**Gating of Long-Term Potentiation (LTP) in the Thalamocortical Auditory System of Rats
by Serotonergic (5-HT) Receptors**

by

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Abstract

The neuromodulator serotonin (5-hydroxytryptamine, 5-HT) plays an important role in controlling the induction threshold and maintenance of long-term potentiation (LTP) in the visual cortex and hippocampus of rodents. Serotonergic fibers also innervate the rodent primary auditory cortex (A1), but the regulation of A1 plasticity by 5-HT receptors (5-HTRs) is largely uncharted. Thus, we examined the role of several, predominant 5-HT receptor classes (5-HT_{1A}Rs, 5-HT₂Rs, and 5-HT₃Rs) in gating *in vivo* LTP induction at A1 synapses of adult, urethane-anesthetized rats. Theta-burst stimulation (TBS) applied to the medial geniculate nucleus resulted in successful LTP induction of field postsynaptic potential generated by excitation of thalamocortical and intracortical A1 synapses. Local application (by reverse microdialysis in A1) of the broad-acting 5-HTR antagonist methiothepin suppressed LTP at both thalamocortical and intracortical synapses, an effect that was mimicked by the selective 5-HT₂R antagonist ketanserin, but not the 5-HT_{1A}R blocker WAY 100635. Interestingly, antagonism of 5-HT₃Rs by granisetron selectively blocked LTP at thalamocortical, but not intracortical A1 synapses. Further, in the absence of TBS delivery, granisetron application resulted in a pronounced increase in fPSP amplitude, suggesting that 5-HT₃Rs play an important role in regulating baseline (non-potentiated) transmission at A1 synapses. Together, these results indicate that activation of 5-HT₂Rs and 5-HT₃Rs, but not 5-HT_{1A}Rs, exerts a clear effect on LTP induction at A1 synapses, allowing 5-HT to act as a powerful regulator of long-term plasticity induction in the fully matured A1 of mammalian species.

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Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
List of Figures.....	vi
Chapter 1 Introduction.....	1
1.1. General Introduction.....	1
1.1.2. Synaptic plasticity: LTP and LTD.....	1
1.1.3. 5-HT Receptors.....	3
1.1.3.1. 5-HT1 Receptors.....	4
1.1.3.2. 5-HT2A Receptors.....	5
1.1.3.3. 5-HT3 Receptors.....	6
1.1.4. The Effects of 5-HT on LTP.....	6
1.1.4.1. 5-HT Effects on Synaptic Plasticity in the Hippocampus.....	6
1.1.4.2. 5-HT Effects on Synaptic Plasticity in the Prefrontal Cortex.....	8
1.1.4.3. 5-HT Effects on Synaptic Plasticity in the Visual Cortex.....	9
1.1.4.4. 5-HT Effects on Synaptic Plasticity in the Auditory Cortex.....	10
1.1.5. Summary, Objectives, and Hypothesis of the Present Thesis.....	11
1.2. Gating of LTP in the Thalamocortical Auditory System of Rats by 5-HTRs	14
Chapter 2 Materials and Methods.....	17
2.1. Subjects.....	17
2.2. Surgical preparation.....	17
2.3. Electrophysiology.....	18
2.4. Drug application.....	19
2.5. Data collection.....	19
2.6. Histology.....	20
2.7 Data Analysis.....	21
Chapter 3 Results.....	23
3.1 fPSP characteristics and LTP in during vehicle (aCSF/saline) application.....	23

3.2 Effects of 5-HTR antagonist application in A1 on LTP.....	24
3.3 Effects of 5-HTR antagonist on baseline (pre-TBS) fPSP amplitude.....	27
Chapter 4 Discussion.....	36
References.....	43

List of Figures

Figure 1. Typical fPSPs recorded in A1 in response to ipsilateral MGN stimulation in the urethane-anesthetized rat.....	22
Figure 2. Effects of cortical application of methiothepin on LTP induction in A1.....	29
Figure 3. Effects of cortical application of WAY 100635 on LTP induction in A1.....	30
Figure 4. Effects of cortical application of ketanserin on LTP induction in A1.....	31
Figure 5. Effects of cortical application of granisetron on LTP induction in A1.....	32
Figure 6. Amplitude of fPSPs in all drug conditions prior to LTP induction.....	33
Figure 7. Effects of cortical application of granisetron on fPSP amplitude in A1.....	35

Chapter 1

Introduction

1.1. General Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter found in the central nervous system (CNS), gastrointestinal tract, and blood platelets (Nichols & Nichols, 2008). The 5-HT signaling molecule is widely distributed throughout the brain and innervates areas that mediate crucial brain functions, such as sensory processing, emotional regulation, and executive functioning. Further, this phylogenetically ancient neurotransmitter has the ability to reconfigure the neural circuitry of sensory and motor systems (Lesch & Waider, 2012; Nichols & Nichols, 2008; Ji & Suga, 2007). More specifically, 5-HT plays an important role in modulating synaptic plasticity, development of axons and dendrites, synaptogenesis, neurogenesis, and cell migration in the CNS (Lesch & Waider, 2012; Nichols & Nichols, 2008; Ji & Suga, 2007; Rao, 2012). As such, the serotonergic system and its receptors drive brain plasticity, which in turn can cause observable behavioral changes. Understanding how the serotonergic system influences neuroplasticity is essential for the understanding of normal and abnormal behaviors, in addition to the emerging concepts of treatment designs for the behavioral and psychological aspects of psychopathologies, ranging from Alzheimer's disease to drug addiction.

1.1.2. Synaptic plasticity: LTP and LTD

The extensive central 5-HT neurotransmitter system that is established for brain 5-HT neuronal signaling is known for its role in modulating cortical synaptic plasticity (Deneris, 2011). 5-HT projections originate from the median and dorsal raphe nuclei and innervate the entire forebrain via the dense network of 5-HT fibers (Lesch & Waider, 2012; Deneris, 2011). Synaptic plasticity is the ability of neuronal circuits and connectivity to either strengthen or

weaken over time. This capability allows the nervous system to adapt behavior, and to anticipate and control executive functions in accordance to the sensory information obtained from the changing environment (Feldman, 2009).

One form of synaptic plasticity is long-term potentiation (LTP), which refers to a long-lasting increase in synaptic strength (Bliss and Lomo, 1973). LTP and long-term depression (LTD; i.e., a long-lasting decrease in synaptic coupling) are two of the probable mechanisms mediating experience-dependent plasticity of the cortex (Rao, 2012). The duration and magnitude of LTP are known to be influenced by various modulators, such as acetylcholine (ACh), 5-HT, noradrenaline, and others (Hasselmo, 1995).

LTP and LTD are forms of neuroplasticity that were first discovered in the hippocampus and later identified to be present at synapses throughout the forebrain, including the prefrontal, auditory, visual, and somatosensory cortices (Bennett, 2000; Malenka and Bear, 2004); both forms of plasticity have been identified as a probable mechanism that mediates CNS memory and learning processes (Lynch, 2004; Martin and Morris, 2002; Massey and Bashir, 2007; Neves et al., 2008). LTP can be defined as the process for the enhancement of synaptic efficacy that requires almost simultaneous activation of presynaptic activity (i.e., transmitter release) and postsynaptic depolarization that involves opening of both N-methyl-D-aspartate receptors (NMDAR) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), along with the increase of intracellular calcium (Bliss & Cooke, 2011; Meunier et al., 2017). The voltage dependent NMDAR is an ion channel protein and belongs to the general class of glutamate receptors (Bliss & Cooke, 2011), while AMPAR act as rapid synaptic neurotransmission mediators in the CNS by acting as ionotropic transmembrane receptors for glutamate (Meunier et al., 2017).

