) is profoundly influenced by various chemical signals released in the nervous system (neuromodulators). Such modulatory effects may be preferential for different types of synapses. For example, cortical acetylcholine (ACh) has been shown to result in a relative enhancement of thalamocortical over intracortical synapses. Here, I tested the hypothesis that field postsynaptic potentials (fPSPs) in the rat primary visual cortex (V1) evoked by single pulse stimulation of the lateral geniculate nucleus (LGN) can be potentiated when LGN stimulation is paired with short bursts of stimuli applied to the basal forebrain (BF), the major source of ACh released in the cortex. Stimulation of the ipsi- and contralateral LGN elicited fPSPs in V1, with fPSPs triggered from the contralateral LGN exhibiting longer latencies and smaller amplitudes relative to fPSPs in ipsilateral projections. Stimulation bursts applied to the BF, paired with single, delayed LGN pulses, resulted in an enhancement of fPSP amplitude (~25%) for contralateral inputs at short (130 ms), but not longer (200-1000 ms) pairing intervals, while ipsilateral fPSPs failed to show significant potentiation over these intervals. The enhancement of the contralateral LGN-V1 fiber system induced by BF pairings was abolished by systemic or V1 application of the muscarinic receptor antagonist scopolamine, while systemic nicotinic receptor blockade was ineffective. These data suggest that there is a differential capacity for plasticity induction between strong, ipsilateral and weaker, contralateral fiber

ii

inputs to V1, with weaker inputs exhibiting greater synaptic enhancement following pairing with BF stimulation to elicit cortical ACh release. This preferential readiness for synaptic potentiation in normally weaker, non-dominant fiber inputs to V1 may facilitate the detection and integration of separate sensory signals originating in thalamic sensory nuclei.

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All work presented within this thesis represents the collective effort of many wonderful minds. Some of those minds have contributed to this thesis directly, with ideas, technical assistance, or by helping with the write-up. Others have helped through less direct means, such as by motivating, inspiring, and supporting me. Enrollment in graduate studies at Queen's has granted me the benefit of a truly enriching environment where I was encouraged to pursue academic and intellectual interests alike. As this phase of my academic life nears completion, it is a great pleasure for me to reflect on it and extend my gratitude to a few of those who made this thesis possible.

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Table of Contents

List of Figures

List of Abbreviations

ACh - acetylcholine

ACx - primary auditory cortex

BF - basal forebrain

CNS - central nervous system

DAG - diacylglycerol

ECoG - electrocorticogram

EEG - electroencephalogram

EPSP - excitatory postsynaptic potential

GABA - gamma-aminobutyric acid

GBS - gamma band synchrony

fPSP - field postsynaptic potential

IP$_3$ - inositol 1,4,5 triphosphate

IPSP - inhibitory postsynaptic potential

LGN - lateral geniculate nucleus

LTP - long-term potentiation

mAChR - muscarinic acetylcholine receptor

nAChR - nicotinic acetylcholine receptor

NBM - nucleus basalis magnocellularis

NMDAR - *N*-methyl *D*-aspartate receptor

PTx - pertussis toxin treatment

TBS - theta burst stimulation

V1 - primary visual cortex

Chapter 1

Introduction

The brain is an intricate network of neurons capable of modulating the way it processes signals and altering the physical nature of its synaptic connections. Armed with these tools, the brain allows organisms to respond to stimuli presented in the environment, and to adapt and change its responses to such external information.

Neuromodulation is the process by which the central nervous system can alter specific signals processed in specific sensory and high-levels pathways (Schacher et al., 1997). One property of neuromodulatory synapses, which makes them distinct from other synapses, is the presence of metabotropic receptors. Neurotransmitters binding to metabotropic receptors exert responses that differ from those elicited through binding to ionotropic receptors. Generally, metabotropic responses can be characterised as having a slower onset, longer duration, smaller contribution to direct membrane depolarization or hyperpolarization, and being capable of affecting nuclear transcription elements (Rodbell, 1995; review Kupfermann, 1979). In contrast, ionotropic receptor responses are mainly responsible for directly contributing to excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs). An important property of metabotropic receptors is the ability to employ intracellular transduction signals through G-protein-dependent mechanisms, thus engaging complex, intracellular signalling cascades within a neuron (Rodbell, 1995). Ultimately, these intracellular metabotropic signals that are initiated by synaptic neuromodulator release converge with, and influence the effectiveness of fast-acting (i.e., ionotropic) synaptic transmission, thus either amplifying

or dampening signal transfer in the central nervous system (Gil et al., 1997). Interestingly, in addition to this acute, online modulation of synaptic transmission, interactions between fast-acting neurotransmitters and neuromodulators also play important roles in the neuroplastic phenomena of the CNS. Neuroplasticity refers to the ability of the brain to alter its physical and synaptic connectivity during initial brain development, and during subsequent life in response to environmental signals (i.e., experience-driven behavioural adaptation). A significant amount of research has been devoted to topics related to neuroplasticity. An interesting phenomenon of neuroplasticity, long-term potentiation (LTP), has received considerable interest in this field.

1.1 Long-Term Potentiaton

Long-term potentiation (LTP) was discovered in Oslo, Norway by Bliss and Lomo (1973) in the hippocampus, although the term LTP was introduced two years later by Douglas and Goddard (1975). Initially, Terje Lomo observed that single pulse stimulation of fibers in the perforant pathway resulted in field EPSPs (fEPSP) recorded in the dentate gyrus of anesthetized rabbits. When brief, high frequency burst stimulation was applied to the perforant pathway, the response to subsequent single stimulation pulses became enhanced, an effect that remained fairly stable for several hours (Bliss & Lomo, 1973). Work in the CA1 area of the hippocampus identified LTP induction as N-methyl D-aspartate receptors (NMDAR) and Ca^{2+} dependent (Bliss & Collingridge, 1993). Additionally, NMDARs were found to be detectors of the degree of correlation between presynaptic and postsynaptic neuronal activity (Bliss & Collingridge, 1993). It was suggested that LTP was a Hebbian process, allowing neurons to detect tightly

2

correlated, coincident activity between pre- and postsynaptic neurons by means of Ca^{2+} currents conducted through NMDARs (Bliss & Collingridge, 1993; Bennett, 2000). Since the initial work by Bliss and Lomo (1973), LTP has been recognized as the primary mechanism underlying various forms of synaptic plasticity present during brain development and into adulthood, making it the leading candidate for experience-dependent brain plasticity, including learning and memory processes (e.g., Morris et al., 1986; Martin & Morris, 2002).

While initial work focused on LTP in the hippocampal formation of rabbits and rodents, subsequent studies have revealed that LTP is a very general phenomenon found in many species, under drastically different experiment conditions (e.g., *in vivo* and *in vitro*), and perhaps all parts and synapses of the nervous system (Bennett, 2000). To date, LTP has been characterized in the amygdala, cerebellum, striatum, and neocortex, among others (Review; Malenka & Bear, 2004). A significant amount of recent work as focused on LTP in the primary visual cortex (V1), where LTP has been proposed to mediate experience-dependent strengthening and maturation of synapses, ocular dominance plasticity, and possibly some aspects of perceptual learning (Sawtell et al., 2003; Lu & Constantine-Paton, 2004; Yao and Dan, 2005; Frenkel et al., 2006; Hofer et al., 2006). LTP has been shown to occur in V1 *in vivo* following theta burst stimulation (TBS) delivered to the lateral geniculate nucleus (LGN) of anesthetized rats. The induction of LTP was reflected in enhanced amplitudes of fEPSPs in V1, resulting from enhancements of current sinks located in cortical layer IV and supragranular (layer II/III) layers (Heynen & Bear, 2001).

1.2 Neuromodulation of Plasticity and LTP: Focus on Acetylcholine

The magnitude of LTP that can be induced at thalamocortical and intracortical synapses in V1 can be influenced by various neuromodulators. For example, *in vitro* studies suggest that serotonin lowers the amount of NMDA-dependent LTP that can be obtained in slices of V1 (Edagawa et al., 2001; Kim et al., 2006). In contrast, acetylcholine (ACh) has been shown to facilitate LTP in V1, as suggested by both *in vivo* and *in vitro* studies (Dringenberg et al., 2007; Brocher et al., 1992).

The neuromodulator ACh is synthesized in terminals of neurons containing the enzyme choline acetyltransferase (Semba & Fibiger, 1989). Each molecule of ACh is formed from two sources, acetyl-CoA, a product of glucose metabolism, and choline, which is imported into the neuron through high affinity Na^+/choline transporters (see Kuhar & Murrin, 1978 for review). ACh differs from other neuromodulating transmitters in that it is enzymatically broken down in the synaptic cleft, rather than being cleared by reuptake pumps. There are two subtypes of acetylcholine receptors (AChRs), nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs). The nAChRs are ionotropic receptors, which function as nonselective cation channels and are characterized by a pentameric structure of considerable molecular diversity in subunits that offers the possibility of a large number of nAChR subtypes (reviewed by Lucas-Meunier et al., 2003). In contrast, mAChRs are metabotropic receptors, which function by coupling to G proteins as their signalling mechanism and can be classified into 5 known subtype families (Lucas-Meunier et al., 2003). It has been shown that, in the neocortex, mAChRs significantly outnumber nAChRs (Krnjevic, 1974; Wake et al., 2000).

