Activation of Nucleus Basalis Facilitates Cortical Control of a Brain Stem Motor Program

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Berg, Rune W., Beth Friedman, Lee F. Schroeder, and David Kleinfeld. Activation of nucleus basalis facilitates cortical control of a brain stem motor program. J Neurophysiol 94: 699-711, 2005. First published February 23, 2005; doi:10.1152/jn.01125.2004. We tested the hypothesis that activation of nucleus basalis magnocellularis (NBM), which provides cholinergic input to cortex, facilitates motor control. Our measures of facilitation were changes in the direction and time-course of vibrissa movements that are elicited by microstimulation of vibrissa motor (M1) cortex. In particular, microstimulation led solely to a transient retraction of the vibrissae in the sessile animal but to a full motion sequence of protraction followed by retraction in the aroused animal. We observed that activation of NBM, as assayed by cortical desynchronization, induced a transition from microstimulation-evoked retraction to full sweep sequences. This dramatic change in the vibrissa response to microstimulation was blocked by systemic delivery of atropine and, in anesthetized animals, an analogous change was blocked by the topical administration of atropine to M1 cortex. We conclude that NBM significantly facilitates the ability of M1 cortex to control movements. Our results bear on the importance of cholinergic activation in schemes for neuroprosthetic control of movement.

INTRODUCTION

Nucleus basalis magnocellularis (NBM) of the basal forebrain forms extensive cholinergic projections throughout cortex (Casamenti et al. 1986; Johnston et al. 1979; Kurosawa et al. 1989; Rye et al. 1984; Saper 1984; Wenk et al. 1980; Woolf et al. 1983). Activation of NBM plays an essential role in the depolarization of cortical neurons (Metherate and Ashe 1993) and in abolishing the overall synchronous activity in cortex that is associated with drowsiness (Buzsaki et al. 1988; Detari et al. 1999; Sarter and Bruno 2000; Wenk 1997). The desynchronized state is associated with mental arousal (Green and Arduini 1954; Vanderwolf 1969, 1990) and attention (Muir et al. 1992) and is correlated with heightened sensory processing (Bringmann and Klingberg 1990; Donoghue and Carroll 1987; Hars et al. 1993; Mercado et al. 2001; Metherate and Ashe 1991; Murphy and Sillito 1991; Sato et al. 1987; Tremblay et al. 1990; Webster et al. 1991), improved working memory (Wrenn et al. 1999), and improved cognition (Baxter and Chiba 1999; Everitt and Robbins 1997). Furthermore, animals in the desynchronized state exhibit sharpened sensory receptive fields during the alert state (Donoghue and Carroll 1987; McCormick and Prince 1986; Sato et al. 1987) and attain a heightened capability for stimulus-induced plasticity (Baskerville et al.

1997; Kilgard and Merzenich 1998; Maalouf et al. 1998; Sachdev et al. 1998). These elastic and plastic effects are mediated at least in large part by the neurotransmitter acetylcholine (Celesia and Jasper 1966; Kanai and Szerb 1965; Perry et al. 1999; Shute and Lewis 1967; Szerb 1967), prevalent in fibers from the NBM. In particular, the impact of pathological conditions like Alzheimer's disease, where the cholinergic cells throughout the basal forebrain degenerate (Geula and Mesulam 1999), emphasizes the central role of NBM in normal brain function.

Despite the presence of extensive cholinergic afferents to motor areas of cortex (Donoghue and Parham 1983; Eckenstein et al. 1988; Jimenez-Capdeville et al. 1997; McKinney et al. 1983; Saper 1984) and the release of acetylcholine during motor activity (Giovannini et al. 2001), a role for NBM in the normal function of motor cortex has received relatively little attention. A recent and notable study showed that animals can complete a forelimb-based retrieval task after pharmacological lesion of their NBM. However, the movements involved in the task were abnormal (Gharbawie and Whishaw 2003). It is possible that both afferent and proprioceptive sensory input, in addition to motor output, are affected. Our approach to dissect the modulation of motor cortex independent from that of sensory cortex is to drive electrical activity in primary motor (M1) cortex and observe a behavioral output. We hypothesize that projections from NBM to M1 cortex could serve to heighten the participation of neurons in sequential motor planning.

The vibrissa system of rat serves as a model preparation to test the above hypothesis. In particular, we ask if the ability of motor cortex to perform cycle-by-cycle control of rhythmic whisking is modulated by the activation of nucleus basalis magnocellularis. Three assays play an essential role in our experiment. 1) A standard measure of arousal may be ascertained from the spectral content of the differential hippocampal field activity (Green and Arduini 1954; Vanderwolf 1969, 1990), denoted the ∇^2 LFP (Fig. 1A). Arousal is signified by rhythmic activity in the 5- to 10-Hz band, or θ -band, while the lack of arousal or attention is signified by irregular activity that manifests itself in the <5-Hz range, or δ -band. We quantify the level of arousal in terms of the integrated power in the 5- to 10-Hz band relative to that in the 0- to 5-Hz band (Fig. 1, B and C). This ratio is at or below 1 for animals in the awake but sessile state but much >1 for animals in the awake and aroused

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FIG. 1. Essential assays of arousal and vibrissa movement and associated \forall EMG. A: power spectra density, S(f), of the hippocampal activity in 2 different states: sessile (black line) and aroused (red line). Spectra were based on a 3-s window of recording (n = 1 animal, 30 trials), with 1 SD from the mean demarcated by the transparent band. B: spectral power in 2 frequency bands: the θ -band, i.e., the power in the 5- to 10-Hz range, and the δ -band, i.e., the power in the 0- to 5-Hz range. C: arousal can be quantified in terms of the ratio of power in the θ -band to that in the δ -band. Note the bimodal distribution (n =143 trials). D: videographs that show the evoked movement of the left vibrissae from a single intracortical microstimulation delivered to the right M1 cortex in the aroused rat. Time between images is 18 ms, and the red triangle in each frame indicates the vibrissa, B1, whose angle was quantified over time. The animal was perched in front of a food tube. E: angular deflection of the vibrissae after the 1st ICMS, averaged over trials and animals, as measured via videography for the animals in the aroused state (red curve, n = 4 animals, 38 trials) and sessile state (black curve, n = 1 animal, 8 trials), evoked from rhythmic ICMS to M1 cortex. Stimuli are indicated at the bottom as a solid bar. Motion consists of a prompt retraction that recovers and, for the case of the aroused state, is followed by a delayed protraction. F: average evoked mystacial VEMG simultaneously recorded from the intrinsic muscles at the time of the videography in D. Scale bar is 100 μ V.

state (Berg and Kleinfeld 2003b) (Fig. 1*C*). 2) A measure of cortical control of motor output is the magnitude and timecourse of vibrissa motion in response to rhythmic intracortical microstimulation (ICMS) to vibrissa M1 cortex (Berg and Kleinