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INPUT-SELECTIVE POTENTIATION AND REBALANCING OF PRIMARY SENSORY CORTEX AFFERENTS BY ENDOGENOUS ACETYLCHOLINE

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Abstract—Acetylcholine (ACh) plays important roles in the modulation of activity and plasticity of primary sensory cortices, thus influencing sensory detection and integration. We examined this in urethane-anesthetized rats, comparing cholinergic modulation of short latency, large amplitude field postsynaptic potentials (fPSPs) in the visual cortex (V1) evoked by stimulation of the ipsilateral lateral geniculate nucleus (LGN), reflecting direct thalamocortical inputs, with longer latency, smaller amplitude fPSPs elicited by contralateral LGN stimulation, reflecting indirect, polysynaptic inputs. Basal forebrain (BF) stimulation (100 Hz) produced a significant (~45%), gradually developing potentiation of the smaller, contralateral fPSPs, while ipsilateral fPSPs showed less enhancement (~15%), shifting the relative strength of dominant/ipsi- and weaker/contralateral inputs to V1. Systemic or local, cortical blockade of muscarinic receptors (scopolamine) reduced potentiation of contralateral fPSP without affecting ipsilateral enhancement, thus preventing the relative amplification of contralateral inputs following BF stimulation. Systemic nicotinic receptor blockade (mecamylamine) resulted in depression of ipsilateral, and reduced enhancement of contralateral fPSPs after BF stimulation. *N*-methyl-D-aspartate receptor blockade (systemic MK-801) abolished ipsilateral fPSP enhancement without affecting contralateral potentiation. Neither drug reduced the amplification of contralateral relative to ipsilateral signals in V1. In a second experiment in the barrel cortex, BF stimulation enhanced multiunit activity elicited by whisker deflection in a muscarinic-sensitive manner. Similar to the observations in V1, this effect was more pronounced for weaker multiunit activity driven by a surround whisker than activity following principal whisker deflection. These experiments demonstrate that ACh release following BF stimulation exerts surprisingly selective effects to amplify non-dominant inputs to sensory cortices. We suggest that, by diminishing the imbalance between different afferent signals, ACh release during states of behavioral activation acts to induce a long-lasting facilitation of the detection and/or integration of signals in primary sensory fields of the cortical mantle.

Key words: basal forebrain, muscarinic receptor, visual cortex, lateral geniculate nucleus, barrel cortex, rat.

Cortical and subcortical (e.g. thalamic) release of acetylcholine (ACh) has long been implicated in the modulation of sensory processing, cognitive and attentional functions, and synaptic plasticity in primate and sub-primate species (McCormick, 1989; McGaughy et al., 2000; Rasmusson, 2000; Sarter and Bruno, 2000; Semba, 2000; Edeline, 2003; Gu, 2003; Thiel, 2007). A primary function of ACh lies in the regulation of the cortical activation state, with elevated levels of ACh facilitating the appearance of high frequency (beta, gamma), low amplitude activity in the electrocorticogram (ECoG) (Buzsáki et al., 1988; Vanderwolf, 1988; Metherate et al., 1992; Détári et al., 1999; Duque et al., 2000). Increases in ACh and the associated ECoG activation during specific behavioral states (waking, rapid eye movement sleep) exert profound effects on cortical sensory transmission. For example, in the primary visual cortex (V1), release or application of ACh, or the appearance of ACh-related ECoG activation typically results in facilitation of neuronal responses in a number of species (rodents, cats, monkeys; Livingston and Hubel, 1981; Sato et al., 1987; Lewandowski et al., 1993; Müller et al., 1993; Meeren et al., 1998; Zinke et al., 2006). Importantly, ACh appears to exert highly selective, input-specific effects in V1, with a profound suppression of intracortical connections, but relatively little effect on thalamocortical inputs (Kimura et al., 1999), a pattern also seen in other sensory fields (e.g. pyriform and primary somatosensory cortex [S1]; Hasselmo and Bower, 1992; Oldford and Castro-Alamancos, 2003). Thus, one of the functions of cortical ACh release and ECoG activation appears to lie in the amplification of thalamocortical sensory signals relative to intrinsic cortical transmission, an effect directly opposite to the disconnection of the cortical mantle from thalamic inputs during states of cortical deactivation (e.g. slow wave sleep; Steriade, 2000).

In addition to this state-dependent, online modulation of sensory and intracortical transmission, ACh also impacts the induction and maintenance of long-lasting plasticity in neocortical networks. Application of exogenous ACh, or release of endogenous ACh by basal forebrain (BF) stimulation can enhance the responsiveness of neurons in S1 to peripheral stimulation for periods of several hours (Metherate et al., 1987; Rasmusson and Dykes,

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Abbreviations: ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BF, basal forebrain; ECoG, electrocorticogram; fPSP, field postsynaptic potential; LGN, lateral geniculate nucleus; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; S1, primary somatosensory cortex; V1, primary visual cortex.

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1988; Verdier and Dykes, 2001; review by Rasmusson, 2000). Elegant work by Weinberger (2004, 2007 for reviews) has shown that the BF cholinergic system mediates receptive field plasticity of rat auditory cortex neurons during associative learning experiences, and equivalent results have been obtained in humans (Thiel et al., 2002; Thiel, 2007). In V1, ACh release results in long-lasting increases of visually evoked responses (Golmayo et al., 2003), similar to its effects in other sensory cortices (see above). Further, ACh plays a permissive role in the expression of ocular dominance plasticity during early postnatal development (Bear and Singer, 1986; Gu and Singer, 1993; review by Gu, 2003), as well as receptive field plasticity, expressed as shifts in orientation tuning of V1 neurons following pairing of sub-optimal orientation stimuli with iontophoretic ACh application (Greuel et al., 1988). Ocular dominance and receptive field (orientation-tuning) plasticity are both thought to be mediated, at least in part, by long-term potentiation (LTP)–like mechanisms to increase synaptic strength and, indeed, ACh release facilitates LTP at synapses in V1 *in vitro* (Bröcher et al., 1992; Kojic et al., 2001) and *in vivo* (Dringenberg et al., 2007).

Those previous data indicate that ACh modulates activity in cortical sensory fields along multiple temporal scales: a dynamic, online regulation of cortical excitability and receptivity to incoming sensory signals, and a slower, long-lasting enhancement of synaptic coupling by amplification of LTP-like mechanisms. For the latter, there currently is little information regarding a possible synapse- or pathway-selective role of ACh to influence synaptic strength in cortical sensory areas. Kojic et al. (2001) showed that, in slices of V1, LTP in layers II/III was enhanced by muscarinic receptor stimulation, while no such effects were seen in the thalamo-recipient layer IV, suggestive of a differential sensitivity of separate classes of synapses to cholinergic modulation. Here, we compare the effect of endogenous ACh release to induce long-lasting increases in synaptic strength in different afferent inputs to V1 *in vivo*. The role of different classes of cholinergic receptors (muscarinic, nicotinic) was assessed by pharmacological manipulations. Also, a potential involvement of *N*-methyl-D-aspartate (NMDA) receptors was examined, given the important role of these receptors in long-lasting synaptic potentiation (e.g. Bennett, 2000), as well as previous observations that NMDA receptors participate in some types of synaptic potentiation elicited by BF stimulation (Verdier and Dykes, 2001). Finally, we carried out additional experiments in S1 (barrel cortex) to evaluate the generality of findings obtained in V1 to other cortical sensory domains.

EXPERIMENTAL PROCEDURES

Subjects and surgical preparation

All procedures were carried out in accordance with the ethical guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committees of Queen's University and Dalhousie University. All efforts were made to minimize the number of animals used and their suffering. Adult, male Long–Evans rats (300–500 g), housed in a colony room (reversed 12-h light/dark cycle) with free access to food and water, were used. Experiments were conducted under deep urethane anesthesia (1.5 g/kg, administered as three i.p. doses of 0.5 g/kg each every 20 min, supplemented as necessary). Rats were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA) and body temperature was maintained between 36 and 37 °C by an electric heating pad (Sunbeam, Shelton, CT, USA) and cotton blankets wrapped around the animal. The skull was exposed and 1 mm holes were drilled at the following coordinates (all in mm from bregma point, midline, and skull surface): BF, AP −1.0, L +2.7, V −7.5; left and right lateral geniculate nucleus (LGN), AP −4.0, L ±3.9, V −4.5 to −5.0; V1, AP −7.5, L +3.5 (i.e. ipsilateral to BF electrode), V ~1.0. Final ventral placements of LGN and V1 electrodes were adjusted to yield maximal amplitude of field postsynaptic potentials (fPSPs) in V1 in response to ipsilateral and contralateral LGN stimulation (see below). Small screws placed in the skull above the cerebellum and right and left frontal cortex served as reference, ground, and stimulation return electrodes, respectively.