Cell bodies of cholinergic neurons, which project throughout the forebrain, can be classified as part of the brain cholinergic system (Fig. 1; Lucas-Meunier et al., 2003). The main source of cholinergic projections to the cortical mantle arises from cell bodies located in the nucleus basalis magnocellularis (NBM) of the basal forebrain (BF; Mesulam et al., 1983). The BF is a large, anatomical complex located ventral to the striatum and contains heterogeneous populations of cell bodies including cholinergic, GABAergic and neuropeptide-containing neurons (Semba, 2000; Zaborszky et al., 1999). Components of the BF that contain cholinergic projection neurons include the medial
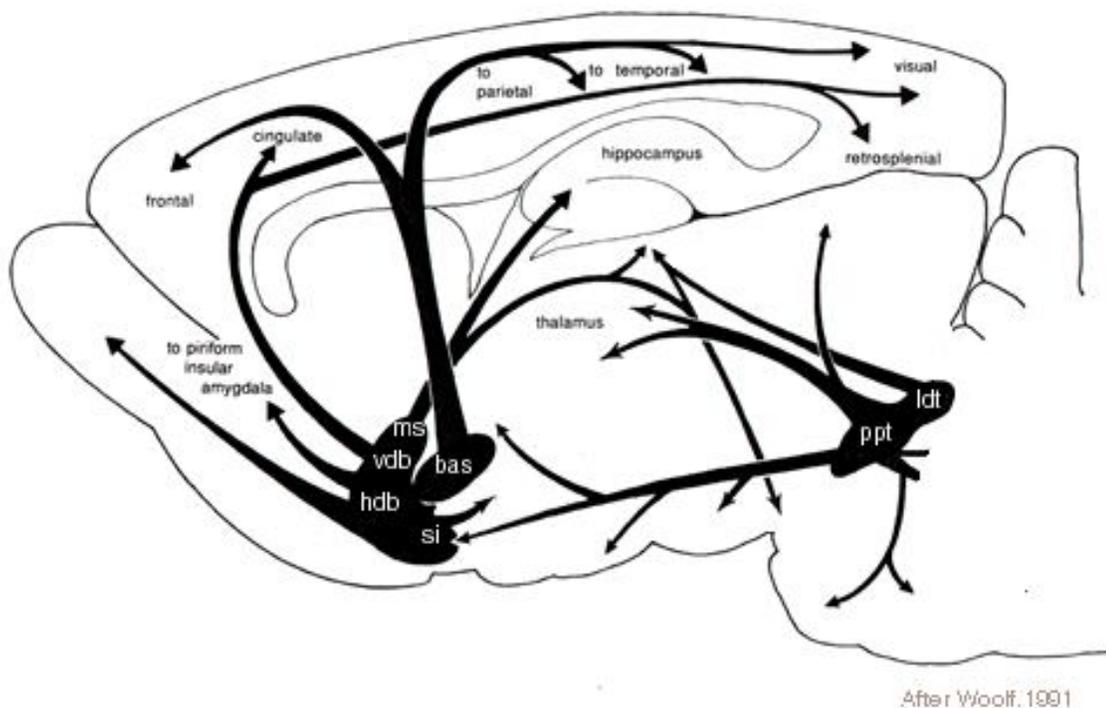

Figure 1: *Cholinergic system of the rat brain including the basal forebrain and cortical projections.* The main nuclei which project cholinergic axons to the cortex include the nucleus basalis (bas) and the substantia innominata (si). Adapted from Woolf. 1991.

septum, the nuclei of the vertical and horizontal limbs of the diagonal band of Broca,

ventral pallidum, substantia innominata, and the NBM (Butcher & Semba, 1989), as listed in rostal to caudal order. The BF sends unilaterally ascending projection fibres to both hemispheres of the cortex, and the two sides of the BF are sparsely connected by localized commissural neurons (Semba et al., 1988). Interestingly, GABAergic and cholinergic ascending fibres co-project and co-distribute throughout the cortex (Woolf et al., 1986). The most significant cholinergic input to the neocortex arises from the substantia innominata and NBM, which together provide the majority of the diffuse cholinergic innervation throughout the cortical mantle (Divac, 1975; Saper, 1984). Consistent with this anatomical work, electrical stimulation of the BF can result in cortical release of ACh (Jiménez-Capdeville et al., 1997). Ascending cholinergic fibres arising from the BF can form synaptic contacts with several elements within the neocortex. These have been suggested to be mainly pyramidal cells, but also include additional excitatory (e.g., spiny stellate cells) and inhibitory (e.g., basket cells) cortical interneurons (Semba, 2000). There have also been reports of cholinergic axon terminals, which do not form synaptic contacts within the cortex (Descarries et al., 1997; Umbriaco et al., 1994). This has led to the idea that in addition to synaptic signalling, cortical ACh may function in a paracrine fashion at extra-synaptic receptors (Descarries et al., 1997).

There are numerous examples of cholinergic modulation of neuroplasticity throughout the cortical mantle. In the somatosensory cortex of anesthetised rats, iontophoretic application of ACh can facilitate responses of neurons to tactile sensory stimuli (Donoghue & Carroll, 1987). In the auditory cortex of anesthetised cats, application of ACh facilitated responses of tones other than the preferred frequency, yet depressed responses to preferred frequencies (McKenna et al., 1989). The facilitation was

suggested to manifest possibly through the result of enhancement at the edges of auditory receptive fields (Rasmusson, 2000), a phenomenon similar to contrast enhancement in the visual system (Ratliff, 1972). ACh released in V1 can facilitate responses of neurons such that specific receptive field properties are often increased. For example, ACh can increase the responses to the preferred direction of movement without corresponding increases in response to non-preferred directions (Sillito & Kemp, 1983; Murphy & Sillito, 1991). In general, cholinergic modulation appears to exert a common effect of facilitating sensory responses by fine-tuning receptive field characteristics of cells populating various modality-specific sensory cortices (Rasmusson, 2000).

In addition, ACh also promotes changes of receptive field characteristics. For example, orientation tuning of V1 neurons can be shifted when presentations of suboptimal orientation stimuli are paired with iontophoretic application of ACh (Greuel et al., 1988). Interestingly, infusions of the mAChR antagonist scopolamine into V1 can inhibit the reorganization of ocular dominance columns seen during monocular deprivation of young cats (Gu & Singer, 1993). Thus, the engagement of cortical AChRs is important to a wide spectrum of neuroplastic phenomena throughout developmental and adult stages of the life cycle.

The cholinergic system is also capable of modulating LTP within the visual cortex. Initially, LTP in V1 was thought to be a temporally limited, developmental phenomenon, present during early postnatal life, but not in the mature cortex (Kato et al., 1991). Brocher et al. (1992) were the first to demonstrate that ACh can enhance this from of LTP studied under *in vitro* conditions, possibly by facilitating NMDAR conductance through activation of mAChRs. Work by Origilia and others (2006), conducted in slices

7

of mouse cortex, found that LTP elicited by TBS was dependent on the presence of both M_2 and M_4 mAChRs. These results suggest the requirement for activation of specific mAChRs subtypes for successful induction of LTP in the visual cortex. In addition, endogenous ACh released through stimulation of the BF was found to facilitate LTP in V1 when BF stimulation occurred within a specific time-window following TBS-induced LTP induction (Dringenberg et al., 2007). This effect was sensitive to scopolamine, again suggesting the importance of mAChRs in modulation of cortical LTP and neuroplasticity. This was the first study examining cholinergic modulation of LTP induction in V1 *in vivo*.

1.3 The Current Study

In this study I tested the hypothesis that field potentials in V1 evoked by single pulse stimulation of the LGN can be potentiated when LGN stimulation is paired with short bursts of stimuli applied to the BF, presumably resulting in cortical ACh release. This work is a continuation of previous experiments on the modulation of cortical LTP *in vivo* by the release of endogenous ACh (Dringenberg et al., 2007). As summarized above, ACh can effectively increase the level of LTP under a variety of experiment conditions. However, in the work published to date, the effectiveness of ACh to induce synaptic potentiation in V1 in the absence LTP induction by means of high-frequency stimulation or TBS has not been examined. There is some evidence that enhancement of ACh release by itself (without direct excitation of glutamatergic synapses, as typically done during LTP induction) can produce LTP-like effect for evoked responses recorded in the somatosensory cortex *in vivo* (Verdier & Dykes, 2001). Whether similar effects can be seen in other cortical areas is not known. For the experiments in this study, paired

stimulation protocols were carried out for a number of intervals between BF and LGN stimulation, and for two types of evoked potentials elicited in V1 by LGN stimulation: (a) more direct, shorter-latency potentials in the ipsilateral fibers between LGN and V1; and (b) long-latency responses that could be elicited in V1 in response to contralateral LGN stimulation. Interestingly, the results show that the long-latency, crossed input to V1 shows significant potentiation following BF stimulation, while little or no synaptic enhancement was apparent in the ipsilateral fiber system. These results demonstrate that different inputs to V1 exhibit varying degrees of sensitivity to cholinergic neuromodulation, a phenomenon that requires integration into existing models of cholinergic regulation of sensory transmission and plasticity in neocortical circuits.

