Research report

Differential modulation of auditory thalamocortical and intracortical synaptic transmission by cholinergic agonist

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Abstract

To investigate synaptic mechanisms underlying information processing in auditory cortex, we examined cholinergic modulation of synaptic transmission in a novel slice preparation containing thalamocortical and intracortical inputs to mouse auditory cortex. Extracellular and intracellular recordings were made in cortical layer IV while alternately stimulating thalamocortical afferents (via medial geniculate or downstream subcortical stimulation) and intracortical afferents. Either subcortical or intracortical stimulation elicited a fast, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)-sensitive, monosynaptic EPSP followed by long-duration, polysynaptic activity. The cholinergic agonist carbachol suppressed each of the synaptic potentials to different degrees. At low concentrations (5 μM) carbachol strongly reduced (>60%) the polysynaptic slow potentials for both pathways but did not affect the monosynaptic fast potentials. At higher doses (10–50 μM), carbachol also reduced the fast potentials, but reduced the intracortically-elicited fast potential significantly more than the thalamocortically-elicited fast potential, which at times was actually enhanced. Atropine (0.5 μM) blocked the effects of carbachol, indicating muscarinic receptor involvement. We conclude that muscarinic modulation can strongly suppress intracortical synaptic activity while exerting less suppression, or actually enhancing, thalamocortical inputs. Such differential actions imply that auditory information processing may favor sensory information relayed through the thalamus over ongoing cortical activity during periods of increased acetylcholine (ACh) release. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cells of the auditory cortex (AC) receive sensory input from thalamic relay cells of the medial geniculate nucleus (MG; extrinsic inputs), and from other cells within the cortex (intrinsic inputs) which themselves can receive either direct or indirect MG input ([8,45,46,55–57,60,72,75] and reviewed in [73,74]). These two sources of input combine to produce sensory evoked responses observed in AC.

The AC also receives non-sensory inputs that modulate evoked responses (see reviews by [34,35]). Prominent among these is the projection from the cholinergic basal forebrain, the major source of ACh to the AC (reviewed by [40,62]). The cholinergic system exerts powerful effects on sensory cortex, influencing cellular excitability, cortical state, and receptive field plasticity [5,11,14,22,29,36,38,41,44,50]. In particular, application of cholinergic agonists or stimulation of the basal forebrain can profoundly modulate cortical EPSPs in sensory cortex both in vivo and in vitro [3,6,11,19,31,42,51].

While the influence of ACh on sensory cortical responses is undisputed, little is known regarding the degree to which it regulates transmission coming from extrinsic (thalamocortical) vs. intrinsic (intracortical) sources. This may be surprising considering the importance of such knowledge for understanding cholinergic regulation of sensation and perception. However, the acquisition of relevant data has been hampered by several factors,

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including the difficulty in conducting appropriate experiments in vivo and the limited availability of in vitro preparations with intact thalamocortical connections [1,14]. In one in vitro study which directly tests this issue, Gil et al. [19] utilized a somatosensory thalamocortical slice to demonstrate that activation of muscarinic ACh receptors suppressed intracortical and thalamocortical EPSPs similarly. In contrast to these findings, muscarnic actions in hippocampus and piriform cortex result in a strong suppressive effect on intracortical synapses with little effect on responses to extrinsic inputs [24,26]. An important question that emerges from these studies is whether the contrasting findings reflect functional distinctions between neocortex [19] vs. archicortex/paleocortex [24,26], or other, possibly methodological, differences. Indirect evidence addressing this issue comes from the recent work of Kimura et al. [30]. They found that ACh suppressed responses to white matter stimulation in upper and lower layers of visual cortex more than in the thalamo-recipient middle layers. The authors propose that muscarinic suppression of intracortical synapses is stronger than that of thalamocortical synapses. This would suggest that the distinction between neocortex and archicortex/paleocortex (hypothesized above) may not be valid.

To address this issue, we have utilized a slice preparation of the AC that, like the somatosensory thalamocortical slice used by Gil et al. [19], preserves both extrinsic inputs from the thalamus and intrinsic, long-range intracortical connections [43]. With this slice, we directly compare cholinergic modulation of thalamocortical and intracortical synaptic transmission. Portions of this work have appeared in abstract form [28].

2. Material and methods

2.1. Preparation of slices

All procedures followed the University of California, Irvine, animal use regulation. Slices were taken from 15 to 43-day-old FVB mice and maintained in vitro (described by Metherate and Cruikshank [43]). Following decapitation under halothane anesthesia, brains were rapidly removed (<60 s) and placed in 0–4°C artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 125, KCl 2.5, KH₂PO₄ 1.25, NaHCO₃ 25, MgSO₄ 1.2, CaCl₂ 2, dextrose 10; bubbled with 95% O₂/5% CO₂. Auditory thalamocortical slices were prepared as described previously [43]. Briefly, slice orientation was near-horizontal, with the anterior end 0–10° above the horizontal plane, and the lateral end 10–25° above horizontal. The auditory cortex was located using landmarks such as white matter fiber tracts running from thalamus to cortex and confirmed during recording by the presence of robust field potentials in layer IV following stimulation of the MG or the thalamocortical pathway. Two to three slices (400–500 μm) were taken from this area and maintained in a holding chamber bubbled with 95% O₂/5% CO₂ at room temperature. Recordings in an interface chamber (32°C, Haas Model, Med. Systems, Greenvale, NY) followed an incubation period of 1–2 h.

2.2. Electrophysiological stimulation and recording

To activate extrinsic thalamic afferents and intrinsic cortical processes, electrical stimuli (200 μs, 5–100 μA) were delivered via concentric bipolar electrodes (200 μm outer diameter; F. Haer) to MG, subcortical (SC) and intracortical (IC) stimulation sites (illustrated in Fig. 1A). Evoked extracellular field potentials and/or intracellular (whole-cell patch) synaptic potentials were recorded with electrodes placed in cortical layer IV of AC. In some simultaneous extra- and intracellular recordings, one recording electrode was placed in lower layer III or upper layer V, and the other in layer IV. Extracellular microelectrodes were pulled on a horizontal puller (P-97, Sutter Instruments Co., Novato, CA) and filled with ACSF. Intracellular patch pipettes (4–6 MΩ) were pulled with the same puller and filled with (in mM): KMeSO₄ 130, NaCl 10, CaCl₂ 0.05, Na-GTP 0.5, Mg-ATP 2, HEPES 10, EGTA 0.16. Neural signals were amplified (CyberAmp AI-401 and Axoclamp 2B, Axon Instruments, Foster City, CA), monitored on a digital oscilloscope (Tektronix, Portland, OR), digitized at 5 kHz and stored on computer (Apple PowerMac). Data acquisition was computer controlled (AxD ata, Axon Instruments) and analyzed off-line (AxoGraph, Axon Instruments).

2.3. Carbachol protocol and receptor pharmacology

In most slices, responses were recorded while alternating stimulation between SC and IC sites (interstimulus interval=15 or 30 s, so that each pathway was stimulated at 30 or 60 s intervals). Following 10–20 min of stable baseline responses, a 2 min bath-applied pulse of carbachol was delivered and the time course of its effect on evoked responses was monitored for ≥30 min. The pulse was limited to 2 min to prevent seizure-like activity that can occur with longer application of carbachol. In some slices, after a complete recovery was observed, the process was repeated with additional doses of carbachol. To determine muscarinic receptor involvement, atropine was applied continuously beginning 20 min prior to the carbachol pulse.

To determine the glutamate receptor involvement in SC- and IC-evoked potentials, we bath-applied the NMDA receptor antagonist (±)-2-amino-5-phosphono pentanoic acid (APV) and the AMPA/kainate (AMPA/KA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). APV, carbachol and atropine were purchased from Sigma Chemical Co. (St. Louis, MO). CNQX was purchased
Fig. 1. Auditory cortex responses to subcortical and intracortical stimulation. (A) Schematic illustration of stimulation sites (grey areas) along thalamocortical (SC) and intracortical (IC) fiber pathways (arrows) to AC. Note that for Experiment 2, stimuli were also delivered in the medial geniculate (MG). (B) Stimulation of SC or IC pathways evoked fast and slow potentials in simultaneous intracellular and extracellular recordings from cortical layer IV. APV (50 μM) reduced the slow potentials and subsequent application of CNQX (10 μM) reduced the remaining fast potentials.

from Research Biochemicals Inc. (Natick, MA). All drugs were dissolved in ACSF from frozen stock solutions and perfused at a rate of 1.0–1.6 ml/min. CNQX stock solution included dimethyl sulfoxide (DMSO, final concentration 0.2%).

In a second set of experiments, we examined the effects of carbachol on layer IV responses evoked by direct MG stimulation while alternating either SC or IC stimulation. Other aspects of the “carbachol protocol” were the same as described above (see also Results).

2.4. Data analysis

Evoked intracellular and extracellular responses consisted of “fast” and “slow” potentials. Fast potentials had a peak latency <10 ms and peak amplitude ≥1 mV or 35 μV (intra- and extracellular, respectively). Slow potentials had onset latencies >10 ms and long durations (typically >100 ms).