For a supplementary experiment, rats were prepared as described above, with the exception that S1 was exposed instead of V1. Further, tactile (whisker) stimulation was employed instead of electrical stimulation of the thalamus. A small piece of bone (about 2×4 mm) overlying S1 was removed, the underlying dura carefully peeled back, and warm oil was applied to the cortical surface to prevent tissue dehydration during the course of the experiment. Subsequently, a microelectrode was lowered into the superficial S1 (~100–300 μm). Tactile stimuli (light pressure to the skin, deflection of whiskers) were used and repeated penetrations were performed until a barrel had been located (i.e. cortical units responded strongly to whisker deflection). Subsequently, the depth of the electrode was adjusted to optimize bursts of multiunit activity to deflection of the principal whisker, as well as a secondary (surround) whisker, which was always located adjacent to the principal whisker. The electrode remained at that location and all other whiskers on the snout contralateral to the S1 recording site were cut, leaving only the principal and one secondary whisker for formal data collection (see below).

The two different electrophysiological assays (field potentials in V1, multiunit activity in S1) were chosen to match the spatial selectivity of the recording technique with the spatial extent of cortical excitation elicited by LGN stimulation and whisker deflection. Electrical stimulation of the LGN produces widespread, synchronized activation of a large extent of V1 (see Heynen and Bear, 2001), making fPSP recordings an appropriate means to measure summed, synchronized currents across V1. In contrast, the much more localized excitation of S1 produced by whisker deflection can be measured more accurately with high-resistance microelectrodes, allowing recordings restricted to neurons within a single barrel in S1 that corresponds to the stimulated whisker.

Electrophysiology

Stimulation of the BF (0.2 ms negative pulses, 1 mA, 100 Hz, applied as 10 0.5 s bursts every 10 s) was provided by a monopolar electrode (125 μm diameter Teflon insulated stainless steel wire) connected to a stimulus isolation unit providing constant current output (PowerLab 4/s system with ML 180 Stimulus Isolator, ADInstruments, Colorado Springs, CO, USA). Stimulation of the LGN (0.2 ms pulses every 15 s, current adjusted to 50%–60% of that eliciting maximal fPSP amplitude in V1) was delivered by two concentric bipolar electrodes (Rhodes Medical Instruments series 100, David Kopf) placed in the left and right LGN. Alternating (every 7.5 s) left and right stimulation was controlled by the same hardware as outlined above. The fPSPs in V1 were differentially recorded (125 μm diameter Teflon-insulated stainless steel wire) against the reference screw placed above the cerebellum. The signal was amplified (Grass P511 amplifiers, Grass Instruments Division of Astro-Med, Inc., West Warwick, RI, USA,

half amplitude filters set at 0.3 Hz and 1 kHz), digitized (10 kHz, AD Instruments 4/s system), and stored for subsequent off-line analysis.

For experiments involving local, cortical drug application, the V1 recording electrode was attached to a microdialysis probe (Mab2.14.4; 2 mm active polyether sulfone membrane, 35 kDa cutoff; SPE Limited, North York, ON, Canada), with the electrode tip located about 1 mm above the probe tip. This electrode–dialysis probe assembly was lowered into V1 so that the recording electrode penetrated the superficial layers (layers I/II), while the probe reached the cortical depth. The dialysis probe was connected to a 2.5 ml Hamilton airtight microsyringe driven by a microdialysis pump (CMA/102, CMA Microdialysis, Solna, Sweden) and perfused continuously with artificial cerebrospinal fluid (aCSF, 1.0 μl/min). The aCSF consisted of, in mM: 124.0 NaCl, 4.4 KCl, 1.2 $MgSO_4$, 1.0 NaH_2PO_4, 2.5 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose.

Multiunit activity in S1 was recorded using tungsten microelectrodes (0.005 in., 2–5 MΩ impedance, A-M Systems Inc., Carlsborg, WA, USA) advanced by means of a microdrive. Signals were amplified, band pass filtered (200 Hz to 1 kHz), passed through a Humbug noise eliminator (Quest Scientific, Vancouver, BC, Canada), monitored on an oscilloscope and audio speaker, and stored for subsequent analysis.

In addition, during the majority of experiments, cortical ECoG activity was recorded before and after BF stimulation, either through the same V1 electrode used for fPSP recordings, or by means of an additional low impedance electrode placed in S1 adjacent to the microelectrode.

Data collection

For experiments in V1, 120 initial baseline fPSPs (every 15 s) were recorded for both ipsi- and contralateral LGN stimulation (alternating between the two LGNs, see above). Subsequently, BF stimulation was applied (parameters as above), after which fPSP recordings continued for another 2 h. For experiments assessing the effects of systemic antagonist treatments, the following drugs were administered (i.p.) 30 min prior to the onset of baseline recordings: scopolamine hydrochloride (5 mg/kg); mecamylamine hydrochloride (10 mg/kg); (+)-MK-801 hydrogen maleate (0.5 mg/kg). For experiments involving cortical drug application via reverse microdialysis, scopolamine hydrochloride (2 mM) was added to the perfusion medium (aCSF) and continuously applied throughout the experiment, starting 30 min prior to the onset of baseline fPSP recordings. Pilot experiments showed that a scopolamine concentration of 1 mM exerted only partial effects, while 5 mM was not more effective than the 2 mM concentration. Consequently, 2 mM was chosen for the formal data collection. Reverse dialysis allows drug molecules to reach neural tissue by means of concentration driven diffusion across the dialysis probe membrane, with drug concentrations outside the membrane estimated to reach approximately 10% of the concentration contained in the perfusion medium (e.g. Oldford and Castro-Alamancos, 2003). All drugs and chemicals were obtained from Sigma/RBI, Oakville, ON, Canada).

For experiments in S1, multiunit activity was recorded in response to deflection (manually using a 1 g von Frey hair) of the principal and secondary whisker (20 deflection each, randomized order). Subsequently, BF stimulation was applied (as above) and whisker deflections were repeated at 5, 30, and 60 min after BF stimulation.

At the end of all experiments, rats were perfused through the heart with 0.9% saline, followed by 10% formalin. Brains were removed from the skull and fixed in formalin for at least 24 h, and standard histological techniques were used to examine the placements of electrodes and microdialysis probes. Data from experiments with inaccurate placements were discarded.

Data analysis

Data are expressed as mean±SEM. All electrophysiological recordings were analyzed by means of automatic signal processing functions in the software packages Scope (v. 4.0.2) and Chart (v. 5.4) running in conjunction with the PowerLab 4/s data acquisition system. The maximal amplitude of fPSPs recorded in V1 was computed off-line (Scope software) and amplitude data were averaged over 10 min epochs. These averages were then normalized by dividing all data for one rat by the average baseline (pre-BF stimulation) amplitude of that animal. For ECoG activity, 10 s epochs recorded before and after BF stimulation were subjected to spectral analyses (Chart software) to determine power in the frequency range of 1.5–30 Hz. Multiunit activity elicited by whisker stimulation was quantified during 1 s time bins following each whisker deflection by an automated event count function (Chart software). For each rat, the threshold for spike detection was adjusted to at least 2× the background cortical activity.

In addition, to assess whether BF stimulation was effective in altering the *relative* strength of responses elicited from the ipsilateral and contralateral LGN, or the principal and secondary whisker, ratios of these responses before and after BF stimulation were calculated, as follows: ipsilateral fPSP amplitude/contralateral fPSP amplitude, or spike count for principal whisker/spike count for secondary whisker. Consequently, a decrease in the ratio is indicative of a relative enhancement of the non-dominant responses (from contralateral LGN and secondary whisker), and vice versa.

For statistical analyses, two-way analyses of variance (ANOVA) were computed using the software package CLR ANOVA (v. 1.1, Clear Lake Research Inc., Houston, TX, USA). When different groups of animals were compared, analyses were conducted with experimental group as between subjects factor and time as within subjects factor. In cases of significant group by time interactions in the initial two-way ANOVA, simple effects tests were computed to determine the time point(s) of the experiment when experimental groups differed from one another. Additional two-way, repeated measures ANOVAs were calculated to assess differences between ipsilateral vs. contralateral fPSPs in V1, or principal vs. secondary whisker responses in S1 in the same animals. For these analyses, the within subjects factors of time and fPSP or whisker were used, with fPSP/whisker nested under time.

