INPUT-SPECIFIC EFFECTS OF ACETYLCHOLINE ON SENSORY AND INTRACORTICAL EVOKED RESPONSES IN THE “BARREL CORTEX” IN VIVO

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Abstract—The somatosensory neocortex processes extrinsic information from the thalamus and intrinsic information from local circuits. We compared the effects of acetylcholine (Ach) on neocortical field potential responses evoked by stimulation of the whiskers and by local electrical stimulation in the upper layers of the neocortex vibrissae representation (“barrel cortex”) of adult rats anesthetized with urethane. In the barrel cortex, the cholinergic system was manipulated using microdialysis by exogenous application of Ach, by increasing the endogenous levels of Ach with physostigmine and by applying specific cholinergic agonists. The results revealed that Ach selectively enhances the sensory response relative to the intracortical response. Thus, pathways in the barrel cortex are differentially regulated by cholinergic inputs. © 2003 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: whisker, rat, sensory processing, nicotinic receptors, muscarinic receptors, acetylcholinesterase.

The somatosensory cortex processes extrinsic information originating from the peripheral environment as well as intrinsic information from within the cortex. Extrinsic information reaches the cortex via thalamocortical synapses, whereas intrinsic information is locally distributed via intracortical synapses. Rodents rely heavily on tactile information provided by the mystacial vibrissae and this information reaches the neocortex via the ventroposterior medial thalamus (VPM). Thalamocortical afferents from VPM terminate in layer IV in discrete cell clusters, which visually resemble barrels (Woolsey and Van der Loos, 1970). Both anatomical (Killackey, 1973) and electrophysiological (Simons, 1978) techniques have demonstrated a one-to-one correspondence between whiskers on the rat’s face and “barrels” in the contralateral somatosensory cortex, such that neurons in a particular barrel respond maximally (shortest latency and largest amplitude) to stimulation of its principal whisker (Armstrong-James and Fox, 1987). In order for normal processing to occur tactile information from neighboring whiskers must be integrated. The horizontal flow of information is primarily achieved through horizontal connections in upper layers II/III and lower layer V, which connect neighboring barrel columns (Hoefinger et al., 1995; Johnson and Alloway, 1996; Kim and Ebner, 1999).

The purpose of the current study is to examine how thalamocortical and corticocortical pathways are modulated in vivo by the presence of acetylcholine (Ach). Ach is an important neuromodulator in the cerebral cortex that has been implicated in many important brain functions, such as arousal (Celesia and Jasper, 1966), attention (Muir et al., 1994; Sarter and Bruno, 2000), learning and memory (Hasselmo, 1999; Weinberger, 1998). Ach is projected from the nucleus basalis of the basal forebrain (Eckenstein et al., 1988; Mesulam et al., 1983; Mesulam and Van Hoesen, 1976) and terminates diffusely throughout the cerebral cortex. Examining the distribution of the catabolic enzyme, acetylcholinesterase, and the synthesizing enzyme, choline acetyltransferase, provides an index of Ach availability. Both staining procedures have illustrated similar laminar patterns of Ach distribution in primary somatosensory cortex, such that high densities of both markers have been observed in layers V and I, whereas lower densities were observed in layers II, III and VI (Houser et al., 1985; Kristt, 1979a,b). Electrical or chemical stimulation of the basal forebrain increases cortical levels of Ach and induces cortical activation (Metherate et al., 1992; Rasmussen et al., 1992; Cape and Jones, 1998). Additionally, the behaviors characterized by strong cortical activation (i.e. arousal and rapid eye movement sleep (REM)) are associated with the highest levels of Ach release (Jasper and Tessler, 1971; Marrosu et al., 1995). Ach acts on both muscarinic and nicotinic receptors. Muscarinic receptors are distributed profusely throughout the cerebral cortex and are generally present in both superficial and deep cortical layers (Lidow et al., 1989; Sahin et al., 1992). In general, the receptors are localized postsynaptically on cortical neurons (Houser et al., 1985; McCormick and Prince, 1985). However, evidence exists for presynaptic localization on intrinsic (Sahin et al., 1992), thalamocortical (Vogt, 1984), and basal forebrain–cholinergic (Mash and Potter, 1986) fibers in the cortex. In fact, electrophysiological studies in vitro have shown that both intracortical and thalamocortical synapses are depressed by activation of muscarinic receptors (Gil et al., 1997; Kirkwood et al., 1999; Hsieh et al., 2000). Studies in vivo also found that activation of muscarinic receptors modulates cortical responsiveness (Metherate et al., 1988; Mc-
Kenna et al., 1988). Nicotinic receptors have been localized to the outer part of layer I, layers III and IV and upper layer VI (Clarke et al., 1985; Sahin et al., 1992). They are found presynaptically on thalamocortical fibers (Sahin et al., 1992; Lavine et al., 1997). In fact, electrophysiological studies have shown nicotine to modulate neurotransmitter release at thalamocortical synapses (Gil et al., 1997; Giovanni et al., 1999), as well as at intracortical synapses (Vidal and Changeux, 1993). Additionally, activation of nicotinic receptors affects inhibitory interneurons in the neocortex (McCormick and Prince, 1985; Xiang et al., 1998).

The current project studies in vivo how the presence of Ach modulates two important pathways: a sensory pathway and an intracortical pathway. We studied a sensory response by mechanically deflecting whiskers on the animals face and recording the evoked response in layer IV of the somatosensory barrel cortex. This is a good way to access thalamocortical fibers, because it isolates a pathway from VPM to barrel cortex. Previous studies that have used electrical stimulation of the thalamus, in vitro (Gil et al., 1997; Hsieh et al., 2000) and in vivo (Giovanni et al., 1999), to investigate the modulation of thalamocortical and intracortical pathways, are plagued by the criticism that stimulation of the thalamus produces antidromic activation of corticothalamic neurons. Since corticothalamic neurons produce intracortical connections in the main thalamocortical-recipient layers (i.e. layers IV and VI), it is not possible to decipher which synapses are being tested. In contrast, the use of sensory stimulation in the current study eliminates the possibility of antidromic activation of corticothalamic fibers. In addition, by activating intracortical fibers with a stimulating electrode placed in layers II/III of the neocortex, we also studied an independent intracortical pathway and compared the intracortical responses with the sensory (i.e. thalamocortical) responses.

EXPERIMENTAL PROCEDURES

Sprague–Dawley rats (250–300 g) were used for the current study. They were anesthetized with urethane and placed in a stereotaxic frame. All skin incisions and pressure points were injected with lidocaine (2%). A small unilateral craniotomy was performed over the whisker representation of the somatosensory cortex. This is a good way to access thalamocortical fibers. Small incisions were made in the dura in order to accommodate the insertion of the necessary probes and electrodes. The surface of the brain was covered with artificial cerebrospinal fluid (ACSF) which consisted of (in mM): NaCl 126, KCl 3, NaH2PO4 1.25, NaHCO3 26, MgSO4 7, H2O 1.3, dextrose 10, CaCl2 2, and H2O 2.5. Body temperature was monitored and maintained at 36–37 °C with a heating pad. All surgical procedures were reviewed and approved by the Animal Care Committee of McGill University.

Electrophysiological recordings

Recordings were made from Teflon-insulated tungsten electrodes and linear 16-channel silicon microelectrodes with 100-μm intersite spacing (Center for Neural Communication Technology, University of Michigan, Ann Arbor, MI, USA). To reduce and equalize the impedance (500 kΩ) of the recording sites on the silicon probes, they were oxidized before use. Data were stored and analyzed on a computer using Experimenter’s Workbench (Data Wave Technologies, Longmont, CO, USA) and origin (Microcal software, Northampton, MA, USA) software. Current source densities (CSDs) were calculated from the voltage recordings made by the 16-channel probes (Castro-Alamancos and Oldford, 2002). Filter settings were set at 1 Hz to 3 kHz.

Microdialysis and stimulation

Drug infusions were made using a microdialysis probe; model CMA11 (CMA Microdialysis, Solna, Sweden). The probe was 2 mm in length and 200 μm in diameter. It was placed in the cortex adjacent to the recording electrode at a depth of 2 mm from the surface of the dura. Application of drugs via microdialysis works by diffusion of the drug through the probes membrane due to concentration gradients. The drug levels achieved vary as a function of the distance from the probe and are estimated to be <10% of the ACSF content of the drug in the area surrounding the probe, and produce effects for about 1 mm around the probe membrane (Gil et al., 1997). In fact, electrophysiological studies have shown nicotine to modulate neurotransmitter release at thalamocortical synapses (Benveniste and Huttemeier, 1990). ACSF was continuously infused through the probe during the experiment at a constant rate of 4 μl/min. Drugs were dissolved into ACSF at specific times and concentrations throughout the experiment. Microdialysis probes and electrodes were placed stereotactically in the somatosensory cortex (Paxinos and Watson, 1982). The single-site recording electrode was placed at the following coordinates from bregma: rostrocaudal, –2.75 mm; mediolateral, 5.5 mm; ventral, 900 μm. Minor adjustments in position and depth were made to locate the response with the largest amplitude and the shortest onset to whisker stimulation. This was accomplished by mapping the cortical region with repeated penetrations. On occasions when the 16-channel electrode was used, it was placed at an angle of 45° so that it was perpendicular to the layers of the cortex (Castro-Alamancos and Oldford, 2002).