For intracellular fast potentials, onset latency was measured from the beginning of the stimulus artifact to the
point where the membrane potential deviated from the resting potential. Amplitude was measured at the first peak (e.g., arrows in Fig. 3C and D). For extracellular fast potentials, onset latency could not be measured because the stimulus artifact often lasted into the beginning of the response. Amplitude was measured at the maximum peak of the field potential. For intracellular and extracellular slow potentials, onset latency was measured at the point where the slow potential deviated from baseline (e.g., Fig. 1B, Subcortical Extracellular Control). In cases where the potential did not recover to baseline after the fast potential (e.g., Fig. 1B, Intracortical Extracellular Control), onset latency was measured at the inflection point after the fast potential. The slow potential’s duration was measured from the onset to the point at which the potential returned to baseline. Magnitude was measured as the area under that curve. Variability is expressed as ±1 Standard Error of the mean. Statistical comparisons are unpaired t-tests except where noted.

3. Results

The results are divided into two sets of experiments. The initial experiments were designed to separately activate extrinsic (subcortical) and intrinsic (intracortical) inputs leading to AC. We placed one stimulating electrode subcortically, within the downstream part of the auditory thalamocortical pathway, and a second stimulating electrode within the middle layers of the cortex lateral to the recording electrode (Fig. 1A). Recordings were made in layer IV of AC at the site of the maximal field response to subcortical stimulation and the effects of carbachol were examined. The results of these first experiments comprise the majority of the dataset in this manuscript. They revealed, among other things, a strong differential cholinergic modulation of responses to subcortical vs. intracortical inputs to AC. We hypothesize that this differential effect could be due to differences between thalamocortical synapses (activated by subcortical stimulation) and intracortical synapses (activated by intracortical stimulation). However, it is possible that the subcortical stimulus may have activated non-thalamic afferents, so we conducted a second, more limited study, in which the auditory thalamus was stimulated directly, thus decreasing the likelihood of activating non-thalamic cortical afferents. The cholinergic modulation of the resulting thalamocortical responses in AC was then compared with that found for the other inputs. Each of the two sets of experiments will be discussed in turn.

3.1. Experiment 1: subcortical vs. intracortical responses and modulation

3.1.1. Responses to subcortical stimulation

Subcortical stimulation within the auditory thalamocortical pathway elicited robust responses in AC (Fig. 1B). Effective stimulation required that the stimulating electrode be placed specifically in the subcortical region anterior to the hippocampus (SC grey area in Fig. 1A; mean distance, measured in a straight line between recording and stimulating electrodes, was 1148±44 μm) that contains the fibers of the auditory thalamocortical pathway [12,43,60,73]. Stimulation of the hippocampus itself or of the striatum at positions greater than about 1 mm anterior to the hippocampus elicited weak or no response. Thus, SC stimulation in the present study likely activated thalamocortical fibers (and possibly corticofugal axons, but see below and Discussion).

For experiment 1, intracellular data derive from 15 slices and extracellular data derive from 35 slices. In intracellular recordings, SC stimulation elicited a fast EPSP followed by a slow, long-lasting depolarization (Fig. 1B, intracellular control trace in response to subcortical stimulation). Simultaneous extracellular recordings revealed corresponding fast and slow negative potentials (Fig. 1B, extracellular control trace in response to subcortical stimulation). Extracellular negativities generally corresponded to intracellular depolarizations in terms of latency, shape, and duration (cf. intracellular and extracellular traces in Fig. 1B), implying a common cellular basis.

Although the fast intracellular EPSP to SC stimulation often consisted of 2 or 3 depolarizing peaks, our aim was to examine thalamocortical, monosynaptic responses; therefore, for quantitative analysis, we measured the first peak with the shortest latency and refer to this as the SC-fast potential. The SC-fast potential had a consistent onset latency (3.1±0.3 ms), initial slope (1.7±0.3 mV/μs), peak latency (6.9±0.7 ms) and amplitude (4.2±0.8 mV) and produces a dominant current sink in layers III/IV [43]. A qualitatively similar potential in AC occurs in response to stimulation of the MG itself ([12,43]; and see Experiment 2 below). These characteristics, along with pharmacological data presented below, suggest that the SC-fast potential is a thalamocortical EPSP. Following the SC-fast EPSP was a slower, long-lasting depolarization with fast fluctuations and spikes. This will be referred to as the SC-slow potential (indicated by arrows in Fig. 1B). It had a more variable latency to onset (33.8±3.1 ms), duration (501.6±38.7 ms) and magnitude (233.1±438.0 mV·μs). This variability was evident in a given slice from trial to trial, as well as between slices. The temporal characteristics and variability of the slow potential indicate that it is polysynaptic (see also [43]).

