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# MODALITY- AND REGION-SPECIFIC ACETYLCHOLINE RELEASE IN THE RAT NEOCORTEX

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**Abstract—The basal forebrain is the major source of acetylcholine in the neocortex, and this projection has been variously described as either diffuse or highly specific. We used *in vivo* microdialysis to examine this discrepancy by collecting acetylcholine release simultaneously from visual, somatosensory and prefrontal cortical areas. Urethane-anesthetized rats were presented with visual and somatosensory stimulation in counter-balanced order and acetylcholine was measured using HPLC. Evoked acetylcholine release was modality-specific, i.e. visual stimulation evoked a large (75%) increase from visual cortex and little (24%) change from the somatosensory area whereas skin stimulation had the opposite effect. No increase was apparent in prefrontal cortex with either stimulation protocol. This experiment extends early studies using cortical cups to collect acetylcholine, and is consistent with the concept of functional specificity within the cholinergic basal forebrain with respect to both its sensory inputs and projections to the neocortex. This functional specificity within the cholinergic basal forebrain might be utilized in the modulation of different cortical regions during selective attention and plasticity.** 

**Key words: acetylcholine, somatosensory, visual, prefrontal, cortex, *in vivo* microdialysis.**

The basal forebrain (BF) is the major source of cholinergic projections to the neocortex (Semba, 2000). These cortically projecting neurons have been implicated in cortical arousal, attention, learning and plasticity (Everitt and Robbins, 1997; Sarter and Bruno, 1997; Jones and Mühlethaler, 1999; Rasmusson, 2000). While cortical arousal can be seen to be a global function affecting the whole cortex at the same time, other proposed functions such as plasticity or selective attention seem to require regional control. This would be necessary if cholinergic input were to modify synapses in one cortical region without affecting the synapses in another, for example. Such specificity would follow if individual cholinergic BF neurons project only to one cortical region (output specificity) and also if they could be activated by selected afferents (input specificity).

In general the anatomical evidence indicates that branching of BF axons is limited (Bigl et al., 1982; Price and Stern, 1983; Saper, 1984; Baskerville et al., 1993), which is consistent with output specificity. Functional experiments, using acetylcholine (ACh) release as a measure of activation of BF cholinergic neurons, have been contradictory. Most early studies, using the cortical cup method to collect ACh from the cortical surface of rabbits, found greater increases in cortical areas corresponding to stimulation of specific sensory modalities (Collier and Mitchell, 1966; Neal et al., 1968; Hemsworth and Mitchell, 1969), consistent with the idea that both the inputs to and outputs from the cholinergic BF are relatively specific. However, some authors using cortical cups (Phillis, 1968) or microdialysis (Sarter and Bruno, 2000) have found similar increases in ACh levels in several regions, and argue that the cholinergic BF activates the cortex in a diffuse and global manner. The early studies need to be taken with caution because of the poor spatial resolution of the cortical cup method. The microdialysis technique is a vast improvement because it has much better spatial resolution. In addition, it is easier with microdialysis to place multiple probes in the same animal so that direct comparisons between cortical regions can be made.

To shed more light on the issue of input and output specificity, we used microdialysis of three cortical sites while stimulating anesthetized rats via visual and somatosensory modalities in counter-balanced order. Two of the collection sites corresponded to the type of sensory input (visual and somatosensory cortices) and the third was a non-sensory area, the medial prefrontal cortex, which has been used in experiments that argue against the specificity idea (Himmelheber et al., 1998). Our findings support the existence of both input and output specificity of the cholinergic BF-to-neocortex pathway.

## EXPERIMENTAL PROCEDURES

Experiments were carried out on 14 adult male Wistar rats (Charles River, St. Constant, Quebec) weighing between 300 and 650 g. The procedures were approved by the Dalhousie University Committee on Laboratory Animals in accordance with the guidelines of the Canadian Council of Animal Care. The animal was anesthetized with urethane (1.4 g/kg, i.p.; Sigma, St. Louis, MO, USA) and placed in a stereotaxic apparatus. The anesthetic was supplemented during the surgery, if necessary, but no additional anesthetic was given once the experiment had begun. Three CMA-12 microdialysis probes (CMA Microdialysis AB, Stockholm, Sweden; 2 mm long, 0.5 mm outer diameter, molecular cutoff 20 kDa) were inserted vertically into the left hemisphere at the fol-

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*Abbreviations:* ACh, acetylcholine; ANOVA, analysis of variance; B, binocular region; BF, basal forebrain; Cg1, cingulate area 1; H, hindlimb region; HPLC, high-performance liquid chromatography; MPFC, medial prefrontal cortex; PrL, prelimbic area; SC, somatosensory cortex; Sk, skin stimulation; V, visual stimulation; VC, visual cortex.