Chapter 2

Methods and Materials

2.1 Subjects

Experiments were conducted on male, Long-Evans rats (250-650 g) weighed using a Nexxtech mini electronic scale (Orbyx Electronics, Walnut, California, USA). The rats were housed in large, transparent plastic cages containing groups of up to four, or smaller cages with room for two. Each cage contained wood shavings for bedding, and a plastic piece of piping for cover. Food and water were freely available. The cages were in a reverse light colony room where lights would turn on at 19:00 h. The colony room was kept at 40-70% humidity and 19-21ºC of temperature. Cage bedding and water were changed twice a week. All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Queen's University Animal Care Committee. Each rat was used only for one experiment.

2.2 Surgery

Animals were deeply anesthetized with urethane (2 g/kg, given intraperitoneally [i.p.] as 4 x 0.5 g/kg doses every 20 minutes, plus additional 0.5 g/kg supplements as needed) prior to being mounted in the stereotaxic frame. The body temperature was maintained between 35-37 ºC by wrapping the rats in a blanket and using an electric heating pad. The skull was exposed and using, stereotaxic co-ordinates, small holes were drilled in the bone overlying the BF (AP -0.7, L +2.7, V -7.7), either one of the ipsilateral (AP -4.2, L +4.0, V -4.5) or contralateral LGN (AP -4.2, L -4.0, V -4.5), and the ipsilateral V1 (AP -8.0, L +4.0, V -1.0). Three additional skull holes were drilled, two

over the cerebellum, to secure ground and reference electrode screws. The third hole was placed contralateral to the BF hole to secure the stimulation return connection for the BF stimulation electrode. The dura mater in the BF and LGN holes was punctured prior to electrode insertion to avoid trajectory deflections upon electrode insertion. Monopolar electrodes (125-um diameter Teflon-insulated stainless steel wire) were lowered into the brain through the centers of the BF and V1 holes (Fig. 2). An additional stimulation electrode (Series 100 concentric bipolar electrode, Rhodes Medical Instruments, David Kopf, Tujunga, CA, USA) was lowered into the hole above the LGN. The final, ventral depths of the LGN stimulation and V1 recording electrodes were adjusted to yield maximal fPSPs in V1 in response to single pulse LGN stimulation.
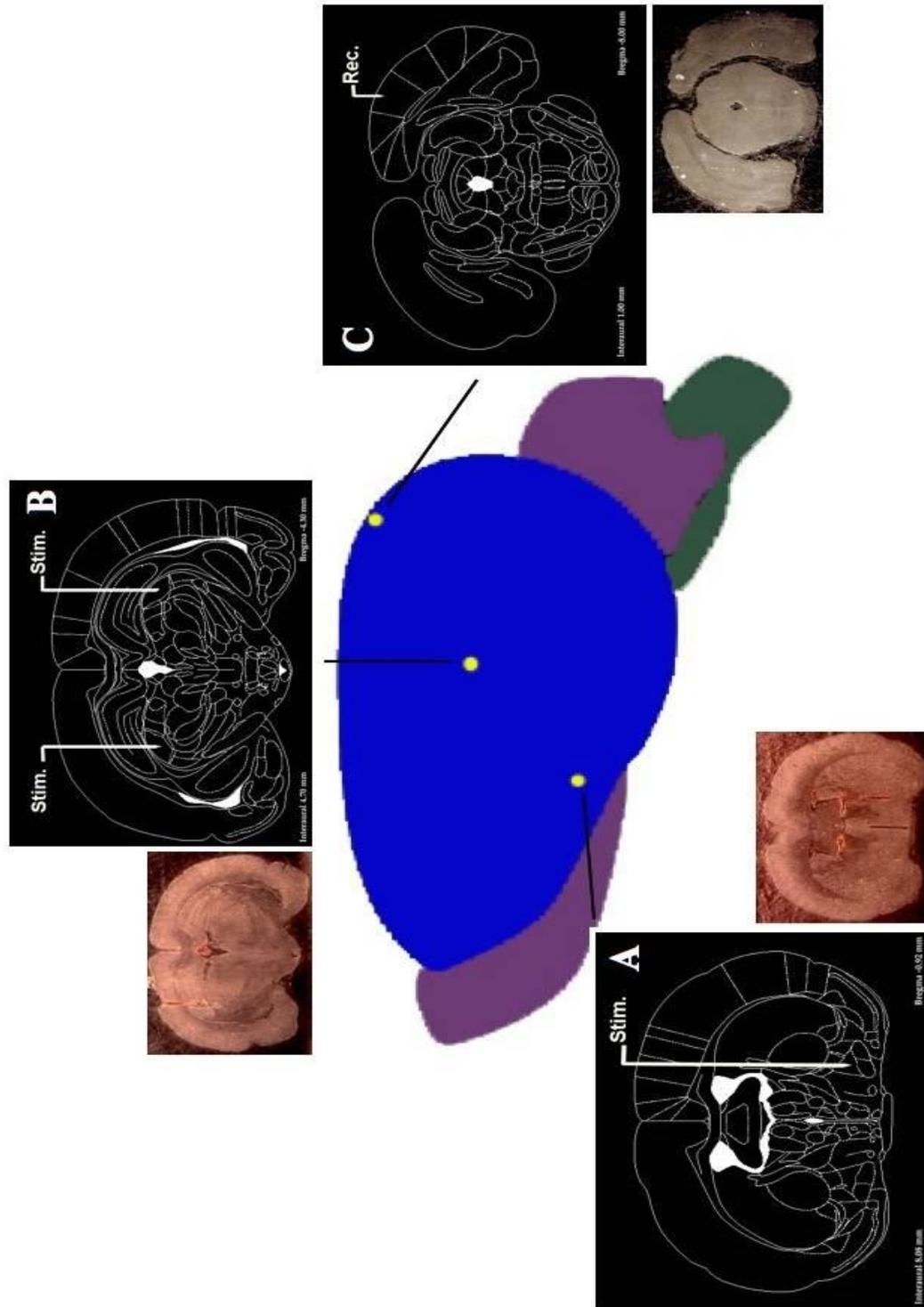
Figure 2: *The locations of stimulation and recording sites*. Stimulation electrodes were placed in the substantia innominata of the basal forebrain (A) and the LGN (B). Recording took place in V1 (C).

2.3 Electrophysiological Procedures

Except were noted otherwise, stimulation of the LGN (single 0.2 ms pulses, intensity adjusted for each animal to yield 50-60% of maximal fPSP amplitude, as outlined below) was delivered by means of the concentric stimulation electrode connected to a stimulus isolation unit (ML 180 Stimulus Isolator, AD Instruments, Toronto, ON, Canada) providing a constant current output.

For the paired BF-LGN stimulation protocol (Fig. 3), the BF electrode was
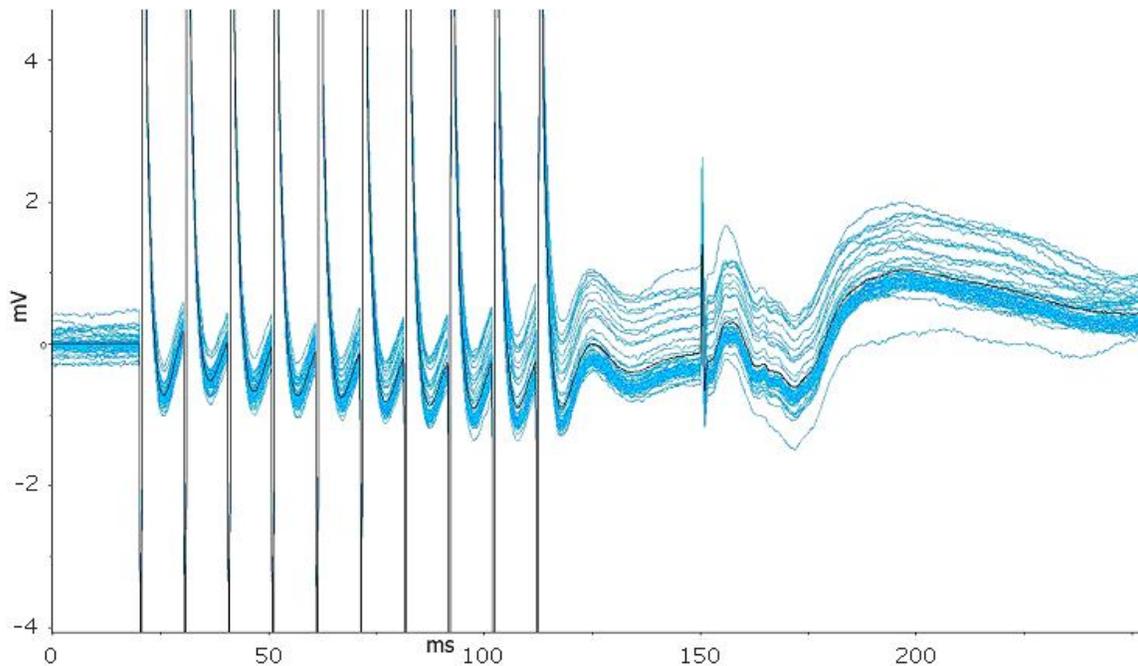
Figure 3: *Example of the paired BF-LGN stimulation protocol.* BF burst stimulation (10 pulses at 100 Hz) followed by single pulse LGN stimulation at a delay of 130 ms from the initial BF pulse (blue are individual sweeps of 50 pairings, black is the average of the 50 sweeps).