The extracellular SC-fast potential had a mean latency to peak of 6.9±0.3 ms, similar to that of the intracellular EPSP. The mean amplitude was 159.1±12.9 μV, and both amplitude and latency displayed trial to trial consistency. In contrast, the extracellular SC-slow potential had a variable onset (25.3±3.9 ms), duration (223.7±23.8 ms) and magnitude (area=13,210±1390 μV·ms). This variability was observed within slices from trial-to-trial, as well as between slices.

The SC-fast and slow potentials were elicited at differ-
ent stimulus intensities, indicating different thresholds for generation. In 26/35 slices (74%) where SC stimulation elicited extracellular fast and slow potentials, low stimulus intensities (e.g., 10–25 μA) elicited the fast potential alone whereas higher intensities elicited both potentials. In the remaining nine of 35 (26%) slices, SC stimuli at intensities up to 100 μA elicited only fast potentials.

Pharmacological manipulations revealed that glutamate receptors contribute to the generation of SC-fast and slow potentials. Bath application of APV (50 μM), an NMDA receptor antagonist, reduced the slow potential nearly completely while having little effect on the fast potential (Fig. 1B, Subcortical APV). Subsequent application of CNQX (10 μM), an AMPA/Kα receptor antagonist, reduced the fast potential (Fig. 1B, Subcortical APV+ CNQX). These data suggest that generation of the slow potential involves NMDA receptor activity and generation of the fast potential involves AMPA/Kα receptor activity. Note, however, that our previous study [43] demonstrated that CNQX alone can also completely reduce the slow potential. Thus, both NMDA and AMPA/Kα receptors likely contribute to generating the slow potential (see Discussion).

3.1.2. Responses to intracortical stimulation

To activate IC afferents, a second stimulating electrode was placed in the middle cortical layers up to 1 mm (mean distance 620±35 μm) lateral to the recording electrode (Fig. 1A). Stimulation above or below the middle layers generally elicited weaker responses, suggesting that intracortical stimuli activated fibers that project horizontally within the middle layers.

The basic physiological and pharmacological properties of the responses elicited by stimulation of the IC pathway were similar to those described for the SC pathway. In intracellular recordings, IC stimulation evoked a fast EPSP followed by a slow, long-duration depolarization (Fig. 1A). In contrast, the IC-slow potential that underlie SC and IC potentials.

3.1.3. Subcortical and intracortical pathway independence

Because the responses elicited by stimulation of SC and IC sites had similar characteristics, it was necessary to demonstrate that SC and IC stimulation activated distinct afferent pathways. To do this, we used a tetanus protocol to fatigue one pathway and then determined the response to stimulation of the other pathway (Fig. 2). For the slice in Fig. 2A, both SC and IC stimuli initially elicited extracellular fast and slow potentials (Fig. 2A (i)). Responses to SC stimulation were then fatigued with a 50 Hz tetanus (Fig. 2A (ii)). During the SC tetanus, a single stimulus pulse delivered to the IC pathway elicited responses similar to control (Fig. 2A (ii); stimulus artifacts from the tetanus appear in both traces). Fifteen seconds after the SC tetanus, both SC and IC responses recovered (not shown). The protocol was then reversed: the IC pathway was fatigued with tetanic stimulation, and a single pulse delivered to the SC pathway elicited responses similar to control (Fig. 2A (iii)). Fifteen seconds after the IC tetanus, both SC and IC responses had recovered (Fig. 2A (iv)). Fig. 2B shows the group effect of this procedure on the amplitudes of the fast and slow potentials (SC tetanus n=4; IC tetanus n=3). Because the tetanus had little effect on responses to stimulation of the non-tetanized pathway, we conclude that the SC and IC pathways are largely independent, at least with respect to the monosynaptic fast potentials (this conclusion cannot be extended to the slow potentials because of their polysynaptic nature, see Discussion). Given this demonstration, we could now determine the sensitivity of IC- and SC-evoked responses to cholinergic modulation.

3.1.4. Effects of cholinergic agonist on intracellular potentials

In intracellular recordings, 10–50 μM carbachol generally had a suppressive effect on all of the synaptic potentials. However, there were significant differences in the degree and manner of this suppression within and between pathways. First of all, within each pathway, carbachol reduced the slow potential significantly more than the fast potential (P’s=0.0002; intracellular data from 10 and 50 μM carbachol were combined for statistical
to make these comparisons for different doses of carbachol, we recorded extracellular potentials while alternating SC and IC stimulation, and applied carbachol at concentrations ranging from 0.5 to 50 μM.