A thalamocortical pathway was accessed by mechanically deflecting two to four posterior whiskers approximately 400 μm using a whisker stimulator. The whisker stimulator consisted of a hollow glass tube mounted onto a mini-speaker. The whiskers selected (usually D1, E1, E2 and E3) were inserted into the glass tube. Intracortical fibers were stimulated using a bipolar stainless steel electrode placed less than 1 mm adjacent to the recording electrode at a depth of approximately 500 μm. This depth was chosen to minimize the activation of thalamocortical fibers. Stimulation intensity was generally 100 μA or less. Whisker stimulation had a duration of 1 ms and intracortical stimulation had a duration of 0.2 ms. The two pathways were stimulated alternatively every 6 s. The number of animals in which each drug was tested is indicated by n in the results section.

Statistical analysis

The statistical tests conducted evaluated differences between baseline and drug conditions on several criteria. 1) Paired t-tests were calculated to determine whether response amplitude evoked from a single stimulus varied as a function of drug application. 2) A two-factor repeated measures ANOVA (baseline/drug-interval) was conducted for each individual drug to evaluate how the form of the evoked response differed at 1-ms intervals poststimulus. The dependent variable was amplitude of evoked response. Simple effects were used to decompose significant main effects and interactions.

RESULTS

Properties of field-potential responses

Extracellular field-potential recordings were made in the barrel cortex in vivo. Two independent pathways were stimulated consecutively, a sensory pathway using whisker stimulation and an intracortical pathway using electrical
stimulation. Fig. 1A shows an example of a sensory (left) and intracortical (right) evoked response when one stimulus is given to each pathway. Whisker stimulation produced a large-amplitude negative field potential (mean±S.E.M.; 1.2±0.1 mV) that had an onset latency of 5.5±0.2 ms and peaked in amplitude at 10.6±0.4 ms (n=13 experiments). Longer latency responses evoked by whisker stimulation were discarded because they were believed to contain an initial intracortical component. Intracortical stimulation evoked a negative field-potential response that had an amplitude comparable to the whisker-evoked response (1.8±0.1 mV) but showed an onset latency of 1.9±0.1 ms and peaked in amplitude at 5.9±0.2 ms (n=13 experiments). In the present study, we consider changes in the amplitude of the evoked response to be of the short-latency component of the response if they occur before the peak amplitude of the response and of the long-latency component if they occur after the peak amplitude. This means changes are considered of the short-latency component if they occur at <10.5 ms poststimulus for the sensory pathway and <6 ms poststimulus for the intracortical pathway. For the sensory pathway, these latency differences reflect the occurrence of early current sinks in layers IV and VI (<10.5 ms) and latter sinks in layers II–III (<10.5 ms) (see CSDs below).

The majority of neocortical Ach originates extrinsically from the basal forebrain (Mesulam et al., 1983). Previous results in vitro have indicated that it has selective effects on different pathways (Gil et al., 1997; Hsieh et al., 2000). In the current study, Ach was applied via microdialysis to the neocortex to examine its effects on the sensory and intracortical pathways. Ach was applied at different doses that we termed low-Ach (1–10 mM), medium-Ach (100 mM) and high-Ach (200 mM). Smaller doses of Ach had no noticeable effects. The average effect of each drug used in the present study is shown in Fig. 2.

Low-Ach depressed both pathways. The response amplitude for the sensory and the intracortical pathways was reduced to an average of 82%±4% (n=6; t-test, P<0.05) and 83%±4% (n=6; t-test, P<0.01) of baseline amplitude, respectively. Low-Ach depressed the sensory and intracortical responses without altering their duration. Depression of the sensory response started 9 ms poststimulus (n=6; ANOVA, P<0.05), which corresponds to the short latency response component. Likewise, depression of the intracortical response began 5 ms poststimulus (n=6; ANOVA,
This corresponds to the short-latency response component for the intracortical pathway. Fast Fourier transforms (FFT) were calculated from every 2 s of spontaneous field-potential activity recorded from the neocortex and displayed as a contour plot. Power of each frequency is color-coded such that an increase in the power is displayed as gray to black and zero is displayed as light gray. (C) Amplitude of evoked responses for both pathways as a function of time. Each point represents the amplitude of the response to a single stimulus, and each pathway was stimulated once every 12 s. The horizontal bar represents the time the drug was applied via microdialysis. Traces are the average of 10 responses.

