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ACETYLCHOLINE RELEASE IS ELICITED IN THE VISUAL CORTEX, BUT NOT IN THE PREFRONTAL CORTEX, BY PATTERNED VISUAL STIMULATION: A DUAL *IN VIVO* MICRODIALYSIS STUDY WITH FUNCTIONAL CORRELATES IN THE RAT BRAIN

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Abstract—By its projections to the primary visual and the prefrontal cortices, the basal forebrain cholinergic system is involved in cognitive processing of sensory stimuli. It has been suggested that visual stimulus-induced cholinergic activation of the visual cortex may exert a permissive role on thalamocortical inputs. However, it is not known if visual stimulation elicits cholinergic activation of high-order brain areas in the absence of attentional need. In the present study, we measured the effects of patterned visual stimulation (horizontal grating) on the release of acetylcholine with dual-probe *in vivo* microdialysis in the visual and the prefrontal cortices of anesthetized rats. We also used retrograde tracing to determine the anatomical relationships of cholinergic neurons with neurons of the visual system and the prefrontal cortex. Finally, we evaluated a functional correlate of this stimulation, namely *c-fos* immunolabeling. Patterned visual stimulation elicited significant increases in acetylcholine release in the visual cortex, accompanied by an increased number of *c-fos* immunoreactive neurons in this brain area. In contrast, in the prefrontal cortex, neither the level of acetylcholine release nor the number of *c-fos* immunoreactive neurons was significantly changed because of the stimulation. Cholinergic basal forebrain neurons projecting to the visual or the prefrontal cortices were both localized within the horizontal limb of the diagonal band of Broca but were not immunoreactive for *c-fos* during visual stimulation. No parts of the visual system were found to directly project to these basal forebrain neurons. These results suggest the differential involvement of cholinergic projections in the integration of sensory stimuli, depending on the level of activity of the targeted cortical area.

Key words: basal forebrain, *c-fos* immunostaining, cholinergic system, sensory stimuli processing, retrograde tracing, vision.

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Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; BF, basal forebrain; ChAT, choline acetyltransferase; DY, Diamino Yellow; FB, Fast Blue; HDB, horizontal limb of the diagonal band of Broca; PBS, phosphate buffer; PFC, prefrontal cortex; SI, substantia innominata; VDB, vertical limb of the diagonal band of Broca; V1, primary visual cortex; V1M, monocular primary visual cortex.

Basal forebrain (BF) cholinergic neurons are involved in the processing of sensory information and task-relevant stimuli through their projections to the cerebral cortex. Regions of cholinergic innervation include both primary cortical sensory areas and high-order cognitive regions (association cortical areas), such as the prefrontal cortex (PFC; Mesulam et al., 1983; Luiten et al., 1987; Gaykema et al., 1990; Woolf, 1991). This implies a role for the BF in both primary as well as a high-order (i.e. PFC) cortical processing of sensory stimuli.

Cortical acetylcholine (ACh) release is enhanced in certain sensory cortical areas by a variety of sensory stimuli (Collier and Mitchell, 1966; Akaishi et al., 1990; Inglis and Fibiger, 1995; Kilgard and Merzenich, 1998). For example, ACh efflux is increased in the rat primary visual cortex (V1) in response to stroboscopic stimulation (Inglis and Fibiger, 1995) and diffuse light (Collier and Mitchell, 1966). Pharmacological and physiological studies suggest a critical role for ACh in cortical facilitation or long-term potentiation of neuronal responses to visual stimuli (Donoghue and Carroll, 1987; Greuel et al., 1988; Lamour et al., 1988; Kirkwood et al., 1999). Moreover, coupling of sensory stimuli with BF stimulation leads to long-term enhancement of neuronal excitability in the somatosensory cortex (Verdier and Dykes, 2001) and dramatic reorganization of cortical maps in the auditory cortex (Kilgard and Merzenich, 1998). It has hence been proposed that ACh released from BF projections may be involved in the processing of thalamocortical inputs in primary sensory cortices.