3.1.5. Dose-dependent effects of carbachol

We determined the effects of 0.5, 1, 5, 10, 25 and 50 μM carbachol on extracellular responses to SC and IC stimuli interleaved at 15–30 s intervals. In 11/35 slices, more than one dose was applied to the same slice. Fig. 4 depicts the time course and magnitude of carbachol-induced effects at each dose and on each of the four synaptic responses (i.e., on SC- and IC-elicited fast and slow potentials). A differential effect on fast vs. slow potentials was first apparent at 5 μM, which reduced the SC- and IC-slow potentials 70.2±6.9% and 63.7±8.1%, respectively (P<0.001) but did not significantly affect the fast potentials (P>0.05). Higher concentrations of carbachol (>10 μM) reduced both slow and fast potentials but the slow potentials were always affected more strongly (P<0.05, paired t-test). In addition, reduction of the fast potential took longer to develop than that of the slow potential. This was quantified for the 50 μM effects dose; the maximal reduction of the slow potential occurred 3.05±0.58 min before that of the fast potential (P<0.001, paired t-test; these differences are not apparent in Fig. 4 because of the variable onset latency of carbachol’s effects across slices). Thus, carbachol reduced the slow potentials at a lower dose, to a greater degree, and more rapidly, than it reduced the fast potentials (Figs. 4 and 5).

Differential cholinergic modulation of the extracellular SC and IC fast potentials (Figs. 4 and 5) was similar to that observed in intracellular experiments (Fig. 3C and D). At 10 μM, carbachol reduced both fast potentials significantly (P<0.05), but reduced the IC-fast potential to a greater degree than the SC-fast potential (SC reduction = 6.6±2.0%; IC reduction = 15.1±3.7%; P<0.05, paired t-test). The differential reduction of fast potentials was enhanced at higher carbachol concentrations (Fig. 5).

Since the reduction of synaptic potentials can occur as a result of membrane depolarization (which would reduce excitatory driving force), we determined the effects of carbachol on membrane potential for two doses. At 10 μM, there was a negligible depolarization of the membrane potential (mean=1.4±1.4 mV; n=6; P>0.3). In contrast, 50 μM carbachol significantly depolarized the membrane potential (mean=4.3±0.9 mV; n=8; P<0.01). However, the carbachol-induced suppression of the synaptic responses remained during repolarization of the membrane potential back to baseline via intracellular current injection (n=3; data not shown).

To determine the subtype of ACh receptors involved in the reduction of synaptic responses, we applied the muscarinic receptor antagonist atropine. Atropine (0.5 μM) blocked the effects of 50 μM carbachol on the evoked...
Table 1
Effects of carbachol on intracellular potentials (% decrease ± S.E.)

<table>
<thead>
<tr>
<th>Subcortical</th>
<th>Intracortical</th>
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<tr>
<td>Fast potential</td>
<td>Slow potential</td>
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<tr>
<td>Amplitude</td>
<td>Slope</td>
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<tr>
<td>10 µM</td>
<td>15.4±6.8</td>
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<tr>
<td>50 µM</td>
<td>14.7±5.5</td>
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synaptic responses (Fig. 5), indicating the involvement of muscarinic ACh receptors in carbachol’s actions.

3.2. Experiment 2: cholinergic modulation of thalamocortical responses

Because the subcortical stimulating electrodes in the above experiments were placed in the auditory thalamocortical pathway rather than in the MG itself, they may have also activated cortical afferents originating outside the auditory thalamus. Stimulating the MG directly would increase the likelihood of activating an uncontaminated population of thalamocortical synapses. To do this, we developed a fully intact thalamocortical preparation in which direct MG stimulation produced robust cortical responses [12,43]. In such intact slices, we compared the effects of 25 µM carbachol on cortical responses evoked by MG stimulation, alternating with SC or IC stimulation (MG vs. SC, n=8; MG vs. IC, n=7).

Results of these experiments are shown in Fig. 6. As before, the slow potentials were suppressed more than the fast potentials for all pathways (P’s < 0.0001). Also, as before, both the IC- and SC-evoked fast potentials were suppressed by carbachol (P’s < 0.01) and this suppression

![Fig. 3. Differential effects of carbachol on synaptic responses.](A) Carbachol (10 µM) strongly reduced the SC-slow potentials (both intracellular and extracellular) but not the SC-fast potentials. All potentials recovered upon wash. (B) Similarly, 10 µM carbachol reduced both the IC-fast and slow potentials, with lesser effects on the IC-fast potentials. (C) and (D) Higher-resolution views of intracellular records in (A) and (B) reveal that carbachol reduced the IC-fast EPSP but not the SC-fast EPSP (arrows indicate peak EPSPs). Data in A, C are from the same cell; data from B, D are from another cell.)
Fig. 4. Dose dependence and time course of carbachol’s actions on fast and slow potentials. Fast potential amplitudes (solid circles) and slow potential magnitudes (open circles) were normalized to mean of 20 responses preceding carbachol (2 min pulse starting at arrow) at concentrations of 0.5/1, 5, 10, 25, or 50 μM. (Neither 0.5 nor 1 μM carbachol had any effect; these data were therefore combined.) For all concentrations, SC fast potential $n=7–12$, SC slow potential $n=5–9$, IC fast potential $n=4–14$, IC slow potential $n=3–6$.