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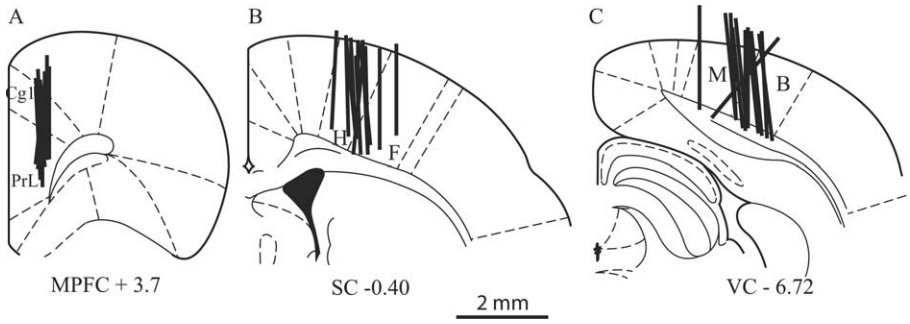


**Fig. 1.** Sites of microdialysis probes in all 14 animals drawn on representative figures from the Paxinos and Watson (1997) atlas with the distance rostral or caudal to bregma indicated in mm. (A) MPFC. (B) SC (F [forelimb] and H regions). (C) VC (B and M [monocular region]). The length of each line represents the 2 mm of exposed microdialysis membrane, based on the histological location of the tip of the probe. The 0.5 mm diameter of the probes is not depicted, however, for clarity.

lowing coordinates (in mm with respect to bregma, Paxinos and Watson, 1997): Medial prefrontal (MPFC): 3.2 anterior, 1.0 lateral; somatosensory (SC): 1.3 posterior, 2.0 lateral; visual (VC): 7.0 posterior, 3.0 lateral. VC and SC probes were inserted 2 mm below the cortical surface, while the MPFC probe was inserted 3 mm down. Placement was confirmed histologically (using Nissl staining) after killing the animal. All efforts were made to minimize the number of animals used and their suffering.

All microdialysis probes were perfused at a rate of 2 μl/min with artificial cerebrospinal fluid (3 mM KCl, 125 mM NaCl, 1.3 mM $CaCl_2$, 1 mM $MgSO_4$) containing 10 μM neostigmine (Sigma) and 10 μM atropine sulfate (Sigma). Neostigmine is necessary for recovery of ACh and atropine increases basal release by blocking presynaptic muscarinic receptors (Dudar and Szerb, 1969). The animals were in a dark room, with minimal visual stimulation. After 80 min of equilibration, a series of 20 min samples was collected and analyzed for ACh content using high-performance liquid chromatography (HPLC; Waters, Mississauga, ON). Details of the HPLC were described previously (Materi and Semba, 2001).

Dialysate was collected for 4 h (12 samples), with sensory stimulation during samples 3 and 8. The sequence of somatosensory and visual stimulation was counterbalanced with seven rats receiving skin and then visual stimulation and seven rats the reverse order. Somatosensory stimulation consisted of a 2 mA, 0.1 s electrical pulse delivered to the right hindlimb once per minute over the 20 min. Visual stimulation was a checkerboard pattern (6×8 black and white squares displayed on computer monitor 30 cm in front and to the right of the animal) that alternated every 3 s throughout the stimulation sample period. Each square subtended approximately 5°. ACh release was normalized for each probe with respect to the mean of the first two (baseline) samples, as in previous experiments (Fournier et al., 2004). The data were analyzed using StatView 5.0 (SAS Institute, Cary, NC, USA) and are expressed as mean±S.E.M.

## RESULTS

The approximate histological locations of the microdialysis probes in all 14 animals are shown in Fig. 1, with each line indicating the 2 mm long membrane of the probe, based on the maximum depth of the track. While this gives the appearance that part of the probe may have been outside the brain, the probe membranes were visually verified to be completely within the brain at the time of insertion. The MPFC probes were situated in cingulate area 1 (Cg1) and prelimbic area (PrL; Fig. 1A). The SC probes were all in primary SC (Fig. 1B), either in or within 1 mm of the hindlimb region (H). The VC probes were centered mainly in the binocular region (B) of primary VC (Fig. 1C).

The baseline release of ACh was approximately three times as large in MPFC (540±41 pmol/sample) as in VC (190±24) or SC (172±11; $n=14$). Due to the wide differences in variance, these values were compared using a non-parametric Friedman test. This confirmed that the regional difference is statistically significant ($P=0.0003$, $df=2$).

The normalized ACh release from each cortical region over the course of the experiment is illustrated in Fig. 2 with skin stimulation (Sk) samples indicated by open bars and visual stimulation (V) samples by black bars. On the left are data from those animals that received Sk first and on the right those that received visual stimulation first. It can be seen that ACh release from SC (Fig. 2A) was increased by the Sk regardless of whether it was presented first or second. Similarly ACh release from VC was clearly increased by visual stimulation (Fig. 2B), whereas MPFC did not show any consistent change with either type of stimulation (Fig. 2C). The results of an analysis of variance (ANOVA) across samples are shown in the upper right for each group, and indicate significant change over time for VC in both groups and for SC in the animals receiving Sk last. ACh release from SC in the animals receiving the other sequence was not significant due to the higher variability across samples. No change across samples was seen in release from MPFC.

When the evoked ACh release during the stimulation samples (stimulation sample/mean of samples 1 and 2) was collapsed across groups, there was no effect of order of stimulation (first: 35±4%; second: 34±3%; $n=14$; $P>0.50$) and no difference between visual and Sk (visual: 34±7%; skin: 35±9%; $n=14$; $P>0.50$). There was, however, a significant difference between cortical regions ($F_{2,14}=11.58$; $P<0.01$) and a significant Cortex ×Stimulation interaction ($F_{2,14}=10.48$; $P<0.01$). This is illustrated in Fig. 3. Sk (Fig. 3A) produced a 78±16% increase in ACh release from SC, only a 21±9% increase from VC, and no change from MPFC (6±11%). Visual stimulation (Fig. 3B) produced a 75±11% increase in ACh release from VC, a 24±9% increase from SC and no change in MPFC (6±10%). Single group *t*-tests revealed that the small increases from sensory cortices with the inappropriate stimulation condition were both significantly