Typically, the induction of LTP is initiated by pre-synaptic glutamate release, causing the activation of post-synaptic AMPAR (Bliss & Cooke, 2011; Meunier et al., 2017). Once the post-synaptic membrane is sufficiently depolarized, magnesium ions will be ejected from the NMDAR ion pore, allowing calcium and other cation to influx into the post-synaptic membrane (Bliss & Cooke, 2011; Meunier et al., 2017). This influx results in the activation of several intracellular, calcium-dependent events, such as calmodulin-dependant protein kinase II (CaMKII), which causes AMPAR phosphorylation and/or AMPAR trafficking to the post-synaptic membrane (Bliss & Cooke, 2011; Meunier et al., 2017).

Synaptic plasticity can be measured through field postsynaptic potentials (fPSPs). fPSPs are extracellular potential recordings of a population of cells (often pyramidal or granule cells in neocortical or hippocampal areas) following synaptic stimulation (Andersen et al., 2007; Lagerlund, 2002). The main advantage of this measurement is that fPSPs can provide detailed information on synaptic activity such as the amplitude, latency, and polarity of synaptic activity (Andersen et al., 2007; Lagerlund, 2002). There are two types of fPSPs: excitatory postsynaptic potentials (EPSP) are caused by inward, depolarizing current flow, that is, an inward (into the neuron) flow of positive ions, which results in negative, local extracellular potentials (Andersen et al., 2007; Lagerlund, 2002); inhibitory postsynaptic potentials (IPSP) are caused by a net outward, positive current flow, or a flow of negative ions into the cell, which results in positive local extracellular potentials (Andersen et al., 2007; Lagerlund, 2002).

1.1.3. 5-HT Receptors

As mentioned, while the initiation of LTP typically involves glutamatergic signalling, neuromodulators such as 5-HT can influence the induction threshold, magnitude, and duration of

LTP. The effects of 5-HT in regulating behaviour, cognition, neuronal activity, and synaptic plasticity are mediated by a number of distinct 5-HT receptors (5-HTRs) that are linked to different signal mechanisms and ion channels (Deneris, 2011; Nichols & Nichols, 2008). 5-HT neuromodulation in rats originates in about 26000 serotonergic neurons, along with 14 5-HT postsynaptic and/or presynaptic receptor subtypes (Deneris, 2011; Basura et al., 2008; Nichols & Nichols, 2008); of these 14 subtypes, the 5-HT1Rs, 5-HT2Rs and 5-HT3Rs subtypes are those that have been studied in most detail.

1.1.3.1. 5-HT1 Receptors

Binding of 5-HT to the 5-HT1R decreases cellular excitability of 5-HT-containing neurons and mediates hyperpolarization of cells in the prefrontal and entorhinal cortices (Araneda et al., 1991; Beique et al., 2004; Grunschlag et al., 1997; Nichols & Nichols, 2008). Neuronal membrane hyperpolarization is the most common function of this G α_i coupled receptor, which leads to the suppression of neuronal firing (Nichols & Nichols, 2008). G protein coupled receptors span the intracellular and extracellular domains through seven transmembrane spanning helices (Nichols & Nichols, 2008). The 5-HT1R is expressed both presynaptically and postsynaptically throughout the brain and can negatively couple to the adenylyl cyclase second messenger system to inhibit cAMP formation (Basura et al., 2008; Sodhi & Sanders-Bush, 2004, Nichols & Nichols, 2008). The 5-HT1R family can be divided into the following subtypes: 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, and 5-HT1F (Nichols & Nichols, 2008). 5-HT1ARs are also located on neurons in the raphe nuclei, where they act as somatodendritic autoreceptors (Nichols & Nichols, 2008). Out of all the 5-HTR subtypes, the structure of the 5-HT1AR is most similar

to adrenergic receptors, which leads 5-HT1ARs to have a relatively high binding affinity for adrenoceptor agents (Nichols & Nichols, 2008).

1.1.3.2. 5-HT2A Receptors

The 5-HT2AR has a major role in neuronal development, cell migration, and neuronal activity regulation, especially in the prefrontal cortex (Guiard & Giovanni, 2015). 5-HT2AR is a type of Gαq coupled receptor (Nichols & Nichols, 2008), which has two main mechanisms of action. The first mechanism is a cascade that begins with the hydrolysis of the membrane phosphoinositides, followed by subsequent formation of the signaling molecules, inositol phosphates and diacyl glycerol (Nichols & Nichols, 2008), activation of protein kinase C and, finally an elevation of intracellular calcium (Nichols & Nichols, 2008). The second mechanism starts with the activation of the Rho signaling pathway, leading to the production of focal adhesion molecules and fibers that regulate structural changes inside the cell (Nichols & Nichols, 2008).

In the human brain, levels of 5-HT2AR are heterogeneously distributed, with particularly high 5-HT2AR densities distributed in cortical layers III and V (Nichols & Nichols, 2008). These cortical areas include the frontal cortex, parietal cortex, temporal cortex, occipital cortex, and entorhinal area (Nichols & Nichols, 2008). 5-HT2ARs have been associated with many physiological functions such as sleep, memory, nociception, reward behaviors, and neuropsychiatric disorders such as depression (Guiard & Giovanni, 2015), depending on the particular location of these receptors.

1.1.3.3. 5-HT₃ Receptors

5-HT₃Rs are ligand-gated cation channels and mediate rapid synaptic brain transmissions (Sodhi & Sander-Bush, 2004; Walstab, Rappold & Niesler, 2010; Lummis, 2004). Structurally, 5-HT₃R have a cation permeable water filled pore surrounded with five subunits (Walstab, Rappold & Niesler, 2010; Lummis, 2004). The permeable ions include calcium, sodium, and potassium (Walstab, Rappold & Niesler, 2010; Lummis, 2004). 5-HT₃R are also part of the Cys-loop family, which includes γ -aminobutyric acid (GABA) A, nicotinic acetylcholine receptors, glycine receptors and Zinc activated cation channel (Walstab, Rappold & Niesler, 2010; Lummis, 2004). Together, accumulating evidence indicates that 5-HT₃R can function as modulators not only of 5-HT release, but also the release of GABA, dopamine, and acetylcholine (Farber et al., 2004; Walstab, Rappold & Niesler, 2010).

1.1.4. The Effects of 5-HT on LTP

The role of 5-HT in synaptic plasticity has been assessed with various methods, mostly in the hippocampus, amygdala, and the visual cortex, as well as a few investigations in the pre-frontal cortex and auditory cortex (Cynader et al., 1980; Persico et al., 2001; Basura et al., 2008). The various methods used to manipulate 5-HT activity include the pharmacological depletion of serotonin or the serotonin precursor, tryptophan; creating serotonergic circuit lesions; and the blockade of specific types of serotonergic receptors.

1.1.4.1. 5-HT Effects on Synaptic Plasticity in the Hippocampus

Hippocampal 5-HT innervation originates in the raphe (the dorsal and median raphe) nuclei of the brainstem (Wyss et al., 1979). 5-HT neurons form nine nuclei clusters along the

rostrocaudal axis and can be divided into three sections, the dorsal raphe nuclei, the median raphe nuclei and the caudal linear nucleus (Dahlstrom & Fuxe; 1964). The rostral section mostly projects to the forebrain and innervates the hippocampus (Gulyás et al., 1999).

Investigations in the hippocampus have found that 5-HT can influence the induction of LTP. 5-HT application to the hippocampus has been shown to activate 5-HT receptors and inhibit LTP induction (Villani & Johnston, 1993; Corradetti et al., 1992). This LTP inhibition can be partially blocked and LTP can be facilitated by 5-HT antagonists that block 5-HT₁, 5-HT₂, or 5-HT₃ receptors (Villani & Johnston, 1993; Reznic & Staubli, 1997; Corradetti et al., 1992; Passani et al., 1994).