RESULTS

Characteristics of fPSPs in V1 elicited by LGN stimulation

Consistent with previous work (Heynen and Bear, 2001; Dringenberg et al., 2007), single pulse stimulation of the LGN (0.39±0.03 mA, intensity to elicit 50%–60% of maximal fPSP amplitude) elicited negative-going fPSPs in the superficial V1 ipsilateral to the stimulation site (Fig. 1, blue trace). These ipsilateral fPSPs had an average amplitude of 1.08±0.12 mV (n=19), with a typical latency to peak of ~15 ms. Applying two stimulation pulses to the LGN (interstimulus interval of 100 ms, tested only during pre-baseline recordings) to elicit two consecutive fPSPs always resulted in an augmentation of the amplitude of the second fPSP (Fig. 1, blue; mean ratio of second/first fPSP was 1.7±0.2). Stimulation of the contralateral LGN (0.32±0.02 mA) also elicited negative-going fPSP (Fig. 1, red trace), characterized by a smaller amplitude (0.4±0.05 mV, n=15) and longer latency to peak (~25 ms). Contralateral fPSPs also showed augmentation to the application of two LGN stimuli (100 ms interval; Fig. 1, red), which

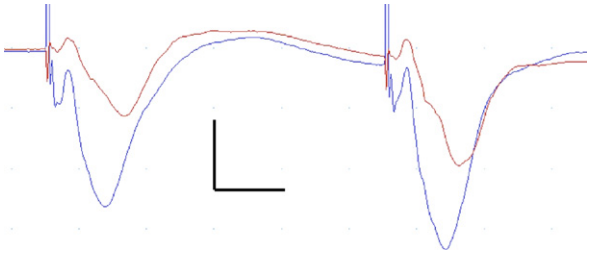

Fig. 1. Examples of fPSPs in the V1 elicited by stimulation of the ipsilateral (blue) and contralateral (red) LGN in urethane-anesthetized rats. Note the larger amplitude and shorter latency of fPSPs evoked by ipsilateral stimulation. Applying two consecutive stimulation pulses (100 ms interstimulus interval) resulted in facilitation of both ipsi- and contralateral fPSPs (traces shown are averages of 10 individual sweeps, scale bar=25 ms, 0.5 mV).

appeared to be more pronounced (mean ratio of second/first fPSP was 2.8±0.4) than that of ipsilateral fPSPs. The difference in amplitude of ipsilateral and contralateral fPSPs (elicited by a single pulse) yielded an ipsilateral/contralateral fPSP amplitude ratio of 2.7 (see Table 1).

Potentiation of fPSPs in V1 by BF stimulation

Initially, the stability of fPSPs elicited by ipsilateral (n=6) and contralateral LGN stimulation (n=5) was assessed in rats that did not receive stimulation of the BF (Fig. 2, open triangles). In these rats, fPSP amplitude stayed relatively stable throughout the course of the experiment, with final amplitudes of 98% and 104% of baseline for fPSPs elicited by ipsi- and contralateral LGN stimulation, respectively (Fig. 2; values are means over the last 30 min of the experiment).

Subsequently, we examined the effect of BF stimulation on fPSP amplitude in pharmacologically untreated rats (Fig. 2, open circles). For fPSPs elicited by the ipsilateral LGN (n=13), BF stimulation (10×0.5 s every 10 s, 100 Hz, 1 mA) resulted in a moderate, but statistically significant increase in fPSP amplitude relative to rats not receiving BF stimulation (Fig. 2; average amplitude over the last 30 min of the experiment was 115% of baseline). Surprisingly, contralateral fPSPs (n=10) showed much greater potentiation (146% of baseline during the last 30 min), which developed gradually over a period of about 80 min following BF stimulation before reaching a plateau that was maintained until the end of the experiment (Fig. 2). This larger potentiation of contralateral fPSPs resulted in a decrease in the ratio of ipsi- to contralateral fPSP amplitude from 2.7 prior to BF stimulation (see above) to 2.14 during the last 30 min of the experiment (Table 1), indicative of a relative amplification of inputs reaching V1 from the contralateral LGN. These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a significant difference between ipsilateral and contralateral fPSPs, $F_{1,9}$=23.8, P<0.001, as well as a significant fPSP by time interaction, $F_{14,126}$=1.7, P=0.013.

Recordings of the ECoG confirmed that BF stimulation altered oscillatory activity in V1 of urethane-anesthetized rats. Spontaneous ECoG activity prior to BF stimulation (n=14) was dominated by large amplitude, low frequency oscillations, with power concentrated at frequencies below 10 Hz (Fig. 3, top). Following BF stimulation, low frequency activity, especially between 1 and 12 Hz, was suppressed and replaced by higher frequency oscillations (Fig. 3, top). This change in background ECoG activity was temporary and, typically, large amplitude activity recovered within 60 s following BF stimulation. Consequently, for the lower frequency bands (1–12 Hz), the power spectrum at the end of the experiment resembled that present during baseline recordings (Fig. 3, top), suggesting that long-lasting changes in low frequency ECoG activity do not account for the increases in fPSP amplitude during the 2 h following BF stimulation. There was, however, some enhancement in power for activity in the 15–25 Hz range that was apparent at the end of the experiment compared to baseline (pre-BF stimulation) ECoG recordings.

Effects of muscarinic, nicotinic, and NMDA receptor blockade on fPSP potentiation

Next, we studied the role of different receptor populations in BF-induced potentiation of fPSPs between LGN and V1 using systemic and local, intracortical drug application. Systemic administration of the muscarinic receptor antagonist scopolamine (5 mg/kg, given i.p. 30 min prior to the onset of baseline recordings) did not significantly affect the relatively small degree of potentiation of ipsilateral fPSPs following BF stimulation (Fig. 2, top; n=7, 112% of baseline amplitude during last 30 min; 115% for untreated rats, see above). In contrast, scopolamine significantly reduced the BF-induced potentiation of contralateral fPSPs (n=6), with fPSP amplitude during the last 30 min of the experiment reaching only 114% of baseline (Fig. 2, top; 146% for untreated rats). Thus, the usual decrease in the ratio of ipsi- to contralateral fPSP amplitude after BF stimulation did not occur in scopolamine-treated rats, with ratios during baseline and the last 30 min of the experiment of 2.34 and 2.30, respectively (Table 1). These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a lack of a significant difference between ipsilateral and contralateral fPSPs, $F_{1,5}$=1.6, P=0.262, as well as a lack of a significant fPSP by time interaction, $F_{14,70}$=1.1, P=0.345.

It is noteworthy that, even though the relative amplification of contralateral inputs did not occur in the presence

Table 1. Summary of changes in the ratio of ipsilateral/contralateral fPSP amplitude induced by BF stimulation

Treatment condition	Before BF stimulation	After BF stimulation	% Change
No drug	2.70	2.14%	−21%
Scopolamine (i.p.)	2.34	2.30%	−2%
Mecamylamine (i.p.)	1.58	1.08%	−32%
MK-801 (i.p.)	2.29	1.55%	−32%
aCSF (local)	2.54	1.89%	−26%
Scopolamine (local)	2.13	2.13	0%

Note the ratio decrease in all conditions except animals that received systemic (i.p.) or local, cortical application of scopolamine (drug doses: scopolamine, 5 mg/kg i.p. or 2 mM, local; mecamylamine, 10 mg/kg i.p.; MK-801, 0.5 mg/kg i.p.).