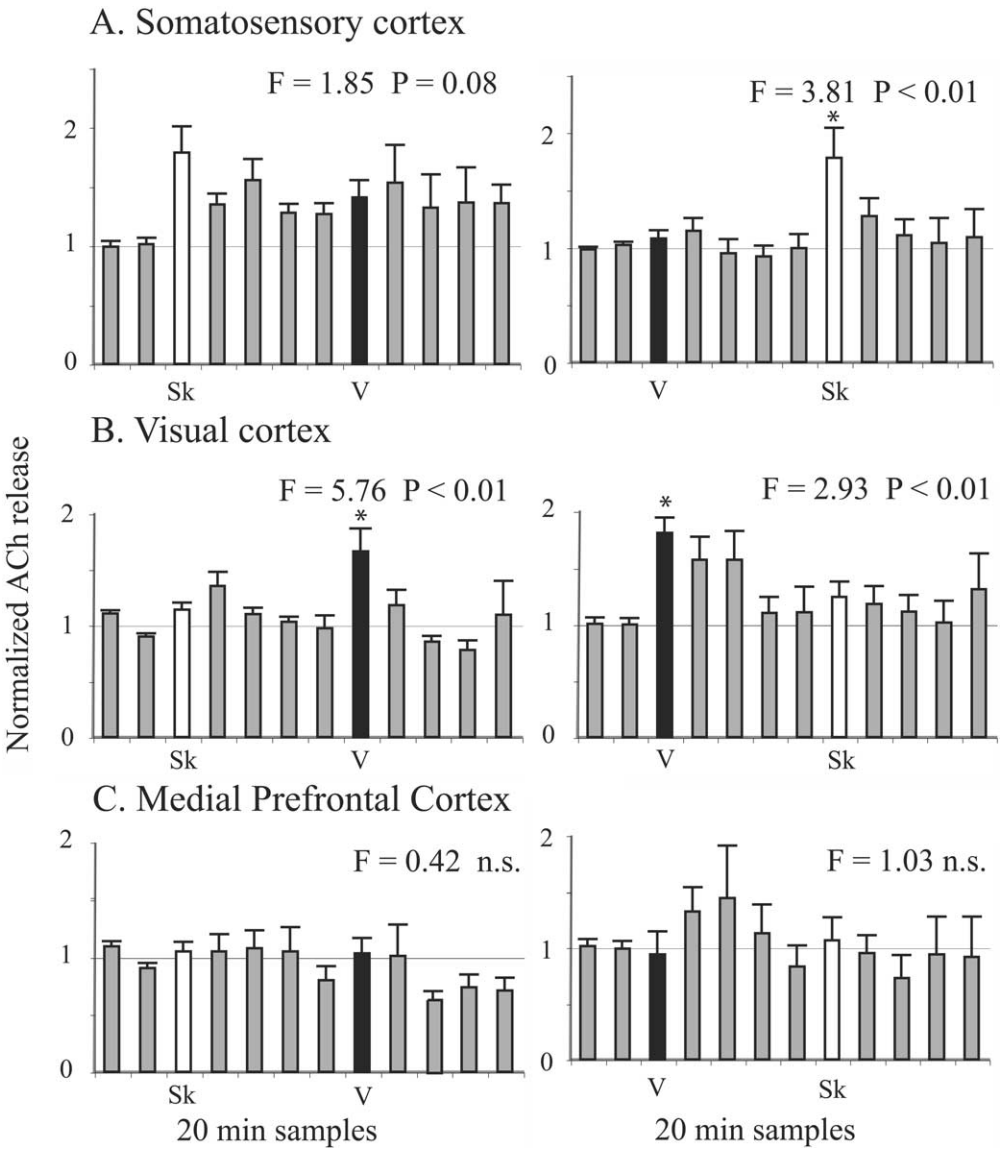


**Fig. 2.** ACh release from the three cortical regions (A–C) over the course of all samples. Left column, results from seven animals that received Sk (open bars) followed by V (black bars). Right column, results from seven animals that received visual stimulation followed by Sk. Mean±S.E.M. normalized to the mean of the two baseline samples. Results of repeated measures ANOVA are shown in upper right of each graph ($df$=11, 70). * $P<0.05$ compared with baseline samples, Tukey post hoc test.

different from 1 (SC with visual stimulation $t_{13}=2.61$, $P=0.024$; VC with Sk, $t_{13}=2.33$, $P=0.042$), but the evoked release from MPFC was not different from 1 ($P>0.5$). In other words, sensory stimulation produced a significant increase from both sensory cortices, but not from MPFC, and the increase was much larger from the sensory area associated with the modality of the stimulation.

It might be argued that the high baseline release from MPFC prevented any further increase. If that were so, there should be a significant inverse relationship between baseline release and evoked release. This was not found to be the case. For MPFC the correlation ($R^2$) between the two measures was +0.032, which was not statistically significant.

## DISCUSSION

While a variety of studies have implicated the BF cholinergic system in complex cognitive tasks, the details of its function and underlying mechanisms remain controversial. An important consideration is how individual cholinergic neurons are activated and how they might influence different cortical regions. Some of the proposed functions of the cholinergic system, such as generalized arousal, could be carried out with little specificity, whereas others, such as selective attention or cortical plasticity, are more logical if different cholinergic neurons could be activated independently. The latter would require both output specificity (discrete projections to different cortical areas) and input specificity (matching of the input of BF neurons to their output