Likewise, release of ACh in the PFC may contribute to high-level cognitive functions by facilitating the final association of sensory stimuli within this cortical area. Lesions of the BF cholinergic system induce decreases in PFC neuronal firing rate (Gill et al., 2000) and impairments of attentional abilities (Muir et al., 1992; Voytko, 1996; Gill et al., 2000; McGaughy et al., 2000; Passetti et al., 2000; Arnold et al., 2002). Moreover, demands for sustained attention are accompanied by increases in ACh efflux in the rat PFC (Gill et al., 2000; Passetti et al., 2000; Dalley et al., 2001) and cholinergic agents modulate performance in attentional tasks (Granon et al., 1995). These data have led to the proposal that BF cholinergic neurons contribute to attentional function through their projections to the PFC.

Although these studies suggest that the BF plays an important role in cortical integration of sensory stimuli, it is not known how ongoing sensory stimuli might induce activity in BF neurons, given that thalamic sensory regions

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apparently do not directly project to the BF (Zaborszky et al., 1991). BF neurons receive input from the PFC (Vertes, 2004) and are activated by electrical activation of the PFC (Golmayo et al., 2003). In relation to these findings, a new perspective is now emerging that suggests cholinergic BF neurons may be activated by PFC neurons receiving convergent sensory inputs. Following input from the PFC, BF neurons may, in turn, modulate activity in sensory areas. As such, the BF may play a role in top-down attentional control of sensory stimuli processing in response to activity in cortical areas associated with higher cognitive functions (Sarter et al., 2001; Golmayo et al., 2003).

We investigated here whether the cholinergic system plays a local or more global role in cortical processing of visual stimuli. We used a dual *in vivo* microdialysis approach to evaluate whether visual stimulation elicits ACh release specifically in the V1 region or in both the V1 and PFC areas. We eliminated attentional demand using anesthetized animals and used patterned visual stimuli that specifically activate visual neurons. Retrograde tracing experiments were conducted to establish possible overlap in cholinergic BF neurons projecting to V1 and PFC. In addition, also with retrograde tracing, we asked whether visual structures (e.g. retina) project directly to BF neurons. Finally, the pattern of visual stimulus-activated V1, PFC and/or the BF neurons was examined using *c-fos* immunoreactivity. Some of these data have appeared in abstract form (Laplante et al., 2002).

EXPERIMENTAL PROCEDURES

Long-Evans rats (275–325 g) were obtained from Charles River Canada (St-Constant, Québec, Canada) and housed individually in a 12-h light/dark cycle with free food access. Distinct groups of rats were used for three different experiments: (1) *in vivo* microdialysis ($n=7$), (2) *c-fos* immunostaining ($n=8$) and (3) retrograde tracing ($n=10$). The effects of monocular visual stimulation on cortical ACh release (*in vivo* microdialysis) and neuronal activation (*c-fos* immunostaining) were investigated. Animal care and protocols conformed to the Canadian Council for Animal Care guidelines on the ethical use of animals. All efforts were made to minimize both the suffering and number of animals used. All experiments were approved by le Comité de déontologie de l'expérimentation sur les animaux de l'Université de Montréal and the McGill University Animal Care Committee.

Visual stimulation

The following monocular visual stimulation paradigm, adapted from Girman et al. (1999) and Porciatti (1999), was used for the *in vivo* microdialysis experiments (after basal level of ACh release had been established in the perfusate) or before *c-fos* immunocytochemistry. Rats were anesthetized with urethane (1.2 g/kg; Sigma Chemical Co., Oakville, Ontario, Canada) to prevent head movements, which ensured consistent ocular stimulation, stress and attention demand. Urethane is commonly used in experiments with rats, since it produces long periods of anesthesia, has a wide safety margin, and has little effect on normal blood pressure and respiration (according to the Guide to the Care and Use of Experimental Animals of the Canadian Council for Animal Care). Rats were positioned in a stereotaxic frame to ensure constant exposure to the stimulus in the desired eye. Animals were then placed into a closed black chamber. Body temperature was maintained at 37 °C with a thermostatically controlled heating pad (FHC, Bowdoinham, ME, USA). Pupil dilation and accommodation paralysis with atropine was not used. Drying of the eye was prevented with a natural eye lubricant (Ophtapharma, Montréal, Québec, Canada). A computer monitor (30×25 cm, Titanium; Apple Computer Inc., Cupertino, CA, USA) was placed 32 cm parallel to the long axis of the rat and centered on the eye. A horizontal sinusoidal grating (contrast 90%, 0.08 c/day, 3.4 Hz) was generated by VPixx software (v 8.5; Sentinel Medical Research Corp., Québec, Canada) and displayed on the computer monitor for 30 min. We selected the orientation, temporal and spatial frequency of the grating based on published values that have been shown to induce an optimal response in most V1 neurons (Girman et al., 1999; Porciatti et al., 1999).