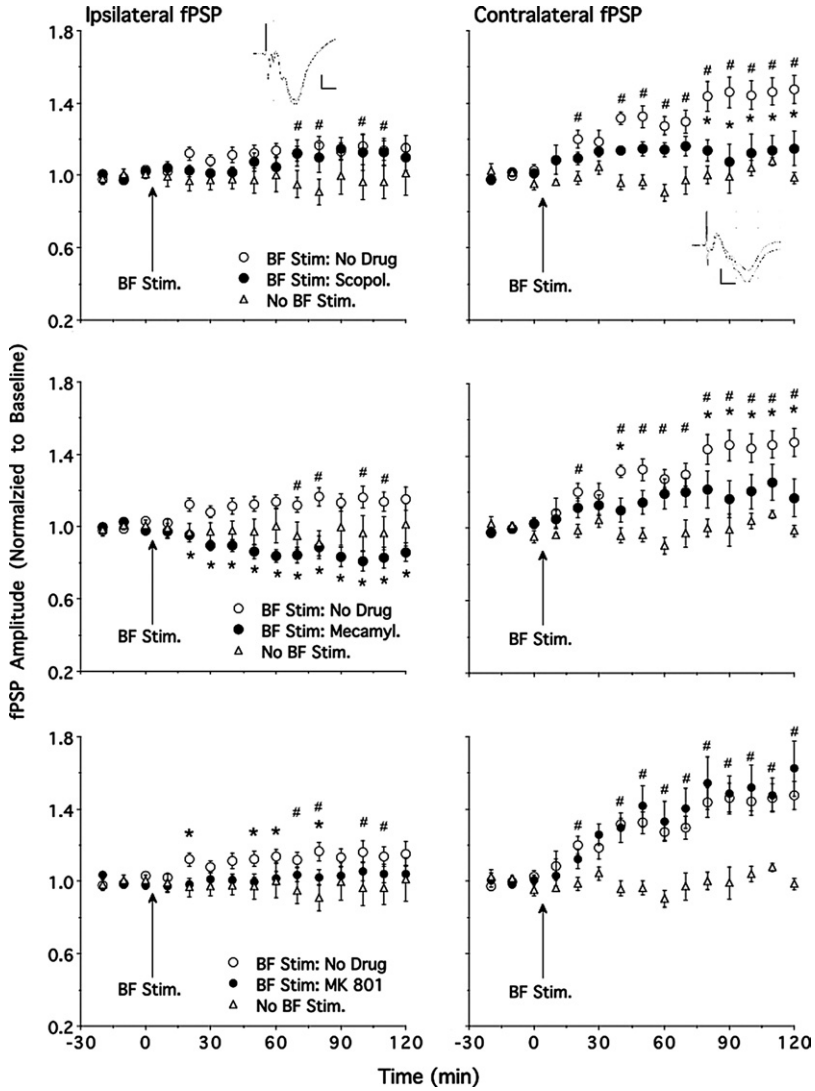

Fig. 2. *Top*: Effects of BF stimulation (BF Stim.) and scopolamine (Scopol, 5 mg/kg i.p.) treatment on ipsilateral (left) and contralateral (right) fPSP amplitude. Rats not receiving BF stimulation (No BF Stim.) showed relatively stable fPSP amplitude throughout the course of the experiment (n=6 and 5 for ipsilateral and contralateral experiments, respectively). BF Stim (No Drug) resulted in a modest enhancement of ipsilateral fPSPs (n=13) relative to No BF Stim. animals (group by time interaction, $F_{14,238}$=2.3, P=0.005). Contralateral fPSPs (n=10) showed a clear, gradually developing potentiation over the 2 h following BF Stim. relative to No BF Stim. animals (group by time interaction, $F_{14,182}$=5.9, P<0.001; # indicates significant (P<0.05) simple effects test comparing No BF Stim. and BF Stim: No Drug groups; note that the same No BF Stim. and BF Stim: No Drug groups are shown in all panels). Insets show typical fPSPs before (smaller amplitude) and 2 h after BF stimulation (larger amplitude; scale bar=0.5 mV and 12.5 ms). Scopol. (BF Stim) had no effect on the ipsilateral fPSP enhancement following BF Stim. (n=7; interaction, $F_{14,252}$=0.9, P=0.554; main effect of group also not significant), but significantly reduced the contralateral enhancement (n=6; interaction, $F_{14,196}$=4.0, P<0.001; group effect also significant; * indicates significant (P<0.05) simple effects test comparing No Drug and Drug-treated groups in all panels). *Middle*: Mecamylamine (Mecamyl, 10 mg/kg i.p.) treatment resulted in significant depression of ipsilateral fPSPs (n=6) relative to BF Stim: No Drug animals (interaction, $F_{14,238}$=8.3, P<0.001, group effect also significant) and reduced potentiation of contralateral fPSPs (n=6; interaction, $F_{14,196}$=2.4, P=0.004). *Bottom*: MK-801 (0.5 mg/kg i.p.) suppressed ipsilateral fPSP enhancement (n=8; interaction, $F_{14,266}$=1.8, P=0.043), while contralateral enhancement (n=8) was intact (interaction, $F_{14,224}$=0.6, P=0.901, group effect also not significant).

of scopolamine, the drug did not abolish all potentiation, given that BF stimulation continued to result in significant increases in fPSP amplitude above levels in rats not receiving BF stimulation (Fig. 2, top). The amplitudes of baseline (pre-BF stimulation) fPSPs elicited by ipsilateral and contralateral LGN stimulation in scopolamine-treated rats were 1.38 and 0.59 mV, respectively.

A separate group of rats received the nicotinic receptor antagonist mecamylamine (10 mg/kg i.p.) 30 min prior to the onset of baseline recordings. In these animals, BF stimulation resulted in a significant depression of ipsilateral fPSP amplitude (n=6) relative to untreated rats (Fig. 2, middle; average amplitude of 83% of baseline during the last 30 min of the experiment). Further, the potentiating effect of BF stimulation on contralateral fPSPs (n=6) was significantly reduced by mecamylamine (amplitude 121% of baseline during last 30 min). Surprisingly, the baseline ratio of ipsi- to contralateral fPSPs was reduced by

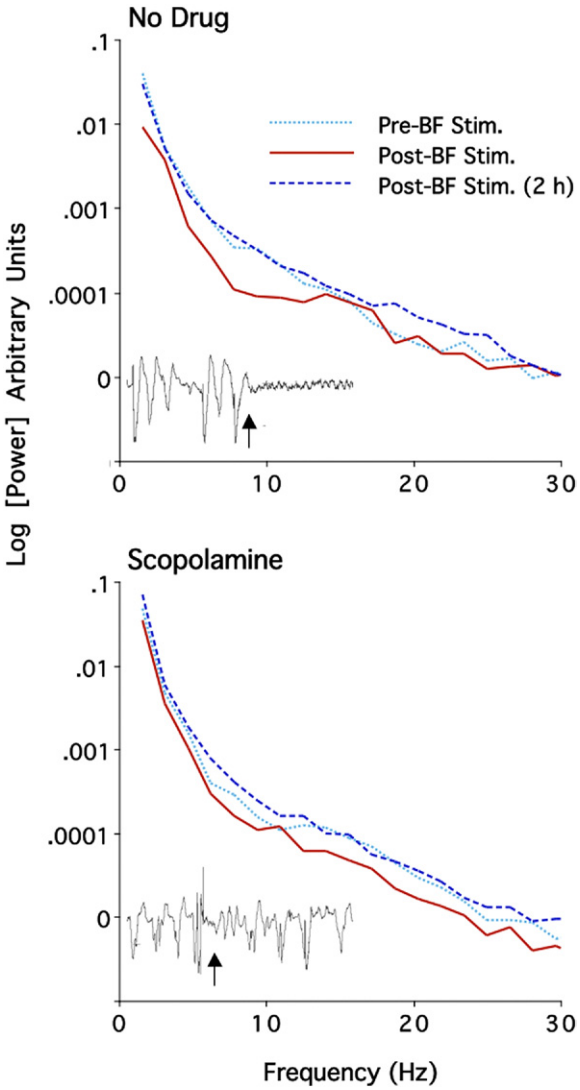

Fig. 3. The effect of BF stimulation on ECoG activity in the urethane-anesthetized rat. *Top*: Power spectral analyses showed that resting ECoG activity (Pre-BF stimulation, Pre-BF Stim.) was dominated by large amplitude, low frequency activity, with power concentrated in the range below 10 Hz. In untreated animals, ECoG activity showed suppressed slow, large amplitude oscillations and power between 1 and 15 Hz immediate after BF stimulation (Post-BF Stim, at arrow). However, at the end of the experiment (Post-BF Stim. Two hours), low frequency power had returned to levels similar to those seen prior to BF stimulation. *Bottom*: Rats treated with scopolamine (5 mg/kg i.p.) showed very little suppression of low frequency power and large-amplitude, slow activity in the ECoG following BF stimulation. Power spectra are averages (n=14 for top, n=8 for bottom) and were computed from 10 s of ECoG epochs obtained immediately prior to, immediately after, and 2 h after BF stimulation. (Duration of ECoG records shown in insets is 17.5 s).

mecamylamine (1.58; Table 1), an effect due to a proportionally higher increase in contralateral baseline fPSP amplitude following drug treatment (1.22 and 0.77 mV for ipsi- and contralateral fPSPs, respectively, before BF stimulation). However, since BF stimulation in mecamylamine-treated rats resulted in ipsilateral depression but only reduced contralateral potentiation, the decrease in ipsi- to contralateral amplitude ratio remained intact under conditions of systemic nicotinic receptor blockade (ratio of 1.08 during last 30 min; Table 1). These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a significant difference between ipsilateral and contralateral fPSPs, $F_{1,5}$=14.9, P=0.012, as well as a significant fPSP by time interaction, $F_{14,70}$=9.8, $P<$ 0.001.