A typical example of a medium-dose application of Ach is illustrated in Fig. 3. Medium-Ach depressed both pathways. The response amplitude for the sensory and the intracortical pathways was reduced to an average of 83%±4% (n=11; t-test, P<0.001) and 82%±2% (n=11; t-test, P<0.005) of baseline level, respectively. Depression of the sensory response started 10 ms poststimulus (n=11; ANOVA, P<0.001) indicating that it was of the short-latency response component. The intracortical response depressed starting at 4 ms poststimulus (n=11; ANOVA, P<0.001) indicating that depression was of the short-latency response component for the intracortical pathway as well. FFT analysis indicates that medium-Ach activated the neocortex, resulting in a reduction of slow oscillatory activity. These changes in spontaneous activity were correlated with depression and completely recovered following removal of the drug.

High-Ach depressed the intracortical pathway but enhanced the sensory pathway. The response amplitude for the intracortical pathway was reduced to an average of 72%±6% (n=11; t-test, P<0.0001) of baseline level whereas the response amplitude of the sensory pathway was enhanced to an average of 136%±9% (n=11; t-test, P<0.005) of baseline level. Depression of the intracortical response began 4 ms poststimulus (n=11; ANOVA, P<0.001) indicating that it was of the short-latency response component. Enhancement of the sensory response began 11 ms poststimulus (n=11; ANOVA, P<0.02) indicating that the enhancement was of the long-latency response component. As well, the duration of the evoked response increased. FFT analysis indicates that high-Ach activated the neocortex causing a reduction in low-frequency oscillatory activity. Also, high-Ach was characterized by the appearance of large-amplitude spontaneous spike-wave complexes in the neocortical activity. In addition, the normal spontaneous activity returned following removal of the drug.

These results indicate that application of Ach has highly specific and dose-dependent effects. The next experiments explored whether specific cholinergic receptor agonists mimicked these effects.

**Effects of cholinergic agonists**

In order to decipher through which receptors the effects of Ach were being mediated, we tested the selectivity of the cholinergic agonists, muscarine and nicotine. A typical example of muscarine application is illustrated in Fig. 4. Application of muscarine (0.5–1 mM) depressed both the sensory and intracortical responses. The intracortical path-
way was reduced to an average of 70%±4% (\(n=7\); \(t\)-test, \(P<0.001\)) of baseline amplitude, while the sensory pathway was reduced to an average of 85%±5% (\(n=7\); \(t\)-test, \(P<0.001\)) of baseline amplitude. The intracortical depression started 4 ms poststimulus (\(n=6\), ANOVA, \(P<0.0001\)), indicating that depression was of the short-latency response component. Likewise, depression of the sensory response started 10 ms poststimulus (\(n=5\), ANOVA, \(P<0.05\)), indicating that it was of the short-latency response component, as well. FFT analysis shows that application of muscarine activated the neocortex, reducing slow oscillatory activity. The changes in spontaneous activity were correlated with depression of evoked responses and activity returned to baseline levels following removal of the drug.

A typical example of nicotine application is illustrated in Fig. 5. Nicotine (1 mM) was found to selectively enhance the sensory pathway, while having no effect on the intracortical pathway. The amplitude of the sensory response increased to 167%±10% (\(n=7\); \(t\)-test, \(P<0.0001\)) relative to baseline. The enhancement began 9 ms poststimulus (\(n=7\); ANOVA, \(P<0.01\)), indicating that the enhancement was of the short-latency response component. As well, the response was not characterized by enhancement of any longer-latency components. FFT analysis indicates that application of nicotine did not produce consistent observable changes in spontaneous field-potential activity.

**Effects of endogenous Ach**

Acetylcholinesterase is an endogenous enzyme responsible for the degradation of Ach released into the synaptic cleft. Inhibiting this enzyme through application of physostigmine results in the gradual accumulation of synaptically located Ach. This method of Ach application permits the study of endogenous Ach localized to its physiological sites of release. Physostigmine was applied at different doses that we termed low-physo (0.5 mM), medium-physo (1–2 mM) and high-physo (5 mM). Low doses of physostigmine did not produce consistent effects on the evoked responses for the sensory and intracortical pathways. On some occasions, drug application was accompanied by depression of the evoked responses for both pathways. However, neither the sensory, nor the intracortical depression was found to be significant (\(P>0.05\) for both). A representative example of a medium-dose application is illustrated in Fig. 6. Application of medium-physo enhanced the sensory pathway to 144%±5%, (\(n=7\); \(t\)-test, \(P<0.005\)) of baseline amplitude. The enhancement started 10 ms poststimulus (\(n=7\); ANOVA, \(P<0.005\)), which corresponds to the short-latency response component. In contrast, the intracortical response was not significantly affected by application of medium-physo. FFT analysis indicates that medium-physo had minimal effects on neocortical spontaneous activity.