In vivo microdialysis

We used a dual-dialysis probe approach to simultaneously measure ACh release in the monocular V1 (V1M) and the medial PFC. The microdialysis procedure was similar to that which has been previously described (Quirion et al., 1994; Day et al., 2001; Laplante et al., 2004), with minor modifications. Surgery was performed 2 days before the *in vivo* dialysis experiment. Rats were anesthetized with a mixture of ketamine (50 mg/kg; Vetrepharm, Belleville, Ontario, Canada), xylazine (5 mg/kg; Novopharm, Toronto, Ontario, Canada) and acepromazine (0.5 mg/kg; Ayerst, Montréal, Québec, Canada) and immobilized in a stereotaxic frame. The skull was exposed through a midline incision and the insertion of the temporal muscle displaced to expose the parietal bone. For the visual cortex, we used a transverse horizontal probe made as described previously (Day et al., 2001). The dialysis membrane (molecular weight cutoff 60 kDa, i.d.= 0.22 mm, o.d.=0.31 mm; Hospal Industry, Lyon, France) was covered with epoxy glue along its whole length except for 3 mm corresponding to the area of dialysis. The transverse probe was inserted into the V1M contralateral to the stimulated eye (mm from Bregma: AP −6.7, L +2.0–5.0, V −1.8; Paxinos and Watson, 1995). The ends of the dialysis tube were connected to a stainless steel cannula with epoxy glue. For the medial PFC, a vertical intracerebral guide cannula (MAB 2.14.G; Scipro, Concord, Ontario, Canada) was lowered just over the medial PFC contralateral to the stimulated eye (mm from Bregma: AP +2.8, L +0.5, V −2.0; Paxinos and Watson, 1995). Steel cannulas were secured on the top of the skull using dental cement. The microdialysis probe (MAB 6.14.4; molecular weight cutoff 15 kDa, o.d. 0.6 mm, 4 mm; Scipro) was prepared according to the manufacturer's instructions and inserted within the vertical intracerebral guide cannula 1 h prior to the *in vivo* dialysis experiment. Each animal was dialyzed only once.

Dialysis probes were connected to a microliter-syringe pump and perfused with a cerebrospinal fluid-like solution containing (in mM) NaCl, 123; KCl, 3; $CaCl_2$, 1.3; $MgCl_2$, 1; $NaHCO_3$, 23 and sodium phosphate buffer (PBS), 10 (pH 7.4). Neostigmine bromide (5 μM), an acetylcholinesterase inhibitor, was added to the solution to increase recovery, as commonly used in anesthetized animals (Fournier et al., 2004; Antoniou et al., 1997; Kurosawa et al., 1989). This concentration of acetylcholinesterase inhibitor does not induce changes in sensory-evoked cortical activity as assessed by field potentials recording (Oldford and Castro-Alamancos, 2003). Moreover it has been demonstrated that varying the concentration of neostigmine in the microdialysis perfusate does not affect significantly the magnitude or the duration of the sensory-evoked ACh efflux (Himmelheber et al., 1998). Probes were perfused at a flow rate of 5 μl/min. After a 1 h wash out, 10 min dialysate fractions were collected for a 1 h period. When the basal level of ACh release stabilized, unilateral visual stimulation began. Dialysates were collected up to 2 h after end of the stimulation to establish the return to basal levels.