was greater for the IC than the SC pathway (Fig. 6B and C; $P<0.05$). More importantly, the effect of carbachol on MG-evoked fast responses was not significantly different from that on SC-evoked responses (Fig. 6A (i) and B; $P>0.2$) supporting the hypothesis that carbachol’s effects on the SC response involves thalamocortical synapses. Despite the similarities, there was one noteworthy difference between the effects of carbachol on MG- vs. SC-evoked responses. In 2/15 slices, application of carbachol resulted in a clear enhancement of the MG-evoked fast potential; this never occurred for SC- or IC-evoked responses in either full or partial thalamocortical preparations. These two cholinergic enhancements contributed to the failure of the average MG data to exhibit significant suppression (Fig. 6A (i); $P=0.90$). Examples of an individual enhancement and a more typical effect are shown in Fig. 6A (ii) and (iii).

4. Discussion

We have examined cholinergic modulation of responses evoked by stimulation of extrinsic (thalamocortical) and intrinsic (intracortical) inputs to auditory cortex. For both pathways, muscarinic modulation suppressed long-duration polysynaptic responses more strongly than it suppressed monosynaptic potentials. Further, intrinsic monosynaptic responses were suppressed significantly more than were extrinsic monosynaptic responses. These differential findings have important implications for understanding auditory processing during periods of increased ACh release.
4.1. Nature of SC-, MG- and IC-evoked potentials

Subcortical stimulation evoked fast and slow potentials in layer IV of AC. Effective stimulation sites were only found within the thalamocortical pathway [12,43,60,73]. Evoked fast potentials had consistent and short onset latencies recorded in the middle layers of AC (the main termination zone of thalamic input) and were mediated largely by AMPA/KA receptors. As expected for monosynaptic activity, the onset latencies of the fast potentials did not change with application of carbachol (despite reduction of response amplitude). These characteristics suggest that the fast potential is a monosynaptic thalamocortical EPSP [1,18,43]. Further supporting evidence for this conclusion comes from our previous and present studies [12,43], showing that stimulation of the MG itself produces short latency potentials and a current sink in the middle cortical layers that are similar to responses evoked by SC stimulation within the thalamocortical pathway. Moreover, the effects of carbachol were not significantly different for responses evoked by SC or MG stimuli (Experiment 2). Therefore, unless otherwise specified, we will consider the effects of carbachol on SC- and MG-evoked responses together.

It is possible that the SC or MG stimuli can activate corticothalamic neurons antidromically, since the slice likely contains corticothalamic fibers [59] and such responses could contribute to our results. However, inves-
tigators using the somatosensory thalamocortical preparation also recognize this problem and argue that the contribution of corticothalamic activity is minimal [1] because thalamocortical fibers reputedly have a lower activation threshold than corticothalamic fibers [1,16,17]. Recently, we have confirmed this notion using direct tests in the auditory thalamocortical slice, demonstrating that the threshold for orthodromic activation of the layer IV field response is about five times lower than the antidromic threshold of layer V and VI neurons [58].

To evoke intracortical responses with minimal direct activation of thalamic afferents we stimulated horizontal (300–950 μm) intracortical pathways. This evoked fast and slow potentials in layer IV of AC. Again, the onset latency of the fast potential was short and consistent. This latency did not change with carbachol application, suggesting that it is monosynaptic.

We established the independence of the SC and IC pathways by demonstrating that complete fatigue of one pathway left responses to stimulation of the remaining pathway relatively unaffected. Thus, at least the monosynaptic SC and IC potentials are independent. However, we have not addressed the independence of the polysynaptic slow potentials. In fact, it is possible that the slow potentials evoked by stimulation at subcortical and intracortical sites are similar phenomena with overlapping circuitry. They both had long latencies and variable durations and magnitudes, suggesting they are polysynaptic in nature. In addition, Metherate and Cruikshank [43] showed that the SC-evoked slow potential propagated very slowly through the AC, implying that it is generated intracortically. Further details on the nature and mechanisms underlying generation of the slow potential have been published [43].