The effect of systemic treatment with the NMDA receptor antagonist MK-801 (0.5 mg/kg i.p., 30 min prior to onset of baseline) was also assessed. In these animals (n=8), the amplitude increase for ipsilateral fPSPs following BF stimulation was largely absent (Fig. 2, bottom; 105% of baseline during final 30 min), but the contralateral fPSP enhancement was intact (154% of baseline). Further, the ratio of ipsi- to contralateral fPSPs decreased from 2.29 during baseline to 1.55 during the final 30 min of the experiment (Table 1), indicative of an intact, relative amplification of the contralateral input. These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a significant difference between ipsilateral and contralateral fPSPs, $F_{1,7}$=15.7, P=0.005, as well as a significant fPSP by time interaction, $F_{14,98}$=5.6, $P<$0.001. Amplitudes of baseline ipsilateral and contralateral fPSPs in MK 801-treated rats were 0.96 and 0.42 mV, respectively.

To localize the effect of scopolamine to reduce BF-induced synaptic potentiation, we performed local application of the drug (2 mM) and its vehicle (aCSF) by means of reverse microdialysis at the recording site in V1. In animals receiving aCSF application (n=7), BF stimulation increased ipsilateral and contralateral fPSPs to 105% and 142% of baseline (means during last 30 min), respectively, confirming the preferential potentiation of contralateral fPSPs (Fig. 4). Consequently, the ratio between ipsi- and contralateral fPSPs declined from 2.54 during baseline to 1.89 at the end of the experiment (Table 1). These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a significant difference between ipsilateral and contralateral fPSPs, $F_{1,6}$=8.6, P= 0.026, as well as a significant fPSP by time interaction, $F_{14,84}$=4.6, $P<$0.001.

Local scopolamine application (n=7) had no significant effect on ipsilateral fPSP amplitude (117%), but reduced the amplitude potentiation of contralateral fPSPs to 117% (Fig. 4). Thus, scopolamine prevented the decrease in ratio between ipsi- and contralateral fPSPs seen in aCSF animals, with ratios remaining virtually identical from baseline to the end of the experiment (both were 2.13; Table 1). These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a lack of significant difference between ipsilateral and contralateral fPSPs, $F_{1,6}<$0.1, P=0.985, as well as a lack of a significant fPSP by time interaction, $F_{14,84}$=0.3, P=0.994. Again, however, note that there was residual potentiation of both ipsi- and contralateral fPSPs during scopolamine application. The amplitudes of baseline fPSPs elicited by ipsilateral and contralateral LGN stimulation during aCSF application were 1.09 and 0.43 mV, respectively, which

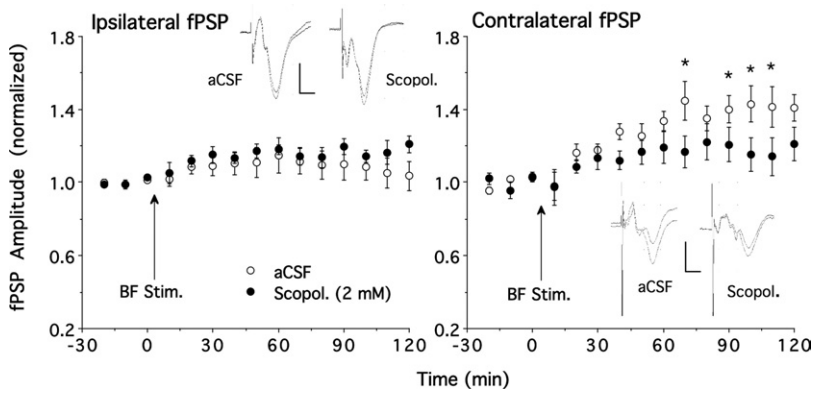

Fig. 4. Effects of local application of aCSF ($n=7$) or scopolamine (Scopol, 2 mM, $n=7$) by reverse microdialysis at the recording site in V1 on BF stimulation (BF Stim.)–induced potentiation of fPSPs elicited by stimulation of the ipsilateral and contralateral LGN. The ipsilateral fPSP enhancement (left) following BF Stim. during aCSF application was resistant to Scopol (group by time interaction, $F_{14,168}=0.8$, $P=0.709$; main effect of group also not significant). In contrast, the contralateral enhancement (right) during aCSF application was significantly reduced by Scopol (interaction, $F_{14,168}=2.5$, $P=0.003$; * indicates significant ($P<0.05$) simple effects test). Insets show typical fPSPs before (smaller amplitude) and 2 h after BF Stim. in the presence of local aCSF or Scopol (scale bar=12.5 ms and 0.5 mV).

were not altered significantly during cortical application of scopolamine (1.0 and 0.56 mV for ipsi- and contralateral fPSPs, respectively).

Consistent with previous work (Vanderwolf, 1988; Metherate et al., 1992; Dringenberg and Olmstead, 2003), muscarinic receptor blockade by systemic or local scopolamine largely abolished ECoG activation elicited by BF stimulation (Fig. 3, bottom). In particular, the BF stimulation-induced suppression of power between 1 and 12 Hz present in untreated rats was absent following scopolamine application ($n=8$), even though there was some suppression of power between 12 and 30 Hz. Neither mecamylamine, nor MK-801 blocked ECoG activation following BF stimulation (data not shown), confirming that nicotinic and NMDA receptors do not play critical roles in activation under the present experimental conditions (see Vanderwolf, 1988; Dringenberg and Vanderwolf, 1996).

Potentiation of barrel cortex multiunit activity by BF stimulation

The experiments summarized above suggest that release of endogenous ACh following BF stimulation acts to rebalance separate inputs to V1, with a relatively greater potentiation of non-dominant signals. We tested the generality of this phenomenon in the barrel cortex by examining the effects of BF stimulation on multiunit responses evoked by stimulation of the principal and secondary (surround) whiskers, constituting the major and non-dominant inputs to a given barrel, respectively. Even though no attempt was made to select cells from specific cortical layers, the majority (>90%) of neurons recorded were located in infragranular layers at depths between 1000 and 1500 μm. Recordings were optimized so that multiunit responses could be reliably triggered by deflection of both principal and secondary whiskers, with a greater number of spikes detected for the principal whisker during the 1000 ms time bin following deflection (Fig. 5; averages of 7.9±1.1 and 5.6±0.9 spikes for principal and secondary whisker, respectively, ratio of 1.41; $n=17$).

In non-drug-treated rats ($n=17$), BF stimulation (same parameters as above) exerted clear effects on the strength of multiunit responses measured immediately after stimulation, with 12/17 sites showing increased discharge for principal whisker deflection, and 14/17 sites showing increases in response to secondary whisker deflection. The remaining sites generally did not show changes, and we did not observe clear inhibitory effects of BF stimulation. When averaging across all recording sites, BF stimulation resulted in a significant facilitation of discharge for both whiskers, with spike counts of 179% and 281% of baseline for principal and secondary whiskers, respectively (Fig. 5). The greater facilitation of secondary whisker responses resulted in roughly equivalent numbers of spikes elicited by principal and secondary whiskers (14.1 and 15.7 spikes, respectively, yielding a principal/secondary ratio of 0.9).

Whisker stimulation was repeated at 30 and 60 min following BF stimulation (Fig. 5). Response strength for multiunit discharge driven by the principal whisker returned to baseline levels at 30 and 60 min (97% and 109% of baseline, respectively). In contrast, responses elicited by the secondary whisker remained elevated at 30 and 60 min (128% and 154% of baseline, respectively), even though they clearly declined from the levels observed immediately after BF stimulation (Fig. 5). The greater potentiation of responses elicited by secondary whisker deflection resulted in a principal/secondary ratio of 1.0 (baseline 1.41) at 60 min after BF stimulation. These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a significant difference between principal and secondary whiskers, $F_{1,16}=18.0$, $P<0.001$, as well as a significant whisker by time interaction, $F_{3,48}=5.5$, $P=0.002$.