High-physo enhanced the sensory pathway and depressed the intracortical pathway. The sensory pathway was enhanced to an average of 234%±21% (\(n=10\); \(t\)-test, \(P<0.0001\)) relative to baseline, whereas the intracortical pathway was reduced to an average of 80%±7% (\(n=10\); \(t\)-test, \(P<0.05\)) relative to baseline. The enhancement of the sensory response started 10 ms poststimulus (\(n=9\), ANOVA, \(P<0.01\)), indicating that the enhancement started with the short-latency response component. In addition, the response was also characterized by enhancement of longer-latency response components, such that the duration of the response increased. Depression of the intracortical pathway started 3 ms poststimulus (\(n=9\); ANOVA, \(P<0.05\)), indicating that the depression was of the short-latency response component. FFT analysis indicates that application of high-physo activated the neocortex, reduc-
ing slow oscillatory activity. In addition, high-physo was characterized by the appearance of large-amplitude spontaneous spike-wave complexes in the neocortical activity. This activity corresponded in time to when the drug was maximally effective. Normal spontaneous activity returned following removal of high-physo.

The effects of all the drugs tested can be summarized by indicating that the short-latency component of the sensory response (<10.5 ms) was depressed following application of exogenous Ach and muscarine and enhanced following application of medium and high doses of endogenous Ach and nicotine. The long-latency component of the sensory response (>10.5 ms) was enhanced following application of high doses of endogenous and exogenous Ach. The short-latency component of the intracortical response (<6 ms) was depressed following application of exogenous Ach, muscarine and high doses of endogenous Ach.

**CSD analysis**

In order to understand how drug application influences laminar current flow and the meaning of the short- and long-latency changes, we conducted a CSD analysis, using 16-channel silicon microelectrodes. These electrodes record voltage concurrently from 16 channels, enabling the calculation of CSDs, which are displayed as contour plots. Sinks are represented by white, sources are represented by black and neutral is represented by gray. Fig. 7A (middle) illustrates a CSD derived following whisker stimulation. Stimulation of the whiskers produced short-latency sinks in layers IV and VI. The largest, located in layer IV had a corresponding current source located in layers II–III, whereas the smaller layer VI sink had a corresponding source in lower layer V. In addition, a small longer-latency current sink was located in upper layers II–III. Fig. 7B (middle) illustrates a CSD derived following intracortical stimulation. Intracortical stimulation produced two short-latency sinks. The largest was located in upper layers II–III with a corresponding current source in the superficial layers. The smaller sink was located in layer IV and it had a current source located in layers II–III. Both sinks are seen to be propagating downwards. In addition, a longer-latency sink was observed in layer VI. Three drugs (high-Ach, nicotine and high-physo) were tested in at least three animals each and the effects on the amplitude of the layer IV and layer III current sinks are displayed in Table 1 and described below.

Fig. 7 shows the effects of high-Ach on laminar current flow evoked by sensory and intracortical stimulation. High-Ach reduced the intensity of the short-latency sensory-evoked current sinks. In particular, the current sinks in layers IV and VI became less intense, indicating that there was a reduction of current flow. On average the amplitude of the layer IV sink in the presence of high-Ach was 64 ± 4% of control ($n=3$; $P<0.0001$). In contrast, high-Ach strongly enhanced the long-latency sink located in layers II–III producing a strong increase in the whisker-evoked current flow in the upper layers (230 ± 11% of control; $n=3$; $P<0.0001$). The intracortical-evoked sinks all became less intense following application of high-Ach (see Table 1). This indicates that the drug reduced the current flow evoked by intracortical stimulation.

Fig. 8 shows the effects of nicotine on laminar current flow evoked by sensory and intracortical stimulation. Nicotine enhanced the intensity of the sensory-evoked current sinks in layers IV and VI. On average the amplitude of the layer IV sink in the presence of nicotine was 182% ± 7% of control ($n=3$; $P<0.0001$). Thus, the enhancement corresponded to the short-latency response component. There was less change of any longer-latency response components. Also, the intracortical-evoked current sinks were not significantly altered by nicotine application.

Table 1. Percent change in current sink amplitude from control in the presence of high acetylcholine (high-Ach), nicotine and high-physostigmine (high-physo) (mean ± S.E.M.)

<table>
<thead>
<tr>
<th></th>
<th>Layer IV Sensory</th>
<th>Layer IV Intracortical</th>
<th>Layer III Sensory</th>
<th>Layer III Intracortical</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-Ach</td>
<td>64 ± 4%*</td>
<td>57 ± 5%*</td>
<td>230 ± 11%*</td>
<td>69 ± 6%*</td>
</tr>
<tr>
<td>Nicotine</td>
<td>182 ± 7%*</td>
<td>102 ± 4%</td>
<td>118 ± 8%</td>
<td>103 ± 3%</td>
</tr>
<tr>
<td>High-physo</td>
<td>189 ± 6%*</td>
<td>69 ± 6%*</td>
<td>221 ± 12%*</td>
<td>78 ± 7%*</td>
</tr>
</tbody>
</table>

* Significant change $P<0.0001$. 

**Fig. 7.** Current source density analysis (CSD) illustrating typical effects of high acetylcholine (high-Ach) on sensory- (A) and intracortical-evoked (B) responses in the barrel cortex. Traces of field-potential responses recorded 900 μm in depth (upper) correspond to the CSD analyses displayed below as contour plots for evoked responses before (middle) and during (bottom) application of high-Ach. White represents current sinks, black represent current sources, and grays are around zero. CSDs and traces are the average of 10 responses.

**Fig. 8.** Shows the effects of nicotine on laminar current flow evoked by sensory and intracortical stimulation. Nicotine enhanced the intensity of the sensory-evoked current sinks in layers IV and VI. On average the amplitude of the layer IV sink in the presence of nicotine was 182% ± 7% of control ($n=3$; $P<0.0001$). Thus, the enhancement corresponded to the short-latency response component. There was less change of any longer-latency response components. Also, the intracortical-evoked current sinks were not significantly altered by nicotine application.

**Fig. 9.** Shows the effects of high-physo on laminar current flow evoked by sensory and intracortical stimulation.
tion. High-physo produced a strong enhancement of the short-latency sensory-evoked current sinks in layers IV and VI. On average the amplitude of the layer IV sink in the presence of high-Ach was \(189\% \pm 6\%\) of control \((n=3; P<0.0001)\). As well, high-physo enhanced the longer-latency sinks located in the upper layers \(221\% \pm 12\%\) of control; \(n=3; P<0.0001\). The enhancement was characterized by an increase in the intensity and size of the sinks, indicating that there was a strong increase in current flow. In contrast, high-physo reduced the intensity of the current sinks evoked by intracortical stimulation (Table 1).

In general, the CSDs revealed that the short-latency changes (<10.5 ms) in the field-potential responses evoked by whisker stimulation were reflected in current flow changes in the thalamocortical recipient layers (IV and VI), while the long-latency changes (>10.5 ms) were reflected mainly in the upper layers (II–III).

**DISCUSSION**

In the current study, a thalamocortical and an intracortical pathway were independently stimulated in vivo. The experiments evaluated how the responses of these pathways are modulated by the presence of Ach. These results demonstrate that Ach has input-specific and dose-dependent effects in the neocortex. Previous studies have shown that Ach differentially modulates input-specific pathways in vivo in the piriform cortex (Hasselmo and Bower, 1992), the hippocampus (Kahle and Cotman, 1989) and the neocortex (Gil et al., 1997; Hsieh et al., 2000). Our results in vivo indicate that Ach differentially modulates the sensory and intracortical pathways. The results suggest that the primary effect of Ach in the barrel cortex during behavior may be a selective enhancement of the sensory pathway relative to the intracortical pathway. Thus, pathways in the somatosensory cortex are differentially regulated by cholinergic inputs. In fact, there appear to be four main effects of Ach in the neocortex: 1) intracortical muscarinic depression, 2) sensory muscarinic depression, 3) sensory nicotinic enhancement and 4) long-latency sensory enhancement. The first three effects involve modulation of the short-latency response component, whereas the forth effect involves modulation of the long-latency response component. These effects can also be segregated based on activation of muscarinic versus nicotinic and synaptic versus extrasynaptic receptors.