4.2. Differential cholinergic suppression of fast vs. slow potentials

Carbachol produced nearly complete suppression of the slow potential while leaving the fast potential relatively unaffected (at a dose of 5 μM) or suppressed to a much lesser degree (≥10 μM). While carbachol is a more effective agonist than ACh, due to its resistance to hydrolysis, it is assumed that the differential effects described here would also occur with the endogenous transmitter, and therefore are functionally relevant. The suppression of the slow potential occurred quite abruptly, whereas reduction of the fast potentials occurred gradually over many trials. A possible explanation for this differential effect is that the fast potential acts to “trigger” the slow potential, and that muscarinic suppression of the fast potential below the trigger threshold will abruptly prevent the appearance of the slow potential. Cholinergic suppression of polysynaptic responses could effectively shorten the response to a sensory stimulus, thus quickly returning the AC to a state of readiness for subsequent sensory events.

4.3. Differential suppression of SC/MG- vs. IC-evoked monosynaptic potentials

The reduction by carbachol of the IC fast potential to a greater degree than the SC/MG fast potential could possibly result from: (1) differential distribution of muscarinic receptors, (2) differential recruitment of inhibition, and (3) differential enhancement via nicotinic receptors. We will discuss each possibility in turn.

Activation of presynaptic muscarinic ACh receptors (mAChRs) can reduce EPSPs by reducing release of glutamate [27,63,64,68]. Thus, differential density of presynaptic mAChRs on SC and IC terminals could differentially reduce fast potentials (discussed by [30]). Sahin et al. [61] combined autoradiography with excitotoxic lesions of thalamic (ventrobasal) or cortical neurons to support such a differential distribution. In their study, thalamic lesions produced no reduction in cortical mAChR binding, suggesting limited presynaptic mAChRs on thalamic afferents. In contrast, excitotoxic cortical lesions produced a dramatic reduction of mAChR binding in layers I, III, IV and VI, indicating abundant mAChRs on elements intrinsic to the cortex. Other studies, including work at the ultrastructural level, have located mAChRs presynaptically on some thalamic afferents [48,70,71]. Thus, presynaptic mAChRs are found at both thalamocortical and intracortical synapses, but may be more prevalent at intracortical synapses. Such a distribution could underlie the preferential muscarinic suppression of intracortical fast potentials in the present study.

Differential recruitment of inhibition by thalamocortical and intracortical inputs also could contribute to preferential suppression of IC potentials. In our slice preparation, thalamocortical inputs activate disynaptic GABAergic IPSPs only weakly [43], whereas IC stimulation activated IPSPs more strongly (unpublished observation). The stronger IPSPs elicited by IC stimulation may underlie the greater reduction of IC fast potentials. Since an overlapping IC-elicted EPSP–IPSP would have a more negative reversal potential than the thalamocortical EPSP (without an overlapping IPSP), it seems possible that the combined potential would be reduced to a greater degree by carbachol-induced membrane depolarization. However, the data do not support this possibility. Not only could carbachol produce differential suppression without membrane depolarization (effects of 10 μM carbachol), but even at higher carbachol doses that depolarized the membrane several millivolts, differential suppression of EPSPs persisted during repolarization of the potential to its original level with intracellular current injection. Thus, a shift in synaptic reversal potential due to IPSPs does not underlie the differential suppression of fast potentials.

Muscarinic agonists can exert indirect suppressive ef-
fects by exciting GABAergic interneurons [36,50]. If cholinceptive GABAergic interneurons inhibit IC-elicited responses preferentially, then such actions could contribute to differential suppression of fast potentials. Gil et al. [19] found that muscarinic actions suppressed thalamocortical and intracortical EPSPs equally. However, Gil et al. [19] continuously infused the region surrounding the recording electrode with a GABA<sub>α</sub> receptor antagonist to explicitly reduce the influence of differentially evoked inhibition [18]. While this manipulation isolated muscarinic actions, it may have masked interactions that produced differential effects in the present study. Other recent studies indicate that cholinergic modulation of GABAergic function may produce complex effects in neural circuits [2,39,52,76]. Clearly, further studies are needed to resolve the role of inhibition in muscarinic suppression of thalamocortical and intracortical pathways.

Finally, activation of presynaptic nicotinic ACh receptors (nAChRs) can enhance cortical EPSPs by increasing release of glutamate [3,19–21,53,69]. In the somatosensory system, Gil et al. [19] observed that nicotinic agonists selectively enhanced thalamocortical EPSPs. Such actions could combine with general muscarinic suppression of EPSPs to produce the differential suppression by carbachol (a mixed cholinergic agonist) in the present study. However, two lines of evidence argue against this possibility. First, bath application of agonist can lead to rapid desensitization of nAChRs [10,65,77] that precludes nicotine-induced enhancement of EPSPs [3]. In support of this, bath application of nicotine to auditory thalamocortical slices from the rat produced no consistent effect on layer IV field responses to SC, IC, or intracortical “on-beam” stimulation (n=18, nicotine concentration 0.5–20 μM, slices obtained from animals aged 8–18 days postnatal; unpublished observations). Second, whereas atropine blocked the suppression produced by carbachol, it did not reveal enhancement of the SC-evoked response, as would be expected if nAChRs were simultaneously enhancing thalamocortical EPSPs (see also [30]). Thus, it is unlikely that nAChRs contribute to the differential actions of carbachol in the present study.