connected to the ML 180 Stimulus Isolator to deliver repeated bursts to the BF, consisting of 10 single pulses at 100 Hz, which were repeated 50 times at 0.5 Hz; 0.2 ms negative pulse, 1.0 mA. During this pairing, single pulse LGN stimulation (parameters as above; intervals between the onset of a BF burst and the single LGN pulse ranged from

13

130 to 1000 ms; i.e., the delays between the last pulse in the BF burst and the LGN pulse ranged from 40 to 960 ms) were provided by an isolated pulse stimulator (Model 2100, A-M Systems, Carlsborg, WA, USA), which was triggered by the ML 180 Stimulus Isolator.

The fPSPs in V1 elicited by LGN stimulation were recorded using a monopolar electrode referenced against a screw in the bone overlying the cerebellum. The recording electrode was connected to an amplifier and A/D converter (PowerLab/4s system running Scope software v. 3.6.5, AD Instruments) allowing the signal to be amplified, filtered (0.3 Hz- 1kHz), digitized (10kHz), and stored for offline analysis.

2.4 Experimental Procedure

Initially, input-output curves were established by stimulating the LGN at increasing intensities (0.1-1.0 mA at 0.1 mA increments). Based on these input-output curves, stimulation intensities for the LGN were chosen that yielded approximately 50-60% of the maximal fPSP amplitude in V1, which were then used for the remainder of the experiment.

Cortical fPSPs (every 30 s) were recorded until 30 min of stable baseline were achieved ($\leq$ 5% difference between successive data points for fPSPs averaged over 10 min epochs). Subsequently, the BF-LGN pairing protocol was delivered (see above). The following intervals (in ms) between BF bursts and LGN pulses were tested; ipsilateral LGN: 130, 150, 200, 500, 750, 1000, 1250, 1500; contralateral LGN: 100, 130, 500, 750, 1000 (the values represent the interval between the first pulse to the BF and the subsequent single LGN pulse). Upon the completion of the pairing protocol, two hours of fPSPs (every 30 s) were recorded to conclude the formal data collection.

As a means to verify correct electrode placements in the BF, the level of BF stimulation-induced ECoG activation was assessed (Dringenberg et al., 2007). The level of ECoG desynchronization was measured after five stimulation bursts to the BF (10 pulses at 100 Hz; 0.2 ms pulse duration, 1.0 mA, bursts repeated at 0.2 Hz). The effectiveness of BF stimulation to induce ECoG activation (i.e., suppression of large amplitude, low frequency activity, see Dringenberg et al., 2007) was used as an additional criterion to confirm the histological verification when selecting for correct BF electrode placements. Subjects displaying no obvious change in ECoG activity after BF stimulation were excluded from the final data analyses.

Cortical fPSPs were recorded, stored, and analyzed using Scope software, while the ECoG activity was recorded using Chart software (Powerlab/4S v. 3.6.5, AD Instruments). All data were stored and recorded using an Apple Macintosh Power Mac G4 computer (933MHz, 512Mb Ram, Mac OS 9.2).

2.5 Pharmacological Characterization

To investigate the role of different receptor populations in the effects of BF stimulation, independent groups of animals received drug treatments of either scopolamine hydrochloride (5 mg/kg), mecamylamine hydrochloride (5 mg/kg), or MK 801 maleate (0.5 mg/kg) to assess the respective roles of muscarinic, nicotinic, and NMDA receptors. All drugs were obtained from Sigma Chemicals (Oakville, Ontario, Canada), prepared in physiological saline (0.9%), and administered i.p. (volume of 1 ml/kg) twenty minutes prior to the onset of baseline recording.

In a further group of animals, the effect of local, intracortical application of scopolamine at the V1 recording site was also examined. For these experiments,

scopolamine (10 mM) was dissolved in artificial cerebrospinal fluid (aCSF) and applied at the V1 recording site using reverse microdialysis. The dialysis probe (MAB 6, 15 000 Dalton cut-off, polyether sulphone membrane, outer diameter 0.6 mm, S.P.E. Limited, Concord, Ont.) was mounted alongside the V1 recording electrode, with the probe extending approximately 1 mm ventrally past the electrode tip. The dialysis probe was connected to a 1 mL Sub-Q syringe (No. 309597, Becton Dickinson & Co., Franklin Lakes, NJ, USA), using FEB microtubing (S.P.E). The syringe was driven by a microdialysis pump (Univentor 801 Syringe Pump, Zejtuin, Malta) at a flow rate of 1 µL/min, with perfusion beginning 20 minutes prior to acquisition of baseline.

2.6 Euthanasia and Histology

At the conclusion of the experiment, the electrodes were withdrawn from the brain, the animal removed from the stereotaxic apparatus, and perfused through the heart. The needle of a standard 35 cc syringe was inserted into the left ventricle and one syringe full of 0.9% saline was injected, followed by 2 syringes full of 10% formalin. The brains were removed and kept in small glass jars containing 10% formalin for a minimum of 24 hours. They were then sectioned using a cryostat into 40 µm slices, which were mounted onto microscope slides. The slides were then inspected using a digital microscope and electrode placements were verified using a rat brain atlas as a reference (Paxinos & Watson, 1998). Data from inaccurate placements was omitted from this study.

2.7 Data Analysis

For each rat, maximum fPSP amplitude was computed using Scope software and averaged over 10 minute intervals. These amplitude values were then normalized by

16

dividing them by the average baseline amplitude of that animal. All data reported in the

text and graphs show means +/- S.E.M., all of which were calculated using Microsoft

Excel. Statistical analysis was conducted by ANOVAs and, where statistically

appropriate, simple effects test using the software package SPSS (ver. 15.0).

Chapter 3

Results

3.1 Characteristics of fPSP in V1 Elicited from Ipsilateral or Contralateral LGN

Stimulation of the LGN resulted in fPSP recorded in V1 ipsilateral to the stimulation site. The fPSP waveform (Fig. 4) consisted of a characteristic downward deflection, which peak in amplitude at latencies of 16-18 ms following the positive-going stimulation artifact. Maximum amplitudes ranged from 1-2 mV. In contrast to ipsilaterally derived fPSPs, stimulation of LGN located contralaterally to the V1 recording site elicited negative-going fPSPs with longer latencies to peak (23-25 ms post stimulation). The maximum amplitudes observed in contralateral fPSPs were smaller

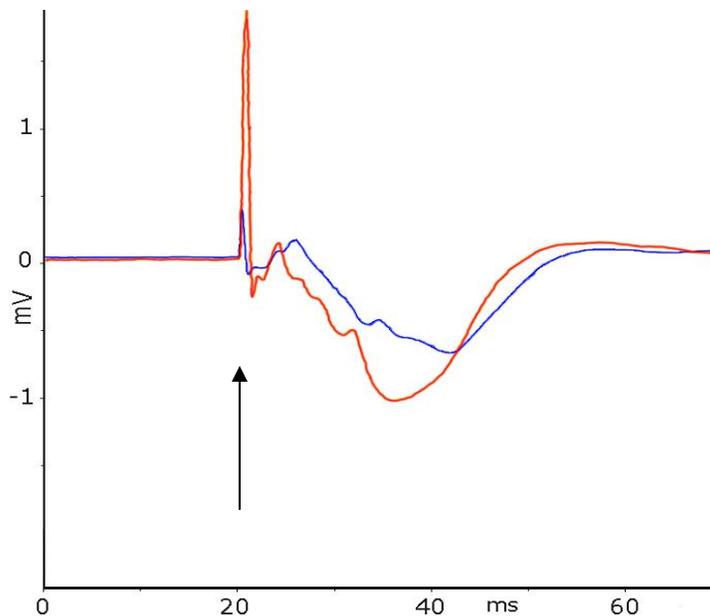**Figure 4:** *Features of fPSPs recorded in V1.* Contralateral (blue trace) fPSPs recorded in V1 were smaller in amplitude and longer in latency relative to ipsilateral fPSPs. The average latencies to peak for ipsilateral (red trace) fPSPs were 16-18 ms and 23-25 ms for contralateral fPSPs. Each trace is an average of 30 individual sweeps, recorded every 30 seconds; stimulation of the lateral geniculate nucleus occurs at arrow.