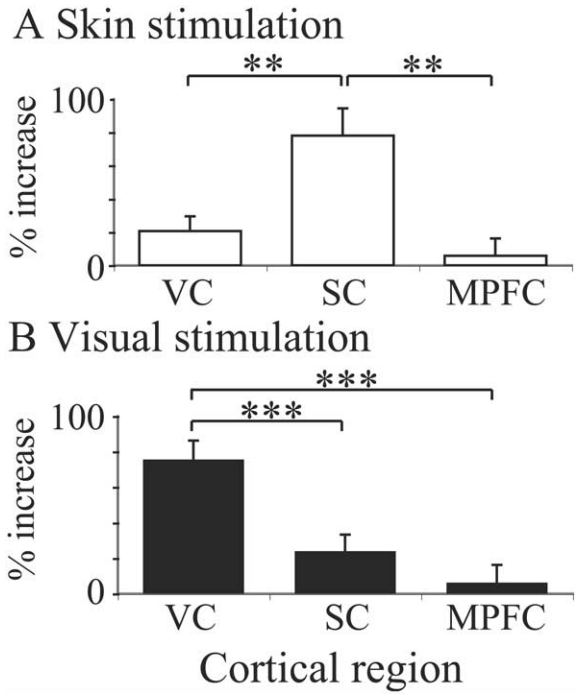


**Fig. 3.** Increase in ACh release from visual (VC), SC and MPFC cortex in response to A) Sk and B) visual stimulation. The values are percentage increase in the stimulation sample compared with the first two samples; mean±S.E.M. *** $P<0.001$; ** $P<0.01$.

projection). Here we examined the hypothesis that BF cholinergic neurons possess input and output specificity using ACh release as a functional measure of their activity.

The microdialysis data in the present study clearly indicate that visual and somatosensory stimulation can evoke ACh release in a modality- and region-specific manner. ACh release was almost doubled in the cortical area corresponding to the sensory input, with only a small (20%) increase in ACh release in the other sensory area and no change from the prefrontal cortex, a non-sensory cortical region. These results confirm and extend early studies by Mitchell's group using the cortical cup technique in rabbits. Collier and Mitchell (1966), for example, found that visual stimulation produced a 330% increase in ACh levels from VC and only a 90% increase from non-visual areas. Neal et al. (1968) reported similar differences with sound stimulation producing a four-fold change in auditory cortex and only a doubling from non-auditory cortex. Electrical stimulation of the sensory pathways produced similar differences in cortical ACh levels (Collier and Mitchell, 1966; Neal et al., 1968; Hemsworth and Mitchell, 1969). However, the cortical cups are large (0.4–1.0 $cm^2$) perhaps encompassing multiple cortical regions, and may only be sampling release from superficial layers of the cortex. Microdialysis probes, on the other hand, can be inserted more precisely into a small region, multiple probes can be placed in the same animal, and they can collect ACh across the full cortical thickness. This is important as cholinergic terminals are distributed through all layers of the cortex and have different laminar densities in different regions (Lysakowski et al., 1989). The present study extends the previous studies by showing, in the same animal, that sensory stimuli evoke greater release from the appropriate sensory cortex than from other regions. The finding that there was a significant, but smaller, increase from the inappropriate sensory region is consistent with the hypothesis that there is a small, non-specific portion of BF neurons that is activated by many inputs in addition to regionally specific relays (Collier and Mitchell, 1966, 1967). The higher baseline release from MPFC is interesting, as this is the only cortical region in rodents with extensive projections to the BF (Záborszky et al., 1997). There may be higher tonic activity in MPFC-projecting cholinergic neurons due to a positive feedback loop. Alternatively, MPFC receives a second cholinergic input, which originates in the mesopontine tegmentum (Vincent et al., 1983) and might contribute to the higher basal release of ACh. It is unlikely that the lack of increase with stimulation is due to a ceiling effect, however, as other studies have seen an increased release of ACh from this area (Inglis and Fibiger, 1995; Fadel et al., 2001).

The simplest explanation of the present results is that a major portion of the cholinergic BF consists of different populations of neurons that can be activated by different sensory inputs. This is supported by the results of electrical stimulation of the BF in which some sites produced greater ACh release from VC than from SC (Jiménez-Capdeville et al., 1997). This is also consistent with many anatomical studies on the BF-to-cortex projection in the rat, which find few or no double-labeled neurons with retrograde tracer injections into different cortical areas (Bigl et al., 1982; Price and Stern, 1983; Saper, 1984; Baskerville et al., 1993; but see McKinney et al., 1983).