These experiments were repeated in animals treated with scopolamine ($n=9$; 5 mg/kg i.p., 30 min prior to onset of data collection). Scopolamine reduced the enhancement in multiunit responses for both whiskers immediately after BF stimulation (Fig. 5), with increases of 114% and 152% of baseline for principal and secondary whiskers,

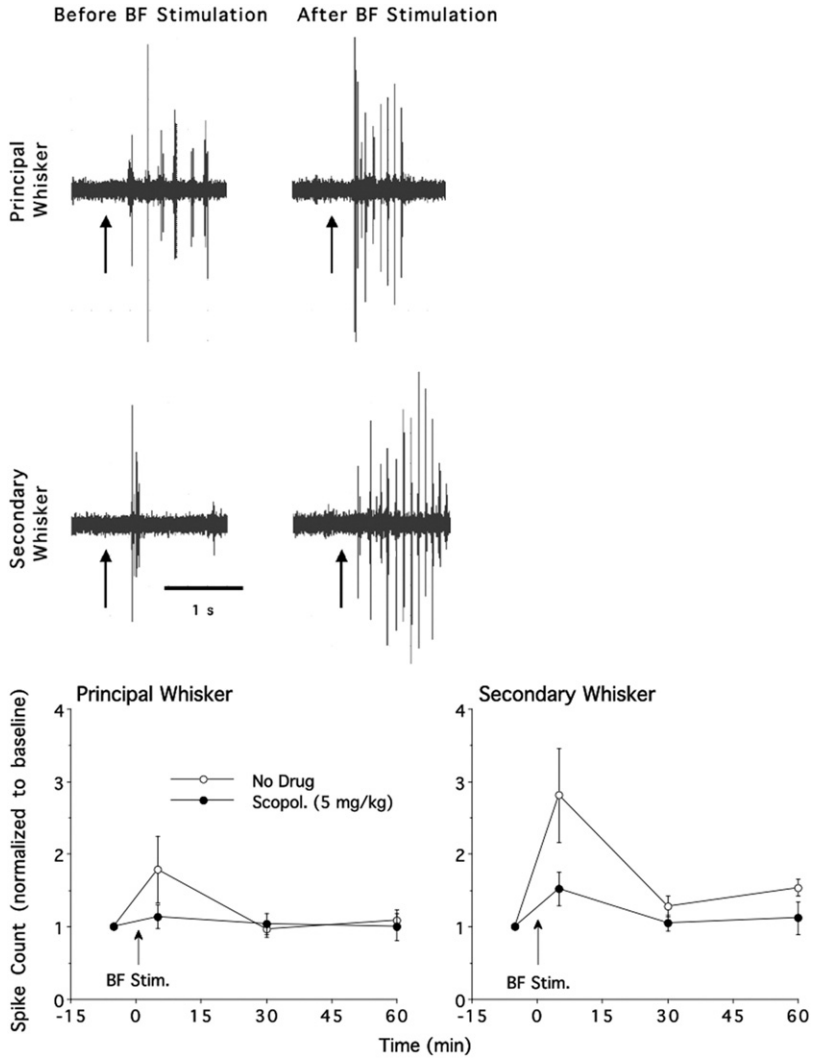

Fig. 5. The effect of BF stimulation (BF Stim.) on multiunit responses in the barrel cortex elicited by deflection of the principal or secondary whisker. *Top and middle*: Representative examples of multiunit responses before and after BF stimulation. Multiunit discharge elicited by either whisker was facilitated after BF stimulation, an effect that was more pronounced for responses elicited by the secondary whisker. *Bottom*: Average spike counts for responses elicited by principal and secondary whiskers in non-drug treated rats (No Drug, $n=17$) and animals given scopolamine (Scopol, 5 mg/kg i.p., $n=9$). Discharge was facilitated immediately after BF stimulation, an effect that was more pronounced and longer lasting for responses elicited by the secondary whisker. For the principal whisker, response facilitation following BF stimulation appeared to be abolished by Scopol, even though this impression was not confirmed by the statistical analysis (group by time interaction, $F_{3,72}=1.3$, $P=0.273$, main effect of group also not significant). The drug appeared to reduce facilitation of responses elicited by the secondary whisker (main effect of group, $F_{1,24}=4.2$, $P=0.052$, group by time interaction, $F_{3,72}=1.4$, $P=0.243$).

respectively (but see statistics in Fig. 5 caption). Further, responses for both whiskers remained close to baseline levels when assessed 30 and 60 min following BF stimulation (Fig. 5; principal whisker: 104% and 100%; secondary whisker: 105% and 112%). The small, but persistent potentiation of secondary whisker responses yielded a small decrease in the principal/secondary ratio from 1.25 during baseline to 1.12 at the end of the experiment (1.41–1.0 in untreated rats). These observations were confirmed statistically by a two-way, repeated measured ANOVA, revealing a lack of a significant difference between principal and secondary whiskers, $F_{1,8}=4.5$, $P=0.067$, as well as a lack of a significant whisker by time interaction, $F_{3,24}=3.0$, $P=0.053$ (note, however, that both effects approached statistical significance).

DISCUSSION

The experiments described here show that stimulation of the BF to elicit release of endogenous ACh can result in long-lasting enhancements of neuronal responses in cortical sensory areas of urethane-anesthetized rats. Surprisingly, this form of synaptic potentiation showed a remarkable degree of selectivity for non-dominant inputs to cortex, which initially produced relatively weak responses of cortical cell populations. Afferents that elicited more pronounced excitatory responses (i.e. ipsilateral LGN-V1 fi-

bers, afferents carrying signal from the principal whisker) showed only minor or transient enhancements following stimulation to the BF. The effect of BF stimulation to preferentially increase weaker cortical responses was largely dependent on activation of cortical muscarinic receptors, with only minor contributions of nicotinic and NMDA binding sites. Ultimately, this ACh-dependent plasticity resulted in a re-adjustment of separate inputs, with more balanced responses elicited by initially dominant and non-dominant inputs to these primary sensory cortices.

Fibers originating in the BF distribute throughout all cortical layers to provide the cholinergic innervation of V1 (Rieck and Carey, 1984; Müller et al., 1993; Zilles et al., 1990; Gu, 2003). This cholinergic input is known to play important roles in the regulation of excitability and plasticity in V1 circuits. While some work focused on relatively short-lasting (50–100 ms) increases in excitability of V1 cells following endogenous ACh release (Lewandowski et al., 1993; Müller et al., 1993), prolonged modifications of cellular and population responses by ACh have also been described. Golmayo et al. (2003) demonstrated that BF stimulation induces a long-lasting (over 30 min) facilitation of visually evoked field potentials in V1 of anesthetized rats, an effect that was abolished by muscarinic receptor blockade. At the level of single neurons, Greuel et al. (1988) found that repeated pairing of a sub-optimal orientation stimulus with iontophoretic ACh application can shift receptive fields of V1 cells, allowing them to become maximally responsive to the paired orientation. The occurrence of a classic type of developmental plasticity in V1, ocular dominance plasticity, also involves ACh. Combined cholinergic and noradrenergic denervation of V1, or chronic blockade of muscarinic (M1) receptors in V1 largely eliminates the shift in ocular dominance normally seen following monocular deprivation in young kittens (Bear and Singer, 1986; Gu and Singer, 1993; Gu, 2003). Finally, in a number of *in vitro* and *in vivo* preparations, the induction of LTP in V1 is facilitated by ACh (Bröcher et al., 1992; Kojic et al., 2001; Dringenberg et al., 2007). Together, these studies emphasize the important, permissive role played by ACh in regulating multiple forms of plasticity in V1 of juvenile and adult animals.

A long-standing, but controversial hypothesis suggests that ACh acts to amplify incoming sensory signals relative to background cortical activity, thereby increasing the signal-to-noise ratio in V1 and other sensory fields (Sillito et al., 1985; Sato et al., 1987). However, other work suggests that ACh reduces stimulus selectivity by broadening receptive fields in the majority of V1 cells (Sato et al., 1987; Zinke et al., 2006). That is, acute effects of ACh in V1 include an increased responsiveness to sub-optimal visual stimuli, rather than further enhancing already strong inputs (i.e. synapses activated by optimal stimuli). Similarly, as discussed above, studies on long-lasting modifications of receptive field properties (orientation, ocular dominance) also demonstrate that the presence of ACh allows a non-optimal input to increase in efficacy, resulting in receptive field shifts (Greuel et al., 1988). By directly comparing the effect of endogenous ACh release on two separate inputs to V1, we found that the longer-latency, smaller amplitude responses mediated by long-range, crossed connections between LGN and V1 showed significantly greater potentiation than the large amplitude evoked responses elicited from the ipsilateral LGN. In the barrel cortex, we also found that weaker responses elicited by the secondary (surround) whisker showed greater and longer-lasting facilitation than responses triggered by the principal whisker. While we acknowledge that the latter experiments require replication using a more standardized method of whisker stimulation than manual deflection using von Frey hairs, these results nevertheless confirm data obtained in V1 and suggest that the phenomenon of afferent selective synaptic enhancement generalizes to several sensory domains. Thus, one of the primary consequences of ACh release is the rebalancing of afferent signals by means of a preferential amplification of non-dominant or sub-optimal inputs to cortical neurons. An important difference between the present and previous work lies in the fact that response enhancement by ACh was elicited in the absence of temporal pairing of sensory input and BF-induced ACh release. Edeline et al. (1994) noted that sound-induced multiunit responses in the auditory cortex increased following repeated pairing of the sound with BF stimulation, while BF stimulation alone was ineffective (also Froemke et al., 2007). Similarly, in S1, pronounced enhancements in evoked potential amplitude evoked by cutaneous stimulation occurred only when it was paired with BF stimulation (Rasmusson and Dykes, 1988; Verdier and Dykes, 2001). It is important to note, however, that this work generally did not explicitly identify sub-optimal sensory inputs and, in fact, often measured cortical responses elicited by peripheral stimuli applied in, or close to, the center of the respective receptive fields (e.g. Rasmusson and Dykes, 1988; Verdier and Dykes, 2001). Thus, previous work noting a requirement for paired sensory-BF stimulation can be viewed as consistent with our data showing only minor changes in the dominant, ipsilateral input from LGN to V1 following unpaired BF stimulation. It is also noteworthy that, for weak sensory stimuli just above the threshold to elicit cortical responses, unpaired BF stimulation alone is sufficient to produce synaptic enhancements in V1 and S1 (Golmayo et al., 2003). Consequently, the temporal contingencies between BF and sensory signals required to elicit synaptic potentiation appear to be less stringent for relatively weak or non-dominant inputs, allowing potentiation even in the absence of paired stimulation during the induction period.