18

relative to ipsilaterally derived fPSPs (usually less than 1 mV).

3.2 The Effect of Pairing Basal Forebrain Burst Stimulation with Single Pulse Stimulation of the Ipsilateral LGN at Various Delay Intervals

Initially, the stability of fPSP recordings over a 2.5 h time period in the absence of any pairing protocol was assessed. Consistent with previous work employing similar control procedures (Dringenberg et al., 2007), these rats showed no significant changes in fPSP amplitude over time (n = 2, data not shown). Similarly, ipsilateral fPSPs remained stable in rats (n = 5) that received burst stimulation of the basal forebrain (BF; 10 pulses at 100 Hz, 0.2ms pulse duration, 1.0mA, repeated 50 times at 0.5 Hz) with no paired stimulation of the LGN during the induction protocol (Fig. 5A), i.e., there was no

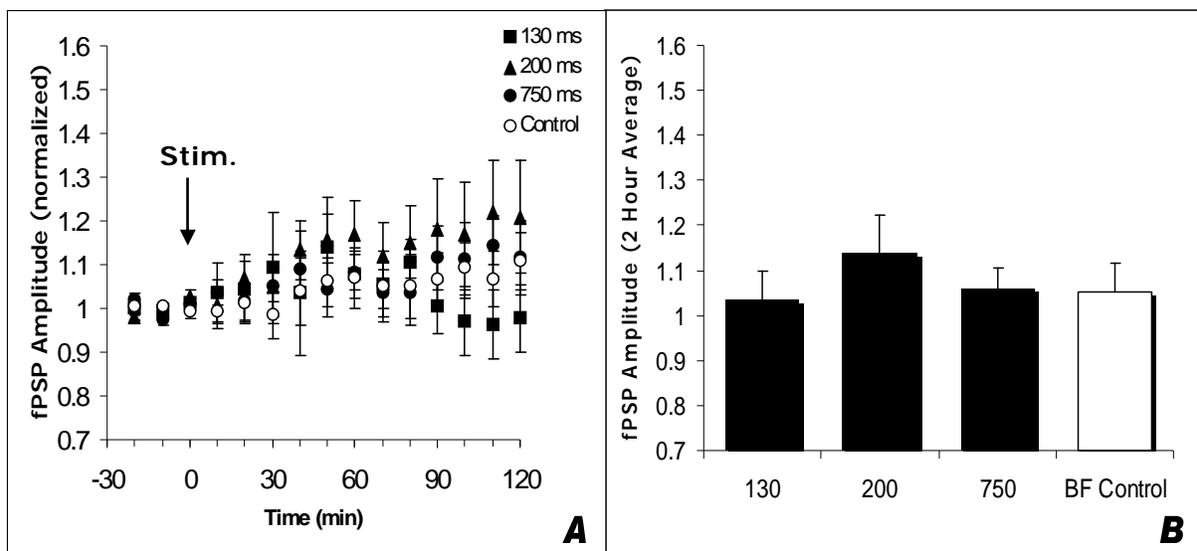**Figure 5:** *Changes to fPSP amplitudes as a result of pairing BF bursts with ipsilateral LGN single pulses* at 130 ms, 200 ms, and 750 ms delay intervals (n = 6, 7, and 6, respectively). A. None of the tested delay interval groups produced values significantly different from control values (BF bursts without paired LGN pulses, n = 5). B. fPSP amplitudes averaged over 2 hours following delivery of the pairing protocol.

19

significant change in fPSP amplitude over time (F(2,8) = 1.418, P = 0.298). Thus, fPSPs between LGN and ipsilateral V1 remained stable in the absence of paired BF-LGN stimulation

Pairing burst stimulation of the BF with a single pulse delivered to the LGN at an interval of 200 ms (n = 7) between the onset of the BF burst and the single LGN pulse resulted in a small, but non-significant enhancement of fPSP amplitude relative to control animals receiving BF stimulation without paired LGN pulses (Fig. 5B; F(2,21) = 2.83, P = 0.81 for ANOVA interaction of stimulation group and time). Decreasing the delay between BF bursts and LGN single pulses to 130 ms (n = 6, Fig. 5A) also did not produce a significant enhancement of fPSP amplitude relative to control animals (F(3,25) = 0.821, P = 0.488 for ANOVA interaction of stimulation group and time). Similarly, no significant difference from controls was seen when delay intervals were increased to 750 ms (Fig. 5A; n = 6; F(2,22) = 2.793, P = 0.75 for ANOVA interaction of stimulation group and time). The inputs to V1 originating in the ipsilateral LGN remained stable across various intervals between stimulation bursts to the BF paired with subsequent single pulse stimulation of the LGN.

3.3 The Effect of Pairing Basal Forebrain Burst Stimulation with Single Pulse Stimulation of the Contralateral LGN at Various Delay Intervals

Similar to the data described above, amplitude of fPSP elicited in V1 in response to single pulse stimulation of the contralateral LGN did not change significantly over

time in control rats (n = 6, Fig. 6A) that received BF burst stimulation in the absence of

paired LGN single pulse stimulation (effect of time, $F_{(3,16)} = 0.507$, $P = 0.690$).

However, pairing basal forebrain bursts with single pulses to the contralateral LGN at a

delay 130 ms (n = 13) between the onset of the BF burst and the LGN pulse resulted in a

significant enhancement of fPSP amplitude relative to controls (Fig. 6A; $F_{(5,83)} = 6.133$,
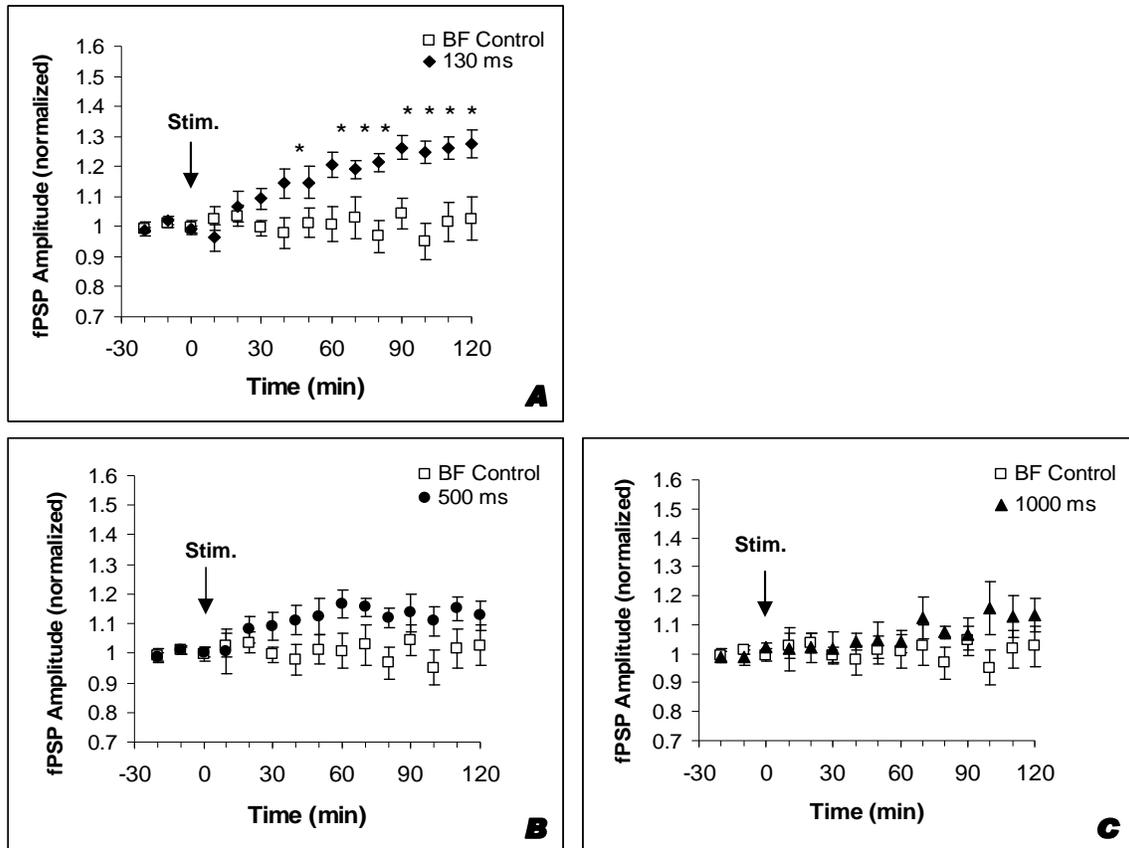
Figure 6: *The effects of pairing BF bursts with delayed single pulse stimulation of the contralateral LGN at three different intervals.* A. Delays of 130 ms (n = 13) produced significant enhancement of synaptic efficacy relative to control animals receiving only BF burst stimulation (n = 6). * P < 0.05 for simple effect comparisons. B and C. Longer pairing intervals of 500 ms (n = 9) and 1000 ms (n = 6), produced little or no synaptic enhancement relative to controls.