The 5-HT₁ARs, which are localized in high density on granular and pyramidal cells, are known to hyperpolarize hippocampal cells (Burnet et al., 1995). By using 5-HT₁AR agonists, Normann and colleagues (2000) demonstrated that 5-HT₁AR agonists impair hippocampal plasticity (LTD) induction. Further, Kojima and colleagues (2003) suggested that 5-HT₁ARs may be responsible for the inhibition of LTP in CA1 seen following administration of a selective serotonin reuptake inhibitor (SSRI). As for 5-HT₂ARs, its agonists have been shown to facilitate LTP in the hippocampus (Wang & Arvanov, 1998). 5-HT₃Rs are located in high densities on interneurons in the hippocampus (Morales et al., 1996; Piguet & Galvan, 1994). Hippocampal 5-HT₃Rs have been associated with the stimulation of GABA release, leading to membrane hyperpolarization of hippocampal neurons (Morales et al., 1996; Piguet & Galvan, 1994). Staubli and Xu (1995) applied 5-HT₃R antagonists onto the hippocampus and discovered that this facilitated LTP, which indicates that activation of 5-HT₃Rs exerts an inhibiting effect on hippocampal LTP inductions; this conclusion is consistent with data by Ohashi et al. (2003), showing that 5-HT acts to limit LTP induction in the hippocampus.

1.1.4.2. 5-HT Effects on Synaptic Plasticity in the Prefrontal Cortex

A dysfunction or downregulation of post-synaptic 5-HT_{1A}R in the PFC has been associated with anxiety disorders, depression, as well as fear disorders and psychosis (Lanzenberg et al., 2007).

Although the specific functions of 5-HT_{1A}Rs in the PFC are still not well understood, it is known that they regulate the excitability of glutamatergic and GABAergic neurons, either on the neuronal targets of 5-HT neurons, as heteroreceptors, or on the serotonergic neurons themselves as autoreceptors, where they exert negative feedback over the release of 5-HT (Puig & Gullledge, 2011). Between 50-60% of post-synaptic 5-HT_{1A}R are expressed in glutamatergic neurons, while 25% are expressed in GABAergic interneurons, which then innervate pyramidal cells in the PFC (Santana et al., 2004).

Recently, Meunier and colleagues (2017) have proposed an intriguing model of how 5-HT_{1A}R may modulate LTP in the PFC. Like other forms of LTP (e.g., in the hippocampus), LTP and LTD in the PFC are thought to involve NMDAR and the subsequent externalization (for LTP) or internalization (for LTD) of AMPA receptors, depending on the magnitude of calcium signalling in the postsynaptic cell (Meunier et al., 2017).

Meunier and colleagues (2017) conducted multiple experiments by decomposing synaptic conductance into its excitatory and inhibitory components. First, Meunier and colleagues (2013) showed that the activation of 5-HT_{1A}R increased the evoked NMDA currents, which indicates a 5-HT_{1A} receptor-induced modulation of NMDA receptors. Subsequently, Meunier and colleagues (2017) experimented on the layer V pyramidal neurons of 5-HT_{1A}R knock-out mice and found that excitatory synapse transmission is modulated by the presence of 5-HT_{1A}R. Further, 5-HT_{1A}Rs are necessary for the activation of glycogen synthase kinase 3 β (GSK3 β),

which shifts the excitation-inhibition balance of synaptic plasticity towards favoring LTD over LTP induction (Meunier et al., 2015; Meunier et al., 2017).

1.1.4.3. 5-HT Effects on Synaptic Plasticity in the Visual Cortex

There are many studies that have examined the role of 5-HT in the gating of plasticity at V1 synapses. Several studies have found that 5-HT impairs plasticity in V1 by blocking or inhibiting LTP induction. Levels of 5-HT in V1 show a pronounced increase over the course of postnatal development, resulting in the activation of 5-HT₁ and 5-HT₂ receptors, both of which have been shown to inhibit LTP induction (Kim et al., 2006; Gagolewicz & Dringenberg, 2016). In particular, perhaps the only in vivo experiment of the influence of 5-HT LTP in V1 has shown an age-dependent switch in the role of 5-HT₁AR in gating V1 plasticity, suggesting that 5-HT₁ARs facilitate LTP in the juvenile brain, but switch to an inhibitory function in the fully matured V1 (Gagolewicz & Dringenberg, 2016); as mentioned, a similar effect of 5-HT to inhibit LTP has also been shown in the hippocampal formation of rodents.

In contrast, other studies have suggested that 5-HT facilitates LTP induction in the visual cortex (Kojic et al., 1997; Kojic et al., 2000; Park et al., 2012) with 5-HT₂ receptor being the major LTP facilitator in layer 4 of the primary visual cortex (Kojic et al., 2001). Other studies have suggested that the facilitation of LTP induction is caused by presynaptic 5-HT₂ receptors (Rozas et al., 2012) that activate protein kinase C and subsequently elevate intracellular calcium (Nichols & Nichols, 2008).

Together, a brief review of the literature suggests that the precise role of 5-HT in LTP modulation in the visual cortex is still not well understood. Factors such as investigating LTP under in vivo vs. in vitro conditions, or the use of rodents of different ages and developmental

status may account for some of the discrepancies in the experimental results. However, it is clear that further studies are required to develop a more complete understanding of the functions of 5-HT in the gating of cortical plasticity, including V1.

1.1.4.4. 5-HT Effects on Synaptic Plasticity in the Auditory Cortex

To date, the role of 5-HT as a regulator of plasticity induction in the primary auditory cortex (A1) has not received a lot of attention. Several studies have assessed the functional consequences of alterations in 5-HT transmission (e.g., 5-HT/tryptophan depletions) on activity in A1 (Ahveninen et al., 2003; Kahkonen et al., 2002a; Kahkonen et al., 2002b; Del Angel-Meza et al., 2002; Hegerl & Juckel, 1993). These studies found evidence to support the notion that 5-HT regulates the processing of auditory stimulus intensity (Kahkonen et al., 2002b; Hegerl & Juckel, 1993) and may also play a role in regulating gender-specific stimulus processing by A1 neurons (Ahveninen et al., 2003; Kahkonen et al., 2002a).

Evidence for a more direct role of 5-HT and its receptors in auditory cortical plasticity was provided by Ji and Suga (2007), who examined tone-induced responses and receptive field plasticity in A1 neurons of bats. Earlier work had shown that exogenous 5-HT application can enhance GABAergic activity and reduce auditory-evoked responses in neurons of the inferior colliculus, the channel for the majority of auditory signals (Hurley et al., 2002). Ji and Suga (2007) extended this work by assessing if 5-HT modulates plasticity directly in A1, and by examining the receptor mechanisms that mediate effects of 5-HT in A1. Using an auditory fear condition paradigm, the bats were exposed to a conditioned tonal stimulus (CS; train of tone bursts) paired with the unconditioned stimulus (US; electric foot shock) applied every 30 s for either a 15 (weak stressed condition) or a 30 (strongly stressed condition) minute conditioning

period. Ji and Suga (2007) found that 5-HT application to A1 is effective in modulating conditioning-induced plasticity (increased neuronal responses to the CS) in A1 neurons. Interestingly, the precise functions of 5-HT changed with the level of stress experienced by the animal, in that 5-HT facilitated A1 plasticity in the mildly stressful condition, but exerted a suppressant effect on plasticity in the strongly stressful condition.

Lastly, the effects of 5-HT on LTP in A1 have been investigated through studies using treatment with a selective serotonin reuptake inhibitor (SSRI). Dringenberg et al. (2014) assessed the effects of chronic (for 2 weeks) treatment with the SSRI fluoxetine on thalamocortical (medial geniculate nucleus to A1) LTP in adult rats. The results showed that fluoxetine treatment suppressed LTP in A1, suggesting that chronic SSRI treatment may inhibit plasticity induction in the neocortex of adult rodents (Dringenberg et al., 2014). At present, it is not known whether this effect involves the serotonergic system, or whether it is mediated by other effects that result from chronic SSRI exposure.