The experiments assessing the effects of mecamylamine treatment suggest that nicotinic receptors also play a role in the fPSP enhancements seen in V1. In the presence of mecamylamine, BF stimulation resulted in suppression of ipsilateral, and reduced potentiation of contralateral fPSPs. Consequently, this effect lacked the selectivity for the contralateral enhancement displayed by scopolamine and, therefore, did not result in a shift in the relative strength of the two inputs to V1. Nevertheless, the data are of interest, given that most forms of long-lasting plasticity in V1 and S1 are mediated by muscarinic receptors (e.g. Rasmusson

and Dykes, 1988; Rasmusson, 2000; Verdier and Dykes, 2001; Golmayo et al., 2003; Gu, 2003; Dringenberg et al., 2007). Nicotinic receptors in sensory cortices play important roles in excitability and on-line gating of sensory signals received from thalamocortical inputs, and may also contribute to experience-dependent postnatal development of neocortical circuits (for review, see Metherate, 2004). The present data indicate that the role of nicotinic receptors to modulate long-lasting plasticity, well established for hippocampal synapses (see Dani and Bertrand, 2007), might also extend to the neocortex, a phenomenon that clearly deserves attention in future investigations.

To us, a surprising finding of the present experiments was that contralateral fPSP enhancement was independent of NMDA receptor activation. The classic NMDA receptor antagonist MK-801, at a dose that blocks pairing-induced, ACh-dependent response potentiation in S1 in urethane-anesthetized rats (Verdier and Dykes, 2001), had no effect on contralateral fPSP enhancement, even though it abolished the smaller enhancement seen in ipsilateral fPSPs. Thus, it appears that fundamentally different cellular mechanisms are involved in mediating plasticity in these two afferent systems. Of course, several forms of NMDA-independent synaptic enhancement have been described, some of which might mediate the effect observed here (e.g. potentiation mediated by metabotropic glutamate receptors, internal calcium release, kainate receptors, co-acting neuromodulators, or voltage-gated calcium channels; see Johnston et al., 1992; Li et al., 2001; Lanté et al., 2006; Huang and Kandel, 2007). Alternatively, the NMDA-independence of the contralateral synaptic enhancement might also be due to a critical role of disinhibition of principal cells in V1. In elegant work, Froemke et al. (2007) have shown that, in the primary auditory cortex, BF stimulation results in an initial suppression of inhibitory cortical synapses, which is followed by a delayed and persistent increase in excitatory synaptic strength. Both effects are dependent on activation of muscarinic receptors, data consistent with our observations. Importantly, Froemke et al. (2007) found that intracortical, but not thalamocortical, synapses are sensitive to these BF-induced changes in inhibitory–excitatory balance. The fact that contralateral fPSPs between LGN to V1 represent long-range, polysynaptic inputs, likely involving a greater number of intracortical synapses (see below), can be expected to make them much more sensitive to the disinhibitory effects of BF stimulation described by Froemke et al. (2007).

It is important to acknowledge that our experiments were carried out in urethane-anesthetized preparations, raising the possibility that the presence of the anesthetic exerted some influence on the cortical responses measured and the experimental effects we characterize. We chose urethane for a variety of reason, including the stable, long-lasting plane of anesthesia provided by this drug, as well as its minimal effects on various autonomic functions (Maggi and Meli, 1986). Detailed analyses of the effects of urethane on cellular properties and synaptic transmission in pyramidal cells of rat V1 have shown minimal or no changes in excitatory or inhibitory transmission, leading to the conclusion that cortical signal transmission is largely intact in the presence of urethane (Sceniak and MacIver, 2006). Likely related to this point, urethane-anesthetized preparations display the full spectrum of natural, endogenous oscillations present in cortical and hippocampal networks, events that are thought to play important roles in the gating of plasticity (Wolansky et al., 2006). Despite these points, we acknowledge that future work using non-anesthetized, immobilized, or freely-moving animals will be required to assess whether the phenomena described here are preserved in the non-anesthetized state.

Field potentials in V1 elicited by electrical stimulation of the ipsilateral LGN have been characterized in previous work. Using an arrangement of stimulation and recording electrodes similar to that employed here (i.e. LGN stimulation, recording at the ipsilateral V1 surface/dura), Heynen and Bear (2001) showed that the large amplitude, surface-negative fPSPs reflect polysynaptic excitation that spreads from thalamocortical synapses (layers IV/deep III) to superficial cortical layers (mostly II/III). The longer latency, small amplitude potentials we observed in V1 following contralateral LGN stimulation have, to the best of our knowledge, not been analyzed in previous work. Preliminary experiments in our laboratory suggest that these negative potentials reflect current sinks in the superficial cortex (200–300 μm depth, layer II), corresponding to a major terminal zone of callosal fibers between homotopic and heterotopic areas of both primary and higher visual cortex areas (Sefton et al., 2004). Consequently, it is possible that the fPSPs elicited by contralateral LGN stimulation reflect multi-synaptic activity that propagates from the contralateral hemisphere to the V1 recoding site via the corpus callosum, even though we do not rule out contributions of other, subcortical routes between the LGN and contralateral V1 (see Sefton et al., 2004).

In rodents, callosal fibers connecting visual areas of the two hemispheres are particularly important for mediating cortical responses to stimulation of the ipsilateral retina and allowing binocular excitation of V1 neurons (Diao et al., 1983; Silveira et al., 1989). Based on evoked response measures, excitation of neurons in V1 is facilitated by simultaneous, binocular stimulation (Silveira et al., 1989; Ates et al., 2006). Similarly, we have noted that concurrent, electrical stimulation of both LGNs (mimicking binocular input) can facilitate fPSPs in V1 (Dringenberg, unpublished observations). Consequently, the preferential, long-lasting enhancement of contralateral LGN inputs reaching V1 (representing mostly signals from the ipsilateral eye) can be expected to facilitate such binocular responses in V1, particularly for stimuli of high contrast and low spatial frequency (see Silveira et al., 1989). The phenomena described here fit well with the increased recognition that long-lasting plasticity can be expressed in the mature visual cortex (Greuel et al., 1988; Heynen and Bear, 2001; Golmayo et al., 2003; Gu, 2003; Dringenberg et al., 2007) and might serve as a mechanism to amplify the detection or integration of visual stimuli by behavioral states related

to high levels of ACh release throughout the cortical mantle.

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REFERENCES

Ates K, Demirtas S, Goksoy C (2006) Binocular interactions in the guinea pig's visual-evoked potentials. Brain Res 1125:26–30.

Bear MF, Singer W (1986) Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature 320:172–176.

Bennett MR (2000) The concept of long term potentiation of transmission at synapses. Prog Neurobiol 60:109–137.

Bröcher S, Artola A, Singer W (1992) Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. Brain Res 573:27–36.

Buzsáki G, Bickford RG, Ponomareff G, Thal LJ, Mandel R, Gage FH (1988) Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. J Neurosci 8:4007–4026.

Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47:20.1–20.31.

Détári L, Rasmusson DD, Semba K (1999) The role of BF neurons in tonic and phasic activation of the cerebral cortex. Prog Neurobiol 58:249–277.

Diao YC, Wang YK, Pu ML (1983) Binocular responses of cortical cells and the callosal projection in the albino rat. Exp Brain Res 49:410–418.

Dringenberg HC, Hamze B, Wilson A, Speechley W, Kuo M-C (2007) Heterosynaptic facilitation of in vivo thalamocortical long-term potentiation in the adult rat visual cortex by acetylcholine. Cereb Cortex 17:839–848.