Anatomical or physiological evidence for *input* specificity to BF neurons is more difficult to obtain. Only a few studies have reported the presence of sensory responses in BF neurons, with the most effective being painful stimuli which may activate many arousal pathways (Détári et al., 1999). Anatomically, there is no evidence for direct projections to the BF from sensory nuclei (Semba et al., 1988; Vertes, 1988; Jones and Cuello, 1989) or from sensory cortices (Záborszky et al., 1991). However, the stimulation of VC and SC has been shown to evoke long-latency responses in BF neurons, possibly through an indirect pathway via the MPFC (Golmayo et al., 2003). These findings suggest that BF neurons might receive a higher-order sensory input indirectly, possibly through the cortex.

The present findings are not consistent with previous microdialysis studies in awake rats. Himmelheber et al. (1998), for example, reported large (100–140%) increases in ACh release from both prefrontal and SC during tactile stimulation. A study of diurnal rhythms in the rat found similar (50%) increases in release from visual and somatosensory regions at the transition to increased motor activity, although a third region (motor cortex) had only a 20% increase (Jiménez-Capdeville and Dykes, 1996). Inglis and Fibiger (1995) tested several different stimulus modalities while measuring ACh release from a transverse probe through frontal and prefrontal cortex. They found significant increases with auditory, olfactory and tactile stimula-

tion but not with visual stimulation. It might be that the differences from the present results are due to the use of anesthesia. However, it is not obvious why the anesthetic would decrease release in one BF projection but not in another. It seems more likely that in the unanesthetized animal the sensory inputs are so diverse that many functionally distinct cholinergic neurons are being activated.

We have considered the possibility that presynaptic mechanisms might account for the differential release. For example, the stimulation paradigm will activate the thalamocortical inputs to a specific cortical region, which are glutamatergic, and it is conceivable that glutamate released by thalamocortical afferents spills over to adjacent cholinergic terminals and enhances ACh release. In fact, glutamatergic synapses are often seen adjacent to cholinergic synapses in cat VC (Aoki and Kabak, 1992). However, administration of glutamate in the rat SC by reverse microdialysis caused a decrease, rather than an increase, in evoked ACh release, apparently via GABAergic mechanisms (Materi and Semba, 2001). While the interaction between glutamate and ACh might be different at the synaptic level, it is our view that any such modulation is relatively minor compared with the selective activation of a BF-to-cortex pathway discussed above. This view is consistent with the anatomical specificity of the BF corticopetal system.

The present findings argue that cholinergic BF projections to the cortex are not diffuse but are modality- and region-specific. Thus distinct cholinergic BF neurons are theoretically capable of modulating different cortical regions. By releasing ACh in a functionally specific manner, the BF cholinergic system has the connectivity necessary to modulate the cortex within the context of ongoing behavior and thus contribute to functions such as plasticity and selective attention.

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## REFERENCES

Aoki C, Kabak S (1992) Cholinergic terminals in the cat visual cortex: ultrastructural basis for interaction with glutamate-immunoreactive neurons and other cells. Visual Neurosci 8:177–191.

Baskerville KA, Chang HT, Herron P (1993) Topography of cholinergic afferents from the nucleus basalis of Meynert to representational areas of sensorimotor cortices in the rat. J Comp Neurol 335:552–562.

Bigl V, Woolf NJ, Butcher LL (1982) Cholinergic projections from the basal forebrain to frontal, parietal, temporal, occipital, and cingulate cortices: a combined fluorescent tracer and acetylcholinesterase analysis. Brain Res Bull 8:727–749.

Collier B, Mitchell JF (1966) The central release of acetylcholine during stimulation of the visual pathway. J Physiol 184:239–254.

Collier B, Mitchell JF (1967) The central release of acetylcholine during consciousness and after brain lesions. J Physiol Lond 188:83–98.

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

Dudar JD, Szerb JC (1969) The effect of topically applied atropine on resting and evoked cortical acetylcholine release. J Physiol Lond 203:741–762.

Everitt BJ, Robbins TW (1997) Central cholinergic systems and cognition. Annu Rev Psychol 48:649–684.