1.1.5. Summary, Objectives, and Hypothesis of the Present Thesis

The summary provided above indicates that 5-HT can influence the induction of plasticity in many areas of the mammalian forebrain (e.g., hippocampus, amygdala, neocortex). The experiments contained in this thesis were designed to further clarify the role of 5-HT in the gating of plasticity induction in the mammalian A1. Specifically, I examined the specific roles of 5-HT1Rs, 5-HT2Rs, and 5-HT3Rs in the induction of thalamocortical LTP between the medial geniculate nucleus (MGN) and A1 of adult rats.

Prior work has demonstrated that serotonin acts to modulate plasticity in the rodent cortex. For the visual cortex, several lines of evidence indicate that 5-HT exerts an inhibitory

effect on LTP induction. In fact, the only *in vivo* study of the primary visual cortex concluded that 5-HT_{1A}R activation exerts an inhibitory role on LTP in adult rats; results from the *in vitro* studies are somewhat contradictory, with different papers reporting facilitating and inhibitory effects on LTP induction following 5-HT receptor stimulation. Since both the primary visual cortex and the primary auditory cortex are neocortical brain regions and primary sensory areas that receive sensory information from primary nerve projections, 5-HT may exert similar effects on LTP in both of these cortical regions. Consequently, I hypothesize that 5-HT will inhibit LTP induction in A1 of adult rats. This inhibitory effect will be removed by blockade of 5-HT receptors, resulting in a facilitation of LTP.

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Karen K.Y. Lee and Hans C. Dringenberg

(Submitted manuscript)

1.2. Gating of LTP in the Thalamocortical Auditory System of Rats by 5-HTs

Synaptic plasticity, or the ability of neuronal connectivity to change over time, plays a crucial role in the ability of the nervous system to adjust responses to incoming sensory stimuli and store information for prolonged time periods (Bliss and Collingridge, 1993; Hebb, 2002; Martin and Morris, 2002). Two widely studied forms of synaptic plasticity are long-term potentiation (LTP) and long-term-depression (LTD), which refer to long-lasting increases and decreases, respectively, of synaptic strength (Bennett, 2000; Malenka and Bear 2004). The interest in LTP and LTD stems, at least in part, from considerable evidence that the synaptic and molecular mechanisms mediating LTP and LTD are similar or identical to those recruited during training and memory formation, indicating that LTP and LTD constitute important mechanism for behavioral plasticity in various animal species (Lynch, 2004; Martin and Morris, 2002; Massey and Bashir, 2007; Neves et al. 2008).

LTP and LTD are readily induced at synapses throughout the forebrain, including the hippocampal formation, amygdala, striatum, and neocortex (Bennett, 2000; Malenka and Bear, 2004; Tsumoto, 1992). An important aspect of LTP is that the induction threshold, magnitude, and duration of synaptic potentiation are strongly influenced by the chemical milieu, particularly the levels of various neuromodulators such as acetylcholine (ACh), noradrenaline (NA), histamine, or serotonin (5-hydroxytryptamine, 5-HT; Dringenberg and Kuo, 2006; Gu, 2002; Kojic et al., 1997). Given that their release shows systematic changes over the sleep-wake cycle (Scammell et al. 2017), fluctuating levels of ACh, 5-HT, NA, and others likely play an important role in mediating the influence of waking and sleep states on the induction/encoding, consolidation, and elimination of synaptic plasticity and memory traces in neuronal circuits (Power, 2004).

The role of neocortical 5-HT release in gating plasticity induction and maintenance has been extensively studied in the visual cortex (V1) of rodents. In slices obtained from kittens or adult rats, bath application of 5-HT can result in a pronounced facilitation of LTP at V1 synapses (Kojic et al., 1997, 2000; Park et al., 2012), while 5-HT depletions have been shown to impair LTP induction, an effect that is mimicked by 5-HT receptor (5-HTR) antagonists acting at either 5-HT_{1A}Rs or HT₂Rs (Inaba et al., 2009). Even though these data suggest that 5-HT exerts a facilitating effect on LTP, there is also evidence that 5-HT can inhibit LTP induction in V1, thereby contributing to the stability of sensory networks (Edagawa et al., 1998, 2000; Gagolewicz and Dringenberg, 2016; Kim et al., 2006). The apparent discrepancy of these experimental results may be due to the differences in age and developmental status of these animals, since recent data suggest that 5-HT acts to facilitate and inhibit plasticity in juveniles and adult animals, respectively (Gagolewicz and Dringenberg, 2016).

Similar to V1, the temporal cortex of mammals, including the primary auditory cortex (A1), is densely innervated by 5-HT fibers originating mainly in the dorsal raphe nucleus (Campbell et al., 1987; Harding et al., 2004; Törk, 1990), and the major classes of 5-HTRs are expressed throughout the temporal/A1 cortex (Barnes and Sharp, 1999; Basura et al., 2008; Clemett et al., 2000; Cornea-Hebert et al., 1999; Garcia-Oscos et al., 2015; Kilpatrick et al., 1987; Leysen et al., 1982; Malgouris et al., 1993). Studies in bats subjected to an auditory fear conditioning paradigm have shown that 5-HT and 5-HTRs exert potent, gating functions over receptive plasticity induction in A1 (Ji and Suga, 2007). Further, 5-HT_{2A} receptors facilitate NMDA receptor (NMDAR)-dependent synaptic potentiation at thalamocortical synapses between the mediodorsal thalamus and prefrontal cortex in mice (Barre et al., 2016).

The thalamocortical auditory system between the medial geniculate nucleus (MGN) and A1 of rodents readily expresses NMDAR-dependent LTP in both juvenile and adult animals (Hogsden 2009a,b; Soutar et al., 2016; Speechley et al., 2007). LTP in this system has been suggested to mediate receptive field plasticity and other learning-related adjustments in synaptic connectivity following auditory experiences (Tzounopoulos and Leão, 2012). To the best of our knowledge, the role of different 5-HTRs on LTP in this system has not been addressed. Thus, we examined the effects of blockade of several classes of 5-HTRs (5-HT_{1A}Rs, 5-HT₂Rs, and 5-HT₃Rs) on the induction of LTP at thalamocortical and intracortical synapses in A1 of adult rats *in vivo*.

Chapter 2

Materials and Methods

2.1. Subjects

The experiments were conducted on adult, male Long-Evans male rats (about 250-450 g; obtained from Charles River Laboratories Inc., Saint-Constant, Quebec, Canada). Each rat was used for one experiment only. Animals were group housed (maximally 4 rats/cage) in standard plastic cages (51 x 40 x 21cm) under a 12:12 reversed light cycle (lights on at 19:00h). Room temperature was kept at $21 \pm 2^{\circ}\text{C}$ and each cage had food and water available ad libitum. All efforts are made to minimize animal suffering and the number of rats employed for all experiments. All experimental procedures were in compliance with the regulations set by the Canadian Council Animal Care Committee and approved by the Queen's University Animal Care Committee.

2.2. Surgical preparation

Rats were deeply anesthetized by means of urethane (Sigma-Aldrich, Oakville, ON., Canada) administration (total dose of 1.5 g/kg, administered as three intraperitoneal (i.p.) 0.5 g/kg injections every 15-20 minutes, with supplements provided as necessary). Following anesthesia induction, the local analgesic Marcaine (bupivacaine, Hospira Healthcare Corporation, Montreal, Quebec, Canada), was administered as 2 mg/kg subcutaneously (s.c.) under the skull skin along the incision line.

Next, the rat was placed in a stereotaxic apparatus, with the skull surface flat between the bregma and lambda points on the skull. The rat's temperature was monitored using a rectal thermometer and maintained at $36-37^{\circ}\text{C}$ throughout the experiment. Subsequently, an incision

was made at the midline of the head exposing the skull and small holes were drilled into the skull over the medial geniculate nucleus (MGN; anterior-posterior (AP): -5.5; medial-lateral (ML): +3.8; ventral (V): -5.4 to -6.4) and the ipsilateral A1 (AP: -4.5; ML: +7.0; V: -3.2 to -5.6; all coordinates were based on the rat atlas by Paxinos and Watson, 2007). Two additional holes in the frontal bone were used to secure a ground and reference electrode (jewelry screws).

2.3. Electrophysiology

A monopolar recording electrode (125 μ m diameter Teflon-insulated stainless steel wire) attached to a microdialysis probe (Mab 2.14.2; 2mm active polyether sulfone membrane, 35kDA cut-off; S.P.E. Limited, North York, ON, Canada; electrode tip was about 1 mm above the probe tip) was lowered into A1. A stimulating electrode (SNE-100 concentric bipolar electrode; Rhodes Medical Instruments, David Kopf, Tujunga, CA) was lowered into the ipsilateral MGN. The final ventral placement for both electrodes was optimized to yield the maximum field postsynaptic potential (fPSPs) amplitude in A1 in response to the single pulse MGN stimulation.

The recording electrode was connected to an amplifier (Model 1800, A-M systems Inc., Microelectrode AC Amplifier) and A-D converter (10 kHz sampling rate; Powerlab 4/s system running Scope Software v4.1.4: AD instruments, Toronto, ON, Canada) to store the signal for subsequent offline analysis. The stimulating electrode was connected to an isolated pulse stimulator (Model 2100, A-M Systems Inc.) delivering constant current stimulation pulses (0.2 ms pulse durations) to the MGN.

2.4. Drug application

The microdialysis probe was connected to a 2.5mL Hamilton airtight micro-syringe driven via a micro-dialysis pump (CMA/102, CMA Microdialysis, Norway). The probe was continuously perfused (flow rate of 1.0 μ l/min) with artificial cerebrospinal fluid (aCSF) composed of: 118.3 mM NaCl, 4.4 mM KCl, 1.2 mM MgSO₄, 1.0 mM NaH₂PO₄, 2.5 mM, CaCl₂, 22.1 mM NaHCO₃ and 10.0 mM glucose. Local drug application in A1 was performed by reverse microdialysis, with drugs dissolved in the aCSF and perfused through the probe and the surrounding brain tissue by means of concentration driven diffusion across the dialysis probe membrane. The following drugs were applied to A1 during different experiments: methiothepin maleate (0.01, 0.1 and 1.0 mM); WAY 100635 maleate (1.0 and 2.0 mM), ketanserin tartrate (0.1 and 1.0 mM), granisetron hydrochloride (1.0 mM). In control rats, aCSF without any drug was continuously applied to A1 throughout the experiment. All drugs were obtained from Tocris Bioscience, Minneapolis, MN, USA.

2.5. Data collection

Following final electrode adjustments, all rats were given at least 10 min prior to the onset of data collection to allow for the stabilization of electrodes and neural tissue. Subsequently, an input-output curve was generated for each rat by successive MGN stimulation with increasing intensities (0.1-1.0 mA in 0.1 mA increments). The stimulation intensity that elicited a fPSP amplitude of approximately 50-60% of the maximum was chosen as the intensity for the subsequent data collection.

Initially, fPSPs were recorded every 30 seconds until a 30 min period of stable (e.g., no systematic increase or decrease in fPSP amplitude) baseline fPSP recordings were collected.

Next, theta burst stimulation (TBS) was delivered to the MGN in the form of pulse bursts (5 pulses at 100 Hz/burst), with bursts repeated 10 times at a rate of 5 Hz. These trains of 10 bursts were repeated four times every 10 sec. Following TBS, fPSPs were recorded (every 30 seconds) for 60 min, after which TBS was applied again. In total, three TBS episodes were delivered for each experiment, each followed by 60 min of fPSP recordings.

For some groups of rats, fPSP were monitored in the absence of TBS delivery to assess whether drug application alone changed fPSP amplitude over the course of the experiment. For these experiments, baseline fPSPs were recorded in the presence of aCSF for 30 min, followed by drug application (or continuous aCSF) for 3 hours.

2.6. Histology

After completion of the electrophysiological procedures, each rat received a supplementary dose of urethane (1 mL, i.p.) and was subsequently perfused through the heart with 60 mL of 0.9% saline, followed by 60 mL of 10% phosphate-buffered formalin. The extracted brain was submerged in formalin for about 24 hours prior to sectioning (40 μ m coronal sections) using a cryostat. The tissue was mounted onto dry gelatin-coated microscopic glass slides and an experimenter who was blind to the data of individual experiments verified the accuracy of electrode placements using standard histological techniques and a digital microscope. Data from experiments with electrode placements outside of the target areas were excluded from the subsequent data analyses.

2.7. Data Analysis

The peak amplitude of the two, predominant negative fPSP peaks (Fig. 1) was detected by the software (Scope, v4.1.4) and the voltage difference between the maximum fPSP peak negativity and the baseline voltage sampled prior to the stimulus artifact was computed. The data for each fPSP were averaged over 10 minute intervals (20 fPSPs/epoch) and normalized by dividing each data point by the averaged (pre-TBS) amplitude for that animal. Data from experiments where fPSP amplitude declined to less than 50% of baseline were excluded from the analysis, due to the non-physiological nature of this decline in synaptic strength.

The data were statistically examined (using SPSS software, v. 23; IBM SPSS Statistics) with a 2-factor, mixed model analysis of variance (ANOVA). This analysis had $\alpha = 0.05$, independent variable (iv) 1 = time (averaged 10 min epochs) as the within subjects (repeated) factor; and iv2 = drug condition (drug vs. aCSF) as the between subjects factor. The Greenhouse-Geisser correction was applied in all cases when the assumption of sphericity was violated, as tested by the Mauchly's test of sphericity (using SPSS software).

Fig. 1. Typical fPSPs recorded in A1 in response to ipsilateral MGN stimulation in the urethane-anesthetized rat.

Typical fPSPs in A1 consisted of two negative-going peaks with latencies to peak of about 6-9 ms (peak 1) and 15-20 ms (peak 2). The blue trace represents a baseline fPSP recording obtained prior to LTP induction; the red trace represents an example of a fPSP obtained following LTP induction.

Chapter 3

Results

3.1. fPSP characteristics and LTP during vehicle (aCSF/saline) application

Single-pulse stimulation of the MGN reliably evoked fPSPs in the ipsilateral A1. These fPSPs consisted of two sequential, negative-going peaks with latencies of about 6-9 and 15-20 ms, respectively (Fig. 1). Prior work using current-source density analyses and pharmacological approaches has suggested that the first and second peak represent the sequential excitation of thalamocortical and intracortical A1 synapses, respectively (Hogsden and Dringenberg, 2009b; Hogsden et al., 2011).

For the pharmacological experiments, two vehicle solutions were used to dissolve various drugs (methiothepin and ketanserin were dissolved in 0.9% saline; WAY 100635 and granisetron were dissolved in aCSF). An initial analysis of data collected in rats receiving local application of saline ($n = 5$) or aCSF ($n = 5$) in A1 by reverse microdialyses did not reveal any statistically significant differences in fPSPs over the course of the experiment between these two vehicle groups (data not shown; main effect of group and group by time interaction all non-significant, $p > 0.3$). Consequently, rats receiving either saline or aCSF in A1 were combined into a single control/vehicle group ($n = 10$) for all subsequent comparisons.

For all experiments, TBS to the MGN was delivered after 30 min of stable fPSP recordings were obtained, and TBS was repeated in hourly intervals for a total of 3 stimulation episodes (each followed by 60 min of recordings). During vehicle application ($n = 10$; see above), TBS resulted in an increase in the amplitude of both fPSP peaks (Fig. 1), which reached 127% (peak 1; Fig. 2A) and 123% (peak 2; Fig. 2B) of baseline levels during the final 30 min of the experiment (main effect of time for **peak 1**: $F(3.56, 32.04) = 5.1$, $p = 0.004$; **peak 2**:

$F(2.39, 21.48) = 3.7$, $p = 0.035$). Thus, consistent with previous results, moderate levels of LTP can be induced by thalamic TBS in the A1 of adult rats (Hogsden and Dringenberg, 2009a; Hogsden et al., 2011).

3.2. Effects of 5-HTR antagonist application in A1 on LTP

Methiothepin: First, we examined the effect of local application of the broad-acting 5-HTR antagonist methiothepin (0.01 mM, $n = 3$; 0.1 mM, $n = 3$; 1.0 mM, $n = 6$) in LTP induction by TBS of the MGN. In the presence of methiothepin, TBS of the MGN resulted in the suppression of fPSP amplitude, rather than the LTP seen during vehicle application (Fig. 2). For peak 1, fPSP amplitude was suppressed to 86%, 82%, and 76% of baseline (means for the last 30 min of the experiment; 127% for vehicle rats, see above) during application of 0.01, 0.1, and 1.0 mM methiothepin, respectively (Fig. 2A). For peak 2, amplitude decreased to 84% (0.01 mM), 93% (0.1 mM), and 89 % (1.0 mM; 123% for vehicle rats; see above) of baseline for the three drug concentrations (Fig. 2B).

Statistical analyses using ANOVA confirmed that, at 1 mM, methiothepin resulted in a significant suppression of amplitude for both fPSP peaks relative to vehicle application (**peak 1:** main effect of drug, $F(1,14) = 11.82$, $p < 0.01$; drug by time interaction, $F(2.92, 40.92) = 6.19$, $p < 0.01$; **peak 2:** main effect of drug, $F(1,14) = 5.57$, $p = 0.03$; drug by time interaction, $F(2.51, 35.17) = 3.33$, $p = 0.038$). At 0.1 mM, methiothepin still resulted in a significant suppression of peak 1 (main effect of drug and drug by time interaction both $p < 0.025$), but not of peak 2. At 0.01 mM, methiothepin, no longer produced statistically significant changes of either fPSP peak compared to the vehicle condition (all p 's > 0.05).

An additional ANOVA was computed to assess whether there were significant changes in fPSP amplitude over time during application the high concentration of methiothepin (1 mM). This analysis did not reveal significant main effects of time for either peak of the fPSP; **peak 1**: $F(1.37, 6.82) = 2.75$, $p = 0.14$; **peak 2**: $F(1.86, 9.30) = 1.43$, $p = 0.29$.

WAY 100635: Next, we examined the effect of selective blockade of 5-HT_{1A} receptors by application of WAY 100635 in A1 (1 mM, $n = 12$). In contrast to methiothepin, WAY 100635 did not exerted significant effects on LTP induction by TBS of the MGN, with peak 1 (Fig. 3A) and peak 2 (Fig. 3B) reaching 113% and 118%, respectively, of baseline during the final 30 min of the experiment.

An ANOVA confirmed the lack of significant effects of 1 mM WAY 100635 on LTP (**peak 1**: main effect of drug, $F(1,20) = 2.71$, $p = 0.12$; drug by time interaction, $F(4.46, 89.27) = 1.33$, $p = 0.26$; note the main effect of time was significant, indicative of successful LTP induction across both conditions, $F(4.46, 89.27) = 9.16$, $p < 0.01$; **peak 2**: main effect of drug, $F(1,20) = 0.28$, $p = 0.60$; drug by time interaction, $F(3.33,66.63) = 0.42$, $p = 0.76$; main effect of time, $F(3.33,66.63) = 8.41$, $p < 0.01$). In a small group of rats ($n = 3$), the effects of 2 mM WAY 100635 were tested, but even this high concentration did not yield clear changes in amplitude of either fPSP peak (Fig. 3 p 's > 0.2 for drug effect and drug by time interaction).

Ketanserin: Application of the 5-HT_{2R} antagonist ketanserin (0.1 mM, $n = 4$; 1 mM, $n = 3$) produced effects very similar to those seen in with methiothepin application. That is, LTP induction was suppressed for both peaks of the fPSPs (Fig. 4). For the lower concentration (0.1 mM), the amplitude of peak 1 and peak 2 decreased to 70% and 92% of baseline, respectively; at 1.0 mM, the two peaks showed a further suppress to 63% and 69% (Fig. 4; means for the final 30 min of the experiment).

ANOVA confirmed that fPSP amplitude during application of either 0.1 or 1.0 mM ketanserin was significant lower compared to vehicle application (**0.1 mM, peak 1:** main effect of drug, $F(1,12) = 21.93$, $p < 0.01$; drug by time interaction, $F(3.83,45.93) = 6.68$, $p < 0.01$; **0.1 mM, peak 2:** main effect of drug, $F(1,12) = 3.29$, $p = 0.10$; drug by time interaction, $F(2.67,31.98) = 3.20$, $p = 0.04$; **1.0 mM, peak 1:** main effect of drug, $F(1,12) = 39.08$, $p < 0.01$; drug by time interaction, $F(3.50,41.94) = 7.60$, $p < 0.01$; **1.0 mM, peak 2:** main effect of drug, $F(1,11) = 11.45$, $p < 0.01$; drug by time interaction, $F(2.71,29.76) = 3.67$, $p = 0.03$).

An additional ANOVA was computed to assess whether there were significant changes in fPSP amplitude over time during application the high concentration of ketanserin (1 mM). This analysis did not reveal significant main effects of time for either peak of the fPSP; **peak 1:** $F(1.68, 5.04) = 4.80$, $p = 0.07$; **peak 2:** $F(1.80, 3.59) = 1.54$, $p = 0.32$.

Granisetron: The last set of LTP experiments evaluated the role of 5-HT₃R by cortical application of granisetron (1 mM, $n = 12$). Interestingly, granisetron resulted in a blockade of LTP induction at thalamocortical synapses (Fig. 5A; fPSP amplitude at 103% of baseline during final 30 min of experiment; 127% for vehicle, see above), while LTP at intracortical synapses remained completely intact (Fig. 5C; amplitude at 126% of baseline; 123% for vehicle, see above).

ANOVA confirmed that granisetron exerted a significant effect on the first peak of the fPSP, while peak 2 was not different between rats receiving application granisetron and vehicle (**peak 1:** main effect of drug, $F(1,20) = 6.51$, $p = 0.02$; drug by time interaction, $F(4.56,91.17) = 3.07$, $p = 0.02$; main effect of time, $F(4.56,91.17) = 7.08$, $p < 0.01$; **peak 2:** main effect of drug, $F(1,20) = 3.42$, $p = 0.08$; drug by time interaction, $F(3.32,66.27) = 2.42$, $p = 0.07$; main effect of time, $F(3.32,66.27) = 12.69$, $p < 0.01$).

3.3. Effects of 5-HT₂A antagonist on baseline (pre-TBS) fPSP amplitude

It is possible that 5-HT₂A receptors in A1 also participate in the regulation of baseline (non-potentiated/depressed) synaptic transmission. Thus, we also measure and compared the fPSP amplitude recorded during the 30 min baseline period of the experiment (i.e., during drug application, but prior to TBS delivery) in all experimental groups.

During vehicle application and prior to LTP induction, the mean amplitude of the first and second fPSP peak was 0.41 mV and 0.39 mV, respectively (Fig. 6). Application of methiothepin, ketanserin, or WAY 100635 did not result in significant changes in the amplitude of either fPSP peak (Fig. 6). However, during granisetron application, the amplitude of both fPSP peaks was much higher compared to the vehicle condition (Fig 6; 0.74 mV and 0.78 mV for peak 1 and peak 2, respectively).

To further investigate this effect, two additional groups of rats were tested that did not receive TBS. In one group (n = 3), fPSPs were monitored during continuous application of aCSF for 3.5 hours, while a second group (n = 5) received aCSF for the first 30 min, followed by 3 hours of granisetron application (1 mM). During continuous aCSF application, fPSPs stayed relatively stable over the course of the experiment, with amplitude at 100.3% and 112% of baseline for the first and second peak, respectively, during the last 30 min of the experiment (Fig. 7). In contrast, granisetron application resulted in a clear increase in the amplitude of both peaks during the 3 hour application period, which reached 126% and 187% of baseline for peak 1 and peak 2, respectively (Fig. 7; peak 1 is based on an n = 4, since this peak could not be resolved in one animal).