**Intracortical muscarinic depression**

Depression of the intracortical response was observed following application of exogenous Ach, high doses of endogenous Ach (i.e. high-physo) and muscarine. For each drug the depression started with the short-latency response component and occurred throughout all cortical lamina. Depression of intracortical-evoked responses following application of Ach or muscarinic agonists has been previously described in the neocortex (Hsieh et al., 2000; Gil et al., 1997; Vidal and Changeux, 1993). Although all
doses of exogenous Ach depressed the intracortical response, only high doses of endogenous Ach were sufficient to produce depression. This discrepancy between endogenous and exogenous Ach application indicates that methodological differences may produce distinct effects due to differential activation of synaptic and extrasynaptic cholinergic receptors. In fact, the cortex contains both synaptic and extrasynaptic cholinergic receptors. The former refers to receptors located in close spatial proximity with cholinergic fibers whereas the latter refers to those not associated with cholinergic fibers (Lidow et al., 1989; Descarries et al., 1997; Spencer Jr. et al., 1986). During normal Ach release, synaptic receptors are preferentially activated. In contrast, the function of extrasynaptic receptors is unknown as they are generally unaffected under these same conditions. In the current study, we enhanced endogenous Ach with physostigmine and we also applied Ach exogenously via microdialysis. Endogenous Ach application mimics physiological conditions, as rising Ach levels are confined to sites of release and therefore selectively activate synaptic receptors. In contrast, exogenous Ach application acts upon both synaptic and extrasynaptic receptors due to diffuse infusion of the drug (Wakade and Wakade, 1983). The exogenous application of Ach may be more effective at extrasynaptic receptors because these are further away from the catabolic enzyme, acetylcholinesterase, which is located at the terminals (Kirst, 1979). Thus, the effects of exogenous application of Ach may be interpreted in the context of a principal effect on extrasynaptic receptors, while the effects of endogenous enhancement of Ach with physostigmine may be interpreted in the context of a principal effect on synaptic receptors.

The finding that only high doses of endogenous Ach could effectively mimic intracortical muscarinic depression suggests that this may not be a sensitive physiological effect. Most likely the high levels of synaptic Ach achieved with high-physostigmine to extrasynaptic receptors. The strong depression observed following exogenous application of Ach and muscarine may also be due to activation of extrasynaptic Ach receptors, or a combination of extrasynaptic and synaptic receptors. This is interesting when considering that intracortical muscarinic depression has been described as a sensitive cholinergic effect by researchers using exogenous application procedures. Thus, the findings obtained with exogenous application of Ach may not necessarily reflect the physiological consequence of Ach release during behavior.

The depressive effects of these drugs may be due to presynaptic or postsynaptic mechanisms. Evidence suggests that muscarinic receptors are localized presynaptically on intrinsic cortical fibers (Sahin et al., 1992) and postsynaptically on cortical neurons (Houser et al., 1985; McCormick and Prince, 1985).

**Sensory muscarinic depression**

Depression of the sensory-evoked response was observed following application of exogenous Ach and muscarine. The depression began with the short-latency response component. Analysis of laminar current flow indicates that both drugs depressed current flow in layers IV and VI. Depression of the sensory pathway by muscarine and exogenous Ach were generally not as strong as that produced in the intracortical pathway. This corresponds with previous findings (Gil et al., 1997; Hasselmo and Bower, 1992; Hsieh et al., 2000) indicating that the suppressive effects of Ach are more selective for intrinsic, rather than afferent fibers in various cortical regions. This has also been described as a relative enhancement of afferent pathways. The fact that enhancing endogenous Ach could not mimic the sensory muscarinic depression suggests that the depression is due to activation of extrasynaptic muscarinic receptors. Therefore, sensory muscarinic depression may be an unlikely consequence of physiological Ach release.

Sensory and intracortical muscarinic depression always occurred together indicating that both effects may have resulted from general changes in postsynaptic cortical excitability. The only exception to this seemed to be high doses of exogenous Ach, which produced an enhancement of the sensory response. However, this sensory enhancement corresponded to the long-latency response component. In fact, analysis of laminar current flow illustrates the co-existence of both (Fig. 7), such that high-Ach produced a depression of the short-latency response component and an enhancement of the long-latency response component.

**Sensory nicotinic enhancement**

Sensory nicotinic enhancement was observed following application of nicotine and endogenous Ach. The enhancement started with the short-latency response component in layers IV and VI and was not characterized by enhancement of any longer-latency components. This is in contrast to the enhancement of the long-latency response component observed following application of high doses of exogenous Ach. The ability of endogenous Ach to mimic the nicotinic enhancement suggests that it results from the activation of synaptically located cholinergic receptors. Therefore, Ach-induced nicotinic enhancement appears to be a sensitive physiological effect and is the most likely primary consequence of Ach release during various behavioral states because it is the first effect observed after enhancement of endogenous Ach. It is also important to consider that physostigmine has been shown to directly modulate nicotinic receptors in hippocampus cells and muscle (Albuquerque et al., 1997). Thus, physostigmine may also facilitate the actions of the enhanced endogenous Ach by modulating nicotinic receptors.