21

P < 0.001 for ANOVA interaction of stimulation group and time). In these rats, fPSP amplitude increased by approximately 25% relative to baseline and this enhancement was maintained for at least 2 hours following the delivery of the BF-LGN pairing protocol.

Increasing the delay between BF bursts and LGN single pulses to 500 ms (n = 9) resulted in a much smaller enhancement of fPSP amplitude, a change that was no longer statistically significant when compared to control values (Fig. 6B; $F_{(5,71)} = 1.599$, $P = 0.166$ for ANOVA interaction of group and time). The enhancing effect of LGN-BF pairing on fPSP amplitude appeared to be completely lost in animals (n = 6) where the BF-LGN delay was further increased to 1000 ms (Fig. 6C; $F_{(3,31)} = 1.228$, $P = 0.318$ for ANOVA interaction of group and time).

Interestingly, decreasing the interval to 100 ms (n = 5, data not shown) resulted in a 15% enhancement of fPSP amplitude two hours following BF and LGN pairing. However, this enhancement was not statistically significant when compared to control group values ($F_{(3,28)} = 1.916$, $P = 0.147$ for ANOVA interaction of group and time). Consequently, it appears that the optimal interval between BF activation and single pulses to the contralateral LGN is around 130 ms. An additional ANOVA compared fPSP amplitude for ipsi- and contralateral fPSPs in animals that received BF-LGN pairings at this 130 ms interval. This analysis confirmed that fPSP amplitude was significantly larger in the contralateral system relative to ipsilateral projections ($F_{(4,70)} = 5.169$, $P = 0.001$ for group by time interaction).

3.4 Pharmacological Characterization of the Contralateral fPSP

Enhancement at the 130 ms Interval

To assess the involvement of acetylcholine in the enhancement seen by pairing BF bursts to single contralateral LGN pulses delayed by 130 ms, we applied the muscarinic cholinergic antagonist scopolamine intraperitoneally (i.p.) at a dose of 5 mg/kg, 20 minutes prior to the onset of baseline recordings. Scopolamine-treated rats (n = 6) did not exhibit an enhancement of fPSP amplitude relative to the BF control group (Fig. 7; $F_{(5,49)} = 2.147$, $P = 0.77$). Additional analyses showed that fPSP amplitudes in scopolamine-treated rats at 130 ms delay intervals were significantly smaller than those in untreated animals receiving pairing at 130 ms (group effect, $F_{(1,17)} = 5.315$, $P = 0.034$; group by time interaction, $F_{(55,93)} = 2.215$, $P = 0.054$).
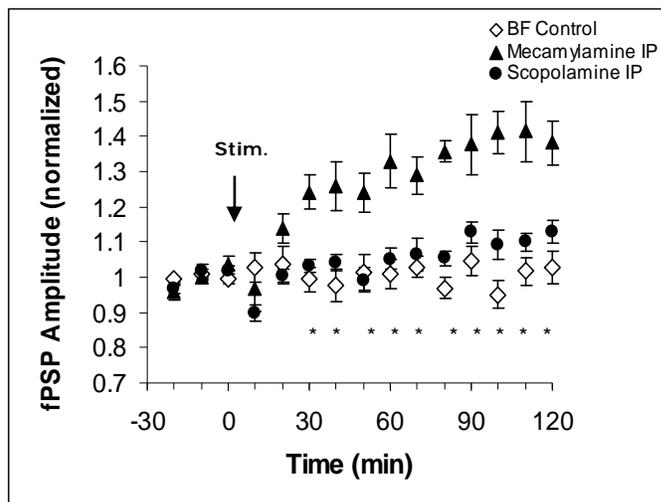

Figure 7: *Systemic blockade of muscarinic-cholinergic receptors with scopolamine* (Scop., 5mg/kg, i.p., n = 6) abolished the fPSP enhancement (130 ms interval), while systemic nicotinic blockade with mecamylamine (Mec., 5mg/kg, i.p., n = 6) was ineffective. * P < 0.05 for simple effect comparisons of nicotinic receptor blockade and BF control (n = 6) groups.

The involvement of nicotinic cholinergic receptors was also investigated through the systemic (i.p.) application of the receptor antagonist mecamylamine (n = 6) at the dose of 5 mg/kg. Interestingly, this group showed 35-40% enhancement in fPSP amplitude two hours post stimulation pairing, and was significantly different from BF control animals (Fig. 7; $F_{(4,39)} = 10.098$, $P < 0.0001$ for group by time interaction). Consequently, pairing-induced fPSP enhancement appears to be dependent on muscarinic, but not nicotinic receptors.

To identify the site of action of scopolamine to block fPSP potentiation following BF-LGN pairing, we infused aCSF containing the antagonist (10 mM, n = 8, Fig. 8) or
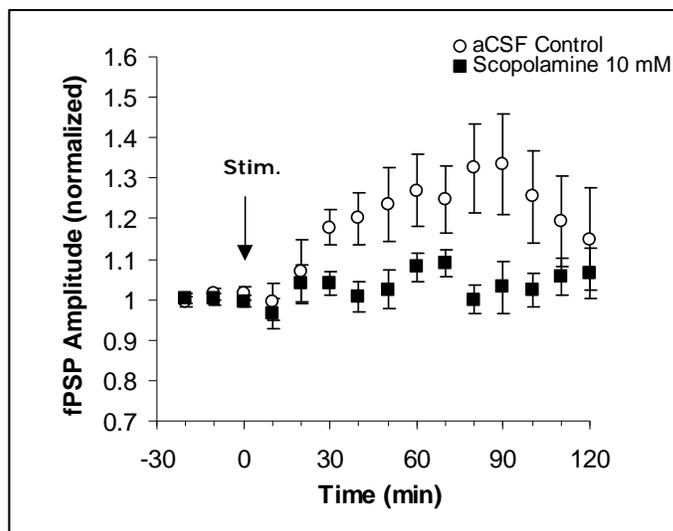
Figure 8: *Local blockade of muscarinic-cholinergic receptors* (scopolamine 10 mM, n = 8) appeared to abolish the fPSP enhancement (130 ms interval) relative to rats receiving only aCSF application (n = 9), even though this effect did not reach statistical significance (see text).

aCSF alone (n = 9) locally using a dialysis probe inserted into the recording site at V1. Local scopolamine blocked the enhancement seen in the 130 ms pairing interval group. However, when compared against the control group (n = 9) employing aCSF only in the dialysis probe, scopolamine treated rats failed to show statistical significance (Fig. 8;

24

F(3,44) = 2.429, P = 0.08 ANOVA for interaction of stimulation group and time). The two groups did show significant interaction if the last 20 minutes of the experiment are omitted (F(3,39) = 3.092, P = 0.044 for interaction of stimulation group and time). Additionally, the aCSF group failed to show a statistically significant change in fPSP amplitude over the time course of the experiment. The results of experiments employing application of aCSF through the dialysis probe proved highly variable.

3.5 Contrasting the Effects of Pairing Burst Stimulation to the Basal Forebrain with Single Pulses to the Ipsilateral or Contralateral LGN when Delayed 130ms

Pairing BF activation through bursts with 130ms delayed single pulses to the LGN produces differing effects depending on if the contralateral or ipsilateral LGN is stimulated. The two groups were found to be significantly different when compared (Fig. 9; F(4,61) = 5.169, P = 0.001 for interaction of stimulation group and time). However, the
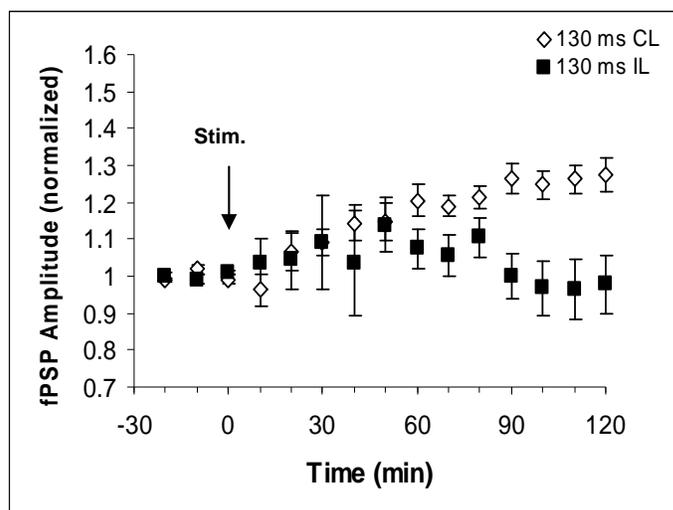
Figure 9: *Comparison of fPSP amplitude in ipsilateral* (IL, n = 6) *and contralateral projections* (CL, n = 13) following paired BF burst and LGN stimulation at the 130 ms delay interval.

fPSP amplitude differences were most notable between 90-120 minutes after the initiation of the pairing protocol.