The ANOVA confirmed that fPSP amplitude was significantly higher during granisetron application relative to the vehicle condition, as indicated by the significant drug by time interaction for both fPSP peaks (**peak 1:** $F(2.36, 11.79) = 4.98$, $p = 0.023$; **peak 2:** $F(1.69, 10.16) = 5.06$, $p = 0.034$).

Fig. 2. Effects of cortical application of methiothepin on LTP induction in A1.

In the presence of vehicle application (Control: $n = 10$; all drugs applied by reverse microdialysis in A1), theta-burst stimulation (at arrows) delivered to the MGN resulted in moderate potentiation/LTP of the first peak (panel **A**) and second peak (panel **B**) of fPSPs recorded in A1. Application of methiothepin (0.01 mM, $n = 3$; 0.1 mM, $n = 3$; 1.0 mM, $n = 6$) suppressed LTP. * indicates significant ($p < 0.05$; simple effects test) difference between control and 1.0 mM methiothepin.

Fig. 3. Effects of cortical application of WAY 100635 on LTP induction in A1.

Application of WAY 100635 (1 mM, $n = 12$; 2 mM, $n = 3$) in A1 did not result in any significant changes in LTP induction of either the first (**A**) or second (**B**) fPSP peak compared to control animals ($n = 10$; same group as shown in Fig. 2).

Fig. 4. Effects of cortical application of ketanserin on LTP induction in A1.

Compared to control animals (same group as in Fig. 2), application of ketanserin (0.1 mM, $n = 4$; 1.0 mM, $n = 3$) resulted in LTP suppression for both the first (A) and second (B) fPSP peak, similar to the effect observed with methiothepin application. * indicates significant ($p < 0.05$; simple effects test) difference between control and 1.0 mM ketanserin.

Fig. 5. Effects of cortical application of granisetron on LTP induction in A1.

Compared to control animals (same group as in Fig. 2), application of granisetron (1.0 mM, $n = 12$) completely abolished LTP of the first fPSP peak (**A**), while LTP of the second peak (**B**) was intact. * indicates significant ($p < 0.05$; simple effects test) difference between control and 1.0 mM granisetron.

Fig. 6. Amplitude of fPSPs in all drug conditions prior to LTP induction.

During vehicle (Control) Mean fPSP amplitude during the 30 min baseline (pre-TBS delivery) recording period was 0.41 mV and 0.39 mV for the first (**A**) and second fPSP peak (**B**), respectively. Methiothepin, ketanserin, or WAY 100635 did not significantly alter baseline fPSP amplitudes, but granisetron application resulted in a pronounced increase in the amplitude of both fPSP peaks (0.74 mV and 0.78 mV for peak 1 and peak 2, respectively). * indicates significant ($p < 0.05$; simple effects test) difference between control and 1.0 mM granisetron.

Fig. 7. Effects of cortical application of granisetron on fPSP amplitude in A1.

Amplitude of the first (**A**) and second fPSP peak (**B**) remained relatively stable during continuous application of aCSF for 3.5 hours ($n = 3$). In contrast, granisetron application ($n = 5$; 1 mM; application start at arrow) resulted in a significant increase in the amplitude of both fPSP peaks (note that these rats did not receive TBS to induce LTD). * indicates significant ($p < 0.05$; simple effects test) difference between aCSF and granisetron.

Chapter 4

Discussion

The present experiments examined the role of several classes of 5-HTRs on plasticity induction in the thalamocortical auditory system of adult, urethane-anesthetized rats. Delivery of TBS to the MGN reliably induced moderate levels of synaptic potentiation of fPSPs recorded in A1. Local, cortical application (by reverse microdialysis) of the broad-acting 5-HTR antagonist methiothepin suppressed LTP at both thalamocortical and intracortical synapses, an effect that was mimicked by the selective 5-HT₂R antagonist ketanserin, but not the 5-HT_{1A}R blocker WAY 100635. Interestingly, blockade of 5-HT₃R by granisetron blocked LTP at thalamocortical, but not intracortical A1 synapses. Further, in the absence of TBS, granisetron application resulted in a pronounced increase of the amplitude of both fPSP peaks, suggestive of an inhibitory role of 5-HT₃R in controlling baseline synaptic transmission at thalamocortical and intracortical A1 synapses. Together, these results indicate that 5-HTRs exert important gating functions by influencing the induction and direction of long-term plasticity at synapses of the fully matured A1 of mammalian species.

The recordings obtained in A1 following single-pulse stimulation of the MGN consisted of fPSPs that exhibited two, distinct peaks, with latencies to peak of about 6-9 and 15-20 ms, respectively (Fig. 1). Both fPSP peaks evoked in A1 by ipsilateral MGN stimulation are negative components with an amplitude of about 0.4 mV. Previous studies employing current source density analysis and pharmacological manipulations have been conducted in order to identify the synaptic mechanisms and origin of these two peaks presented in fPSPs recorded in the A1 of anesthetized rats.

In one investigation (Hogsden & Dringenberg, 2009b), fPSPs were recorded throughout the entire dorsal-ventral extent of A1 (in 100-micron steps) and current source density analysis was used to identify the current sinks (i.e., positive current entering cells, creating negative extracellular potentials; see Vanderwolf, 1990) present at different cortical depth and layers. The results of this study indicate that the first fPSP peak corresponds to a current sink in deeper cortical layers (around layer IV) that receive direct inputs from thalamic terminals, while the second fPSP peak is driven by current sinks in layers II/III of A1, which receive inputs from deeper cortical layers (Hogsden & Dringenberg, 2009b). Subsequent pharmacological experiments have confirmed and extended these observations; local application of pharmacological agents that inhibit intracortical transmission while preserving activity of thalamocortical terminals (a combination of the GABA receptor agonist muscimol to inhibit cortical activity; and the GABA-B receptor antagonist SCH 50911 to protect thalamocortical terminals from the effects of muscimol) results in a preferential suppression of the second fPSP peak, consistent with the involvement of intracortical mechanisms in its generation (Hogsden et al., 2011). It is also noteworthy that both fPSP peaks are largely abolished by local application of CNQX (Hogsden & Dringenberg, 2009), suggesting that these fPSPs are mediated by excitatory, AMPA-receptor mediated currents in A1.

We were surprised that application of WAY 100635, a highly selective antagonist at 5-HT_{1A}Rs (Forster et al., 1995), did not affect LTP induction in A1. 5-HT_{1A}Rs act as somatodendritic autoreceptors that suppress 5-HT release, but are also present at postsynaptic locations in the forebrain (Barnes and Sharp, 1999; Nichols and Nichols, 2008). Activation of 5-HT_{1A}Rs has been implicated in plasticity/LTP modulation in several forebrain areas, including the hippocampus, visual cortex, and prefrontal cortex. For example, in V1, 5-HT_{1A}Rs have been

shown to inhibit LTP induction *in vitro* and *in vivo* (Dringenberg and Gagolewicz, 2016; Edagawa et al., 1998; Kim et al., 2006), suggesting that 5-HT may participate in the stabilization of synaptic connectivity in the mature cortex. Similarly, in the prefrontal cortex, 5-HT_{1A}Rs activation can inhibit calcium influence through NMDA channels, thereby reducing the probability of LTP induction (and favouring LTD; Meunier et al., 2017). Based on the present results, 5-HT_{1A}Rs do not perform similar functions at either thalamocortical or intracortical synapses in the mature A1, at least under the present, experimental conditions.

Application of the broad-acting 5-HTR antagonist methiothepin (Bard et al., 1996; Hoyer et al., 1994; Peroutka, 1990) suppressed the TBS-induced response of both thalamocortical and intracortical A1 synapses, an effect that was mimicked by the selective 5-HT₂R antagonist ketanserin (Leysen et al., 1982; Peroutka, 1990). Thus, 5-HT₂Rs appear to play a critical role in LTP induction at neocortical synapses. Prior work is consistent with this interpretation, in that 5-HT₂R stimulation has been shown to enhance LTP induction at synapses in the basolateral amygdala and hippocampal CA1 field *in vitro* (Chen et al. 2003; Rozas et al. 2012).