The increase in evoked responses produced by nicotine and endogenous Ach may involve either presynaptic or postsynaptic mechanisms. Evidence suggests that nicotinic receptors are located presynaptically on thalamocortical fibers (Sahin et al., 1992; Lavine et al., 1997). As well, electrophysiological evidence indicates that nicotine modulates glutamatergic transmission at thalamocortical synapses (Gil et al., 1997; Gioannini et al., 1999).
Long-latency sensory enhancement

Application of high doses of endogenous and exogenous Ach both produced an enhancement of the sensory response. In contrast to the sensory nicotinic enhancement, this enhancement was of the long-latency response component (>10.5 ms), such that the duration of the response increased. Analysis of laminar current flow indicates that the enhancement occurred primarily in the upper layers of the cortex. In addition, it was generally accompanied by the appearance of large-amplitude spikes in the spontaneous field-potential activity. The enhancement produced by high doses of endogenous and exogenous Ach were not identical. High endogenous Ach produced an enhancement, which included both short- and long-latency response components. Specifically, it appeared to be a combination of sensory nicotinic enhancement and long-latency enhancement.

The long-latency enhancement may be due to a reduction of inhibitory circuits. Thalamocortical fibers recruit strong feed-forward inhibition (Porter et al., 2001; Swadlow, 1995), which can be observed intracellularly as a long-latency IPSP following a brisk EPSP (Carvell and Simons, 1988). Disruption of this inhibition could partially or fully account for enhancement of the long-latency response component produced by high doses of Ach. Diminishing inhibitory influences could result in a net enhancement of excitation. Ach has been reported to hyperpolarize FS inhibitory interneurons through activation of muscarinic receptors (Xiang et al., 1998). In the current study, large-amplitude spontaneous spikes in the cortex generally accompanied the long-latency enhancements. This epileptiform activity may be indicative of a loss of GABAergic inhibition. In fact, application of a GABA<sub>A</sub> antagonist in vivo enhances ventrobasal-evoked thalamocortical responses (Castro-Alamancos, 1997) with the appearance of long-latency response components.

Functional considerations

Our results indicate that methodological issues play an important role in the differential effects of Ach reported on thalamocortical pathways. Specifically, we found that exogenous and endogenous application procedures produce effects which are opposite in nature. Most studies in vitro have reported sensory muscarinic depression of afferent fibers following bath application of muscarinic agonists (Gil et al., 1997; Hsieh et al., 2000). This may result from preferential activation of extrasynaptic receptors. Another in vitro study (Vidal and Changeux, 1993) reported that Ach consistently depressed the intracortical-evoked response unless it was co-applied with physostigmine. During these conditions, the depression was reversed to a nicotinic enhancement. They interpreted the physostigmine-induced enhancement as evidence that high concentrations of Ach are necessary to activate nicotinic receptors. However, the results may also reflect the need to enhance synaptically located Ach.

Ach seems to activate mechanisms within the cortex, which adjust the relative strength of input-specific pathways. Differential effects on input-specific pathways likely reflect the distinct distribution of cholinergic receptors. In particular, the preferential distribution of nicotinic receptors in the vicinity of synapses relaying sensory information would modulate thalamic inputs while not affecting others. In fact, enhancement of the short-latency component of the sensory response was the most sensitive effect produced by endogenous Ach, and this was mimicked by nicotine. This suggests that the most likely consequence of Ach release during behavior is the selective enhancement of the sensory pathway through activation of nicotinic receptors.

What is the importance of modulating the relative strength of thalamocortical and intracortical inputs? Perhaps the importance may be elucidated when considering that the majority of synapses in the neocortex originate from cortical neurons. In particular, only a minority of synapses on layer IV thalamocortical-recipient cells are thalamic in origin (i.e. approximately 5–10%), whereas the remaining are intracortical in origin (Douglas et al., 1995; White, 1989). This suggests that under normal conditions, intracortical pathways probably dominate cortical processing. Therefore, Ach release during various behaviors may serve to facilitate the numerically inferior thalamocortical pathways in order to maximize transfer of information from the periphery to the cortex, while perhaps higher levels of Ach may also suppress the dominant intracortical pathway.

REFERENCES

Gil Z, Connors BW, Amitai Y (1997) Differential regulation of neocor-