3.6 ECoG Activation Through Basal Forebrain Stimulation

To determine successful BF activation, at the end of each experiment we recorded ECoG activity at the V1 site (Fig. 10), while stimulating the BF. When the electrodes were successful in targeting the BF, stimulation resulted in the change of cortical ECoG activity from a state of large amplitude, synchronized waves, to a state of small amplitude, desynchronized waves.
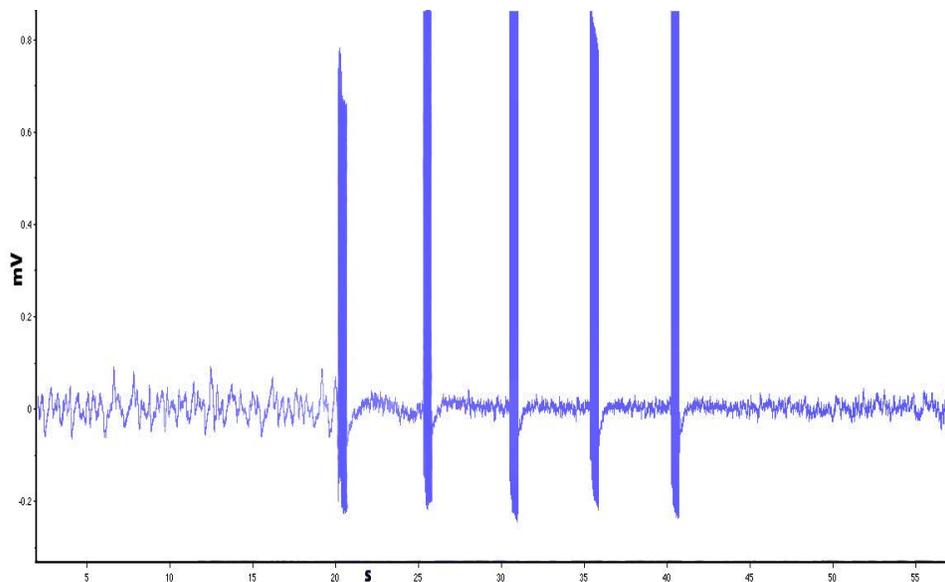
Figure 10: *The effect of BF stimulation on the electrocorticogram.* Solid vertical lines represent stimulation to the BF (50 pulses at 100 Hz, 0.2 ms pulse duration, 1.0 mA, 5 stimulation bursts at 0.2 Hz).

Chapter 4

Discussion

The present experiments demonstrate that different inputs to V1 exhibit varying degrees of sensitivity to cholinergic neuromodulation. The long-latency, crossed inputs between LGN and V1 exhibited significant potentiation when paired with BF stimulation. In contrast, the more direct, shorter-latency ipsilateral fiber system exhibited little or no synaptic enhancement with this type of stimulation protocol. Interestingly, field potentials evoked in V1 through stimulation of the contralateral LGN appeared to be optimally enhanced when the pairing delay between BF burst stimulation and trailing LGN single pulses was set to 130 ms. This enhancement was sensitive to the local and systemic application of scopolamine, suggesting an effect dependent on the activation of cortical mAChRs. The major source of ACh in V1 and throughout the cortex is the BF (Semba, 2000; Vaucher et al., 2003; Fournier et al., 2004). Previous studies have shown that stimulation of the BF results in increased levels of ACh throughout the cortex (Casamenti et al., 1986). Therefore, we assume that stimulation of the BF resulted in ACh release and mAChR stimulation in V1, thus leading to this neuromodulatory facilitation. A previous study implicated the involvement of mAChRs in the enhancement of LTP induced by TBS of fibres between the LGN and ipsilateral V1 (Dringenberg et al., 2007). Thus, there is considerable evidence that the release of ACh has the potential to exert important, regulatory effects on synaptic plasticity in the V1 of rats.

4.1 The Significance of mAChRs Antagonism within V1

Pharmacological studies have identified four mAChR receptor subtypes in the cortex (M_{1-4}; Caullfield & Birdshall, 1998). Molecular techniques have found an additional fifth subtype, the M_5 (Weiner et al., 1990). The mAChRs are metabotropic receptors and act through intracellular secondary messengers via coupled G-proteins. Coupling to different types of G proteins is commonly used to classify receptors into larger groups or families. The M_2 and M_4 receptors have been shown to couple with PTx (pertussis toxin treatment)-sensitive G_i+G_o proteins, while the M_1 and M_3 receptors activate PTx-insensitive $G_{q/11}$ and G_{13} proteins (Arneric et al., 1995; Ehlert et al., 1995; Felder 1995). The significance of this is that M_2 and M_4 receptor activation results in inhibition of adenyl cyclase, which leads to lower intracellular cAMP levels (review Wess, 1996). Binding of ACh to M_1 and M_3 receptors leads to the activation of phospholipase C and D, which mediate intracellular effects such as the relase of IP_3 (inositol 1,4,5 triphosphate) and DAG (diacylglycerol). Increasing levels of IP_3 and DAG in the postsynaptic cell leads to the release of Ca^{2+} from intracellular stores. The effect of M_1 or M_3 receptor activation to result in an increase in intracellular levels of Ca^{2+} is reminiscent of the Ca^2 increase seen following opening of NMDA channels, even though the mechanisms mediating these effects are highly distinct.

The various mAChR subtypes are also located on different elements of the synapse, with M_1 receptors found mostly on the postsynaptic membrane, while M_2 receptors primarily occupy the pre-synaptic side (Levey, 1996), where they function as an autoreceptor responsible for controlling ACh release. Much less is known about the precise, cellular location of M_3, M_4, and M_5 receptors.

Our study employed scopolamine, which acts to non-selectively antagonize mAChRs in V1. The most abundant mAChR in the rat visual cortex appears to be M_1 (Levey, 1993), but at present it is unknown whether this or another subtype is responsible for the enhancement seen in the contralateral LGN-V1 fibre system. It would be perilous to attribute a neuromodulatory effect within the complex circuitry of V1 to a specific receptor based on receptor numbers alone, especially when the receptor in question was not the specific target of the antagonist. Origlia and colleagues (2006) reported that LTP could be effectively induced in V1 slices of M_1-M_3 knockout mice, while it was abolished in M_2-M_4 knockout mice, suggesting a critical role of M_2 and/or M_4 receptors. Nevertheless, results obtained with genetically altered animals do not necessarily generalize to functions in an unaltered, normally developed nervous system. Clearly, the question regarding the relative roles of different types of mAChR remains to be addressed with future work.

The cellular mechanisms mediating the synaptic enhancement in the crossed fibre system between LGN and V1 are currently unknown. Cholinergic axons originating in the BF terminate on several cell-types within the cortex, each of which presumably contains different distributions of mAChRs. Cholinergic fibers contact different populations of neurons in V1, including pyramidal and spiny stellate cells. These contacts are mostly on dendritic shafts and less often on spines (Wainer et al., 1984). Inputs to GABAergic interneurons have also been described (Beaulieu & Somogyi, 1991), but details of the precise nature of these connections are not well established. While the effects of ACh application on the membrane potential of cortical pyramidal cells can be complex, common responses include a short-lasting hyperpolarization, followed by a

longer depolarization, as well as a reduction of the inhibitory after-hyperpolarization

following discharge (Rasmusson, 2000). The initial hyperpolarization was explained as

the result of activation of GABAergic interneurons, while the depolarizing effects and

after-hyperpolarization reduction are due to suppression of outward potassium currents

(McCormick & Prince 1986; Rasmusson, 2000). Interestingly, both of these effects can

be blocked by the mAChR antagonist scopolamine (Bernardo & Prince, 1982;

McCormick & Prince 1986).

4.2 The Temporal Relationship Between Bursts of the BF and Pulses in the LGN

Electrical stimulation of the BF has been shown to result in the release of ACh

throughout the neocortex (Casamenti et al., 1986). The nature of the stimulation is

important, because continuous stimulation trains appear to result in different patterns of

ACh release when compared with protocols consisting of brief, repeated stimulation

bursts. When continuous stimulation was applied to the BF for 10 minutes, stimulation

frequencies of 20-50 Hz resulted in the greatest release of ACh, while 100 Hz stimulation

was less effective, and frequencies between 5-10 Hz elicited little or no increase in

cortical ACh (Kurosawa et al., 1999). If BF stimulation consisted of pulse trains repeated

once every second, the highest cortical ACh release occurred at a stimulation frequency

of 100 Hz (Rasmusson et al., 1992). Based on the latter finding, was used 100 Hz

stimulation for our experiments, aiming to achieve maximal levels of cortical ACh

release.