5-HT₂Rs are widely distributed throughout the neocortex and most cortical layers (layers II to VI; Hamada et al., 1998; Jakab et al., 2000). Interestingly, some types of 5-HT₂Rs (e.g., 5-HT_{2A}Rs) are located on both cortical pyramidal cells and GABAergic interneurons (Jakab et al., 2000), as well as presynaptically on terminals of thalamocortical axons (Barre et al., 2016). Thus, there are a number of complementary mechanisms by which these receptors can modulate cellular excitability and plasticity induction in cortical networks. Activation of 5-HT₂Rs in neocortical pyramidal cells often (but not always) elicits excitatory effects, including increases in spontaneous excitatory postsynaptic currents, cellular depolarization, and inhibition of the after-hyperpolarization current that follows neuronal discharge (Aghajanian and Marek, 1997;

Araneda and Andrade, 1991; Celada et al., 2013; Davies et al., 1987; Newberry et al., 1999). Blockade of 5-HT₂Rs by ketanserin can result in a facilitation of inhibitory responses to 5-HT application (Lakoski and Aghajanian, 1985). Recent work has shown that activation of presynaptic 5-HT_{2A}Rs on thalamocortical fibers (terminating in the prefrontal cortex) acts to facilitate NMDA-mediated transmission (Barre et al., 2016). The precise mechanisms mediating the LTP suppression seen during methiothepin or ketanserin application remain to be elucidated; however, the observation that LTP at thalamocortical A1 synapses involves a presynaptic component (as measured by changes in paired-pulse ratios; Soutar et al., 2016) is consistent with a potential, presynaptic (at least in part) locus of this effect.

Application of granisetron, a potent antagonist of 5-HT₃Rs (Plosker and Goa, 1991; Sanger and Nelson, 1989) exerted a surprising, selective effect on plasticity induction by blocking LTP of the first, but not the second peak of cortical fPSPs. Thus, it appears that 5-HT₃Rs play an important role in the gating of plasticity at thalamocortical, but not intracortical synapses in A1. 5-HT₃Rs are ligand-gated ion channels mediating rapid neuronal depolarization (Barnes and Sharp, 1999; Celada et al., 2013) and are expressed widely throughout the forebrain, including the neocortex (Barnes and Sharp, 1999; Kilpatrick et al., 1987; Morales et al., 1998). Since many 5-HT₃Rs are located on GABAergic interneurons in the hippocampus and neocortex (Jakab et al., 2000; Morales et al., 1996), they are able to profoundly shift the excitatory-inhibitory balance of cortical circuits. At present, it is not known if the impairment of LTP induction by granisetron involves changes in inhibitory tone on A1. It would appear that a blockade of 5-HT₃Rs on GABAergic cells should result in cortical disinhibition and enhanced plasticity induction, rather than the LTP impairment observed in our experiments. Future experiments measuring both excitatory and inhibitory currents and LTP in principal cells and

different classes of interneurons are required to more fully elucidate the mechanisms involved in LTP gating by 5-HT₃Rs in the rodent A1.

We also noted that the application of granisetron resulted in a clear enhancement of baseline (i.e., non-potentiated) fPSP amplitude, which reached 126% and 187% of baseline levels for the first and second fPSP peak, respectively (it is noteworthy that these levels of facilitation are greater than those seen during the induction of LTP in A1). Prior work has demonstrated the involvement of 5-HT_{1A}Rs and 5-HT_{2A}Rs in modulating inhibitory transmission in layers II/III of A1 (Garcia-Oscos et al., 2015). The present data extend these findings by showing that 5-HT₃Rs also exert a powerful role in controlling A1 synaptic activity by inhibiting thalamocortical and intracortical A1 synapses, an effect that was relieved during granisetron application. It is possible that this inhibitory role of 5-HT₃Rs is directly related to the presence of these receptors on GABA-containing interneurons in A1, as discussed previously.

In summary, the role of 5-HT and different types of 5-HTRs in gating plasticity induction in neocortical circuits remains unclear, with variable and seemingly contradictory effects reported for LTP induction in cortical sensory fields (e.g., V1, enhanced LTP: Inaba et al., 2009; Kojic et al., 1997, 2000; Park et al., 2012; impaired LTP: Edagawa et al., 1998, 2000; Gagolewicz and Dringenberg, 2016; Kim et al., 2006). The use of different species, ages of experimental subjects, induction protocols, and electrophysiological recordings obtained in different cortical layers likely all contribute to the heterogeneous results obtained in previous studies.

The results of the present set of experiments reveal a consistent picture in that the blockade of two main classes of 5-HTRs (5-HT₂Rs and 5-HT₃Rs) impairs LTP. Thus, it appears that the tonic activation of these receptors exerts a permissive and/or facilitating influence over

LTP induction in the A1 of adult rodents. Pharmacological treatments targeting serotonergic transmission (e.g., selective serotonin reuptake inhibitors) have shown promise in enhancing synaptic plasticity in the mature nervous system, with potential therapeutic effects, such as the restoration of visual functions in animal models of amblyopia (Ruiz-Perera et al., 2015; Vetencourt et al., 2008). Given that LTP in A1 is most pronounced in juvenile animals and declines into adulthood (Hogsden and Dringenberg, 2009a), some of the effects described here may be relevant to pharmacological strategies for restoring heightened (perhaps juvenile-like) levels of synaptic plasticity in the fully mature nervous system of adult mammals (e.g., Bavelier et al., 2010).

The experiments summarized in this thesis add to the growing body of literature demonstrating that the auditory system of adult animals retains a significant potential for the induction of synaptic plasticity (Tzounopoulos et al., 2012). For, example, LTP and LTD have been observed in the fully matured auditory cortex of rodents, where they likely play a role in learning related plasticity, such as experience-induced changes in the tonotopic organization of A1 (Tzounopoulos et al., 2012). The experimental results reported here show that 5-HTRs can act on plasticity induction in A1. As such, this work may have important implications for attempts to enhance plasticity in the mature brain, e.g., by restoring heightened, juvenile-like levels of LTP in the mature A1.

Synaptic plasticity is now known as an essential contributor to the success of cochlear implants to restore auditory perceptual functions in patients suffering from hearing loss (Fallon et al., 2002). However, the success cochlear implants is variable, given that implants provide patients only with an abnormal, impoverished acoustic input (Fallon et al., 2002). Also, the effectiveness of cochlear implants often decreases with age, an effect that likely relates to the

loss of plasticity in the central auditory pathway, which cannot adapt to the acoustic signals provided by the implant. If implanted under 12 months of age, the communication skills of congenitally deaf children will improve and begin to match those of children without hearing impairments (Fallon et al., 2002). In contrast, patients receiving cochlear implants as young adults often continue to show perceptual deficits, including poor speech perception and abnormal pitch perception (Fallon et al., 2002).

Work in rats has shown that, even though the mature A1 does exhibit LTP, there is a clear, age-related decline in LTP over postnatal life (Hogsden et al., 2009). Based on the assumption that a loss of central auditory plasticity reduces the effectiveness of cochlear implants, it is tempting to speculate that an enhancement of plasticity could improve the clinical outcomes for patients receiving implants, particularly if this happens in adulthood or later life. The present set of experiments has shown that 5-HT₂R and 5-HT₃R activation exerts a clear effect on LTP induction at A1 synapses. Thus, it is possible that 5-HT₂R and 5-HT₃R agonists can be used to enhance the LTP levels in the A1 of the adult or aged brain. Ultimately, identifying novel (pharmacological and/or others) strategies to enhance synaptic plasticity in the mature auditory cortex may help adults/older adults to better adapt to cochlear implants and, consequently, facilitate the perceptual improvements following the implantation procedure.

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