Dringenberg HC, Olmstead MC (2003) Integrated contributions of basal forebrain and thalamus to neocortical activation elicited by pedunculopontine tegmental stimulation in urethane-anesthetized rats. Neuroscience 119:839–853.

Dringenberg HC, Vanderwolf CH (1996) Cholinergic activation of the electrocorticogram: an amygdaloid activating system. Exp Brain Res 108:285–296.

Duque A, Balatoni B, Détári L, Zaborszky L (2000) EEG correlation of the discharge properties of identified neurons in the basal forebrain. J Neurophysiol 84:1627–1635.

Edeline J-M (2003) The thalamo-cortical auditory receptive fields: regulation by the states of vigilance, learning and the neuromodulatory systems. Exp Brain Res 153:554–572.

Edeline J-M, Hars B, Maho C, Hennevin E (1994) Transient and prolonged facilitation of tone-evoked responses induced by basal forebrain stimulations in the rat auditory cortex. Exp Brain Res 97:373–386.

Froemke RC, Merzenich MM, Schreiner CE (2007) A synaptic memory trace for cortical receptive field plasticity. Nature 450:425–429.

Golmayo L, Nuñez A, Zaborszky L (2003) Electrophysiological evidence for the existence of a posterior cortical-prefrontal-basal forebrain circuitry in modulating sensory responses in visual and somatosensory rat cortical areas. Neuroscience 119:597–609.

Greuel JM, Luhmann HJ, Singer W (1988) Pharmacological induction of use-dependent receptive field modifications in the visual cortex. Science 242:74–77.

Gu Q (2003) Contributions of acetylcholine to visual cortex plasticity. Neurobiol Learn Mem 80:291–301.

Gu Q, Singer W (1993) Effects of intracortical infusions of anticholinergic drugs on neuronal plasticity in kitten striate cortex. Eur J Neurosci 5:475–485.

Hasselmo ME, Bower JM (1992) Cholinergic suppression specific to intrinsic not afferent fiber synapses in rat piriform (olfactory) cortex. J Neurophysiol 67:1222–1229.

Heynen AJ, Bear MF (2001) Long-term potentiation of thalamocortical transmission in the adult visual cortex in vivo. J Neurosci 21:9801–9813.

Huang YY, Kandel ER (2007) Low-frequency stimulation induces a pathway-specific late phase of LTP in the amygdala that is mediated by PKA and dependent on protein synthesis. Learn Mem 14:497–503.

Johnston D, Williams S, Jaffe D, Gray R (1992) NMDA-receptor-independent long-term potentiation. Annu Rev Physiol 54:489–505.

Kimura F, Fukuda M, Tsumoto T (1999) Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. Eur J Neurosci 11:3597–3609.

Kojic J, Gu Q, Douglas RM, Cynader MS (2001) Laminar distribution of cholinergic- and serotonergic-dependent plasticity within kitten visual cortex. Dev Brain Res 126:157–162.

Lanté F, de Jésus Ferreira MC, Guiramand J, Récasens M, Vignes M (2006) Low-frequency stimulation induces a new form of LTP, metabotropic glutamate (mGlu5) receptor- and PKA-dependent, in the CA1 area of the rat hippocampus. Hippocampus 16:345–360.

Lewandowski MH, Müller CM, Singer W (1993) Reticular facilitation of cat visual cortical responses is mediated by nicotinic and muscarinic cholinergic mechanisms. Exp Brain Res 96:1–7.

Li H, Chen A, Xing G, Wei M-L, Rogawski MA (2001) Kainate receptor-mediated heterosynaptic facilitation in the amygdala. Nat Neurosci 4:612–620.

Livingston M, Hubel DH (1981) Effects of sleep and arousal on the processing of visual information in the cat. Nature 291:554–561.

Maggi CA, Meli A (1986) Suitability of urethane for physiopharmacological investigations in various systems. Part 1: general considerationsExperientia 42:109–114.

McCormick DA (1989) Cholinergic and noradrenergic modulation of thalamocortical processing. Trends Neurosci 12:215–221.

McGaughy J, Everitt BJ, Robbins TW, Sarter M (2000) The role of cortical cholinergic afferent projections in cognition: impact of new selective immunotoxins. Behav Brain Res 115:251–263.

Meeren HKM, Van Luijtelaar ELJM, Coenen AML (1998) Cortical and thalamic visual evoked potentials during sleep-wake states and spike-wave discharge in the rat. Electroencephalogr Clin Neurophyiol 108:306–319.

Metherate R (2004) Nicotinic acetylcholine receptors in sensory cortex. Learn Mem 11:50–59.

Metherate R, Cox CL, Ashe JH (1992) Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. J Neurosci 12:4701–4711.

Metherate R, Tremblay N, Dykes RW (1987) Acetylcholine permits long-term enhancement of neuronal responsiveness in cat primary somatosensory cortex. Neuroscience 22:75–81.

Müller CM, Lewandowski MH, Singer W (1993) Structures mediating cholinergic reticular facilitation of cortical responses in the cat: effects of lesions in immunocytochemically characterized projections. Exp Brain Res 96:8–18.

Oldford E, Castro-Alamancos MA (2003) Input-specific effects of acetylcholine on sensory and intracortical evoked responses in the "barrel cortex" *in vivo*. Neuroscience 117:769–778.

Rasmusson DD (2000) The role of acetylcholine in cortical synaptic plasticity. Behav Brain Res 115:205–218.

Rasmusson DD, Dykes RW (1988) Long-term enhancement of evoked potentials in cat somatosensory cortex produced by co-activation of the basal forebrain and cutaneous receptors. Exp Brain Res 70:276–286.

Rieck R, Carey RG (1984) Evidence for a laminar organization of basal forebrain afferents to the visual cortex. Brain Res 297:374–380.

Sarter M, Bruno JP (2000) Cortical cholinergic inputs mediate arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. Neuroscience 95:933–952.

Sato H, Hata Y, Masuio H, Tsumoto T (1987) A functional role of cholinergic innervation to neurons in the cat visual cortex. J Neurophyiol 58:765–780.

Sceniak MP, MacIver MB (2006) Cellular actions of urethane on rat visual cortical neurons in vitro. J Neurophysiol 95:3865–3874.

Sefton AJ, Dreher B, Harvey A (2004) Visual system. In: The rat nervous system, 3rd ed. (Paxinos G, ed), pp 1083–1165. San Diego: Elsevier Academic.

Semba K (2000) Multiple output pathways of the basal forebrain: organization, chemical heterogeneity, and roles in vigilance. Behav Brain Res 115:117–141.

Sillito AM, Salt TE, Kemp JA (1985) Modulatory and inhibitory processes in the visual cortex. Vis Res 25:375–381.

Silveira LC, Heywood CA, Cowey A (1989) Direct and transcallosal contributions to the cortical evoked response in rats. Behav Brain Res 31:291–294.

Steriade M (2000) Corticothalamic resonance, states of vigilance and mentation. Neuroscience 101:243–276.

Thiel CM (2007) Pharmacological modulation of learning-induced plasticity in human auditory cortex. Restor Neurol Neurosci 25: 435–443.

Thiel CM, Friston KJ, Dolan RJ (2002) Cholinergic modulation of experience-dependent plasticity in human auditory cortex. Neuron 35:567–574.

Vanderwolf CH (1988) Cerebral activity and behavior: control by central cholinergic and serotonergic systems. Int Rev Neurobiol 30: 225–340.

Verdier D, Dykes RW (2001) Long-term cholinergic enhancement of evoked potentials in rat hindlimb somatosensory cortex displays characteristics of long-term potentiation. Exp Brain Res 137: 71–82.

Weinberger NM (2004) Specific long-term memory traces in primary auditory cortex. Nat Rev Neurosci 5:279–290.

Weinberger NM (2007) Associative representational plasticity in the auditory cortex: a synthesis of two disciplines. Learn Mem 14: 1–16.

Wolansky T, Clement EA, Peters SR, Palczak MA, Dickson CT (2006) Hippocampal slow oscillation: a novel EEG state and its coordination with ongoing neocortical activity. J Neurosci 26:6213–6229.

Zilles K, Wree A, Dausch N-D (1990) Anatomy of the neocortex: neurochemical organization. In: The cerebral cortex of the rat (Kolb B, Tees RC, eds). Cambridge, MA: MIT Press.

Zinke W, Roberts MJ, Guo K, McDonald JS, Robertson R, Thiele A (2006) Cholinergic modulation of response properties and orientation tuning of neurons in primary visual cortex of anaesthetized marmoset monkeys. Eur J Neurosci 24:314–328.

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