4.4. MG vs. SC modulation

Although the effects of carbachol on the SC- and MG-evoked responses were not statistically different from each other, some potentially important observations were noted. First, unlike the SC pathway, the average fast response evoked by MG stimulation was not suppressed significantly by carbachol. As stated in the Results, a large contribution to this lack of suppression came from 2/15 slices that exhibited increases in MG-evoked response; such increases never occurred for the SC- (or IC-) evoked responses. It may also be noteworthy that two additional slices showed large carbachol-induced increases in MG-evoked responses that were sustained beyond 30 min. Because the latter did not recover to baseline levels, they were excluded from the analysis (see Material and methods). However, as with the two cases that did recover, the onset of facilitation was time locked to carbachol infusion, the slow potentials evoked by MG stimulation were suppressed normally, and responses along the control pathways within the same slices (e.g., SC or IC) behaved normally (e.g., Fig. 6A (iii)). This supports the possibility that the increases were caused by specific cholinergic modulation of the thalamocortical pathway and not a general change in state or health of the slice.

Obviously the increases represent a minority of the total, and the majority of MG-evoked responses were either suppressed or not affected by carbachol, similar to SC-evoked responses. Thus, it is likely that the SC and MG stimuli activated a largely overlapping population of thalamocortical synapses. It is possible that the small number of enhanced responses to MG stimulation may result from carbachol-induced increased excitability of some MG soma [13,37,47]. This in turn may have enhanced the presynaptic input to the cortex during carbachol infusion for some slices in Experiment 2. In contrast, since axons of the MG cells would not be excited by carbachol, no cholinergic enhancement would be expected for SC stimuli. The transient and sustained increases in MG-evoked response are of great interest, especially in contrast to the suppression of intracortical synapses, and warrant further study.

4.5. Functional implications and relevance to previous in vitro and in vivo studies

Studies of cortical neurons in vivo have generally found that muscarinic actions increase responsiveness to sensory stimuli [15,42,44,54,67], whereas, in striking contrast, in vitro studies of cortical neurons have generally found suppression of EPSPs [2,4,27,64,69]. We propose two explanations for these conflicting findings, based partly on the present work. First, sensory stimuli in vivo activate cortical neurons via thalamocortical inputs, whereas electrical stimuli in vitro generally activate intracortical synapses (most cortical slice preparations either do not contain thalamocortical connections or do not allow for selective stimulation of thalamocortical inputs). Given the present results on preferential suppression of intracortical EPSPs, it is likely that in vitro studies typically demonstrate strong suppression of EPSPs due to their dependence on intracortical stimulation. The relatively weak suppression, or outright enhancement of thalamocortical EPSPs in the present study more closely resembles in vivo findings.

A second factor is that neurons recorded in vivo have more depolarized membrane potentials than neurons in vitro, and as a result may have activated voltage-dependent K<sup>+</sup> currents. Muscarinic blockade of voltage-dependent K<sup>+</sup> currents can profoundly increase postsynaptic excitability and responsiveness [7,9,32,33,36]. Such post-
synaptic actions are less pronounced in vitro because of more hyperpolarized membrane potentials. The lack of postsynaptic muscarinic actions could leave presynaptic actions relatively unopposed, resulting in net reduction of EPSPs. Thus, in the present study, thalamocortical EPSPs are generally only “enhanced” relative to the stronger suppression of intracortical EPSPs, whereas an analogous in vivo study could reveal greater EPSP enhancement due to increased postsynaptic excitability (cf. [42]). A similar proposal stems from the work of Hasselmo and colleagues in piriform cortex [24,26,30]. Differential presynaptic suppression, combined with increased postsynaptic excitability, would presumably result in an enhanced ability for extrinsic inputs to generate spikes while decreasing the effectiveness of intrinsic inputs [25,52].

Thus, the present results support the concept that cholinergic modulation serves to favor responses to external stimuli over ongoing cortical activity. Such selective processing of thalamocortical inputs during periods of increased ACh release (e.g., during behavioral arousal or attention), could underlie the widely hypothesized function of ACh to increase the “signal-to-noise” ratio of sensory responses over ongoing cortical activity (reviewed in [23,66]).

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