4.3 Heterosynaptic Plasticity Induced by ACh

In order for BF stimulation to modify inputs to V1 or any other part of the cortex, ACh levels should be elevated when a particular input is active. This would mean that enhancement should occur at an optimal temporal window, or interval, between BF stimulation and the arrival of a stimulus. The interval would depend on factors such as speed of conduction along BF efferents, duration of neurotransmitter release at the terminals, duration of ACh binding to the receptors and the time required for mAChRs to induce intracellular changes (Rasmusson, 2000). Our experiments have shown that long lasting synaptic enhancement occurs when the BF was activated 130 ms prior to a stimulation of the contralateral LGN. Longer and shorter delay intervals did not reliably produce enhancement in this fiber system. The optimal time characterized in the present experiment window fits reasonably well with previous evidence showing that responses elicited in somatosensory cortex by tactile stimulation show maximal enhancement when the tactile stimulus followed BF stimulation by 20-200 ms (Rasmusson & Dykes, 1988). It is important to note that synaptic enhancement was not simply caused by activation of the BF alone, since applying BF bursts without subsequent LGN single pulses did not result in significant synaptic enhancement. Consequently, the effect requires co-activation of two separate inputs to V1, typically of other forms of synaptic plasticity, especially heterosynaptic forms of LTP (e.g., Dringenberg et al. 2007). Similar results have been described in other areas of the neocortex. In the somatosensory cortex of rats, BF stimulation paired with cutaneous electrical stimulation of the hind-paw, produced long-term enhancement of sensory-evoked potentials, an effect that is sensitive to

blockade by atropine (Verdier and Dykes 2001). This would again suggest the importance of mAChRs in enhancement of cortical synaptic plasticity.

4.4 Receptive Field Plasticity and Cholinergic Neuromodulation

The results described above are reminiscent of examples of receptive field plasticity induced by mAChR activation in visual and other sensory cortices. In the visual cortex, neurons respond to bars of light presented in the visual scene at different orientations. Different neurons respond optimally to preferred orientations of the bars of light. These preferred responses are subject to neuromodulatory influence. In V1, iontophoretic application of ACh paired with visual stimulation results in facilitation of modifications to receptive fields of cortical neurons (Gruel et al., 1988). When sub-optimal visual stimuli were presented to cells and paired with iontophoretic application of ACh to the cortex, responses to the sub-optimal orientations became stronger while at the same time responses of the same cell to the originally preferred orientation became weaker (Gruel et al., 1988; Gu, 2003). Similar results have been obtained in the primary auditory cortex (ACx). ACh or AChR agonists, iontophoretically applied to the ACx, have been shown to produce long-lasting shifts in the frequency tuning of individual neurons of awake cats. This was reflected by increased responses to non-preferred frequencies of individual neurons, and corresponding decrease in response to initially preferred frequencies (McKenna et al., 1989; Ashe et al., 1989; Metherate & Weinberger, 1990). These studies suggest that muscarinic receptor activation in ACx produces enhancements in responses to weaker, non-optimal sensory input. It was also suggested that endogenous ACh functions in reshaping ACx neuronal receptive fields in accordance with stimuli in the environment of behavioral significance (Ashe et al., 1989). These

studies were later extended to show that pairing of tetanic BF stimulation with single pulses to the thalamus, could facilitate fPSPs recorded in the auditory cortex of urethane anesthetized rats (Metherate and Ashe, 1991). Together, the work summarized above is consistent with the data reported in this thesis and clearly highlights the important, permissive role played by ACh on plasticity in several cortical sensory domains.

4.5 Cortical Oscillations, Synchrony and Signal Processing

Activation of the ECoG induced through stimulation of the BF is suppressed by the administration of mAChR antagonists (Metherate et al., 1992). Stimulation of the BF with current delivered through electrodes results in ACh release in the cortical mantle (Casamenti et al., 1986). In urethane-anesthetized rats, cortical ECoG activation was described as the suppression of lower frequency bands (delta, theta, alpha) and enhancement of higher (beta) bands (Dringenberg et al., 2007), while changes in other bands were not reported. These effects resulting from stimulation of the BF were brief and reported to last less than 60 seconds following stimulation (Dringenberg et al., 2007).

Anesthesia induced through a variety of substances results in the appearance of continuous, large amplitude, slow oscillations in the ECoG. It is known that these large-slow oscillations contain embedded high frequency waves in different bands. For example, gamma waves are prominent during deep negative components of slow neocortical oscillations but suppressed during deep positive components (Vanderwolf, 2000). It is also known that cortical neuronal cell discharge occurs during the deep negative components of slow oscillations and suppresses during the deep positive components (Vanderwolf, 1998). These observations would suggest that gamma bursts correlate with neuronal cell firing and gamma suppression occurs during epoch lacking

33

neuronal cell discharge. Furthermore, gamma waves result from the synchronized, oscillatory firing of inhibitory interneurons (Whittington et al., 1995; Wang and Buzsaki, 1996). Their rhythmic IPSP activity has been suggested to be responsible for synchronized firing of pyramidal cells (Traub et al., 1997). Application of scopolamine via iontophoresis in the V1 of cats was shown to interfere with cortical gamma wave activity (Rodriguez et al., 2004). Moreover, it has been suggested that a steady activation of mAChRs is a prerequisite for synchronization of visual responses in the gamma range (Rodriguez et al., 2004). Increased gamma band activity has been linked to cortical processing of visual stimuli (Keil et al., 1999). Interestingly, the degree of synchrony among oscillatory discharges of interconnected neurons has been implicated as a critical variable in controlling the occurrence of synaptic modification (review by Singer, 1993). It is currently unknown if synchrony within the gamma band is important to the enhancement described in our results.

4.6 Anesthetic Effects of Urethane

It is important to consider the effects of the anesthetic used in this experiment. Urethane is chosen for many studies as it is generally believed that it exerts less general, suppressive effects on neocortical activity than many other anesthetic agents, as well as providing long lasting, surgical plane anesthesia with minimal effects on the cardiovascular and autonomic systems (Magi and Melli, 1986; Soma, 1983). It has been reported that urethane is able to potentiate responses of nAChRs, at least in oocytes expressing surface receptors, as well as modestly enhancing $GABA_a$ receptor (Hara and Harris, 2002). Although there appears to be general agreement that the urethane doses required for surgical plane are suitable for effective electrophysiological experiments, the

authors cautioned that excessive doses have substantial effects on receptor systems within the CNS (Hara & Harris, 2002).

Urethane anesthesia can also affect neuronal activity in the BF, generally by reducing discharge of BF neurons (Detari et al., 1997; Detari & Vanderwolf, 1987). However, it was shown that even in surgical plane urethane anesthesia, there remained a basal release of ACh on the cortex (Rasmusson, 1992). The effect on the cortical EEG under deep urethane anesthesia seen in our experiments was the occurrence of synchronized, slow oscillations in V1. The fact that BF stimulation can effectively elicit EEG/ECoG activation under deep urethane anesthesia shows that the well-characterized activating effect of the BF-cholinergic system is intact in the deeply anesthetized urethane preparation.

4.7 Future Directions and Recommendations

There are several questions, which were not addressed in this study and may require additional attention in the future. For instance, although the enhancement was sensitive to mAChR antagonists, it was not shown which subtype of receptor was critical for this effect. Currently, antagonists are available for 4 out of the 5 mAChR subtypes and only the M_5 receptor cannot be selectively blocked. It would be useful to determine the exact mAChR subtypes to more fully characterize this phenomenon described here. Additionally, it is currently unknown how long this enhancement potentially lasts. All experiments performed have only measured synaptic strength for two hours following the induction protocol. It would be useful to run longer-lasting experiments to determine if this enhancement is maintained over time. Several studies have reported increased Gamma Band Synchrony (GBS), in the ECoG following stimulation of the BF, it would

be interesting to see if enhanced GBS was a prerequisite for the enhancement observed as a result of this type of induction protocol. The specific timing interval important to the enhancement, and the involvement of mAChRs are significant clues to understanding the mechanisms governing the results of this study. However, the nature of the contralateral fPSP also requires attention. As there are no direct connections between the contralateral LGN and V1, it is difficult to determine the route along which the signals travel. It would be extremely interesting to locate the path and neurons which are involved in generating the recorded fPSPs. This step is also crucial to understanding the mechanism of this effect and in allowing some interpretation of how this phenomenon fits into signal processing in V1.

4.8 Conclusions

The results of these experiments show that different inputs to V1 exhibit varying degrees of sensitivity to cholinergic neuromodulation. The long-latency, crossed inputs between LGN and V1 exhibit significant potentiation when paired with BF stimulation. In contrast, the more direct, shorter-latency ipsilateral fiber system exhibit little or no synaptic enhancement with this type of stimulation protocol. Activation of mAChRs in V1 was important for the observed effect.

The several billion neurons within the cortical mantle generate a constant rumble of electrical activity regardless of our behavioural states and beyond the reaches of our awareness. This neuronal system of immense connectivity has the capability to tune into specific signals, amplify them, and allow precise processing of stimuli in the environment by focusing the sensory information from various modalities upon relevant neuronal circuits. This task if effectively carried out by neuromodulatory systems, which are found

throughout the brain and function along in concert with classical excitatory and inhibitory synaptic transmission. An improved understanding of the mechanisms and functions of cortical neuromodulation will aid greatly in furthering our knowledge of general cortical processing in normal states, as well as diseases characterized by significant dysfunctions of these neuromodulatory inputs.

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