Fadel J, Sarter M, Bruno JP (2001) Basal forebrain glutamatergic modulation of cortical acetylcholine release. Synapse 39:201–212.

Fournier GN, Materi LM, Semba K, Rasmusson DD (2004) Cortical acetylcholine release and electroencephalogram activation evoked by ionotropic glutamate receptor agonists in the rat basal forebrain. Neuroscience 123:785–792.

Golmayo L, Nunez 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.

Hemsworth BA, Mitchell JF (1969) The characteristics of acetylcholine release mechanisms in the auditory cortex. Br J Pharmacol 36: 161–170.

Himmelheber AM, Fadel J, Sarter M, Bruno JP (1998) Effects of local cholinesterase inhibition on acetylcholine release assessed simultaneously in prefrontal and frontoparietal cortex. Neuroscience 86: 949–957.

Inglis FM, Fibiger HC (1995) Increases in hippocampal and frontal cortical acetylcholine release associated with presentation of sensory stimuli. Neuroscience 66:81–86.

Jiménez-Capdeville ME, Dykes RW (1996) Changes in cortical acetylcholine release in the rat during day and night: differences between motor and sensory areas. Neuroscience 71:567–579.

Jiménez-Capdeville ME, Dykes RW, Myasnikov AA (1997) Differential control of cortical activity by the basal forebrain in rats: a role for both cholinergic and inhibitory influences. J Comp Neurol 381:53–67.

Jones BE, Cuello AC (1989) Afferents to the basal forebrain cholinergic cell area from pontomesencephalic-catecholamine, serotonin, and acetylcholine-neurons. Neuroscience 31:37–61.

Jones BE, Mühlethaler M (1999) Cholinergic and GABAergic neurons of the basal forebrain: role in cortical activation. In: Handbook of behavioral state control: cellular and molecular mechanisms (Lydic R, Baghdoyan HA, eds), pp 213–233. Boca Raton: CRC Press.

Lysakowski A, Wainer BH, Bruce G, Hersh LB (1989) An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. Neuroscience 28:291–336.

Materi LM, Semba K (2001) Inhibition of synaptically evoked cortical acetylcholine release by intracortical glutamate: involvement of GABAergic neurons. Eur J Neurosci 14:38–46.

McKinney M, Coyle JT, Hedreen JC (1983) Topographic analysis of the innervation of the rat neocortex and hippocampus by the basal forebrain cholinergic system. J Comp Neurol 217:103–121.

Neal MJ, Hemsworth BA, Mitchell JF (1968) The excitation of central cholinergic mechanisms by stimulation of the auditory pathway. Life Sci 7:757–763.

Paxinos G, Watson C (1997) The compact rat brain in stereotaxic coordinates, 3rd ed. San Diego: Academic Press.

Phillis JW (1968) Acetylcholine release from the cerebral cortex: its role in cortical arousal. Brain Res 7:378–389.

Price JL, Stern R (1983) Individual cells in the nucleus basalis-diagonal band complex have restricted axonal projections to the cerebral cortex in the rat. Brain Res 269:352–356.

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

Saper CB (1984) Organization of cerebral cortical afferent systems in the rat. I: Magnocellular basal nucleus. J Comp Neurol 222:313–342.

Sarter M, Bruno JP (1997) Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res Rev 23:28–46.

Sarter M, Bruno JP (2000) Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent

regulation of the basal forebrain by telencephalic and brainstem afferents. Neuroscience 95:933–952.

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

Semba K, Reiner PB, McGeer EG, Fibiger HC (1988) Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. J Comp Neurol 267:433–453.

Vertes R (1988) Brainstem afferents to the basal forebrain in the rat. Neuroscience 24:907–938.

Vincent SR, Satoh K, Armstrong DM, Fibiger HC (1983) Substance P in the ascending cholinergic reticular system. Nature 306:688–691.

Záborszky L, Cullinan WE, Braun A (1991) Afferents to basal forebrain cholinergic projection neurons: an update. In: The basal forebrain (Napier TC, ed), pp 43–100. New York: Plenum.

Záborszky L, Gaykema RP, Swanson DJ, Cullinan WE (1997) Cortical input to the basal forebrain. Neuroscience 79:1051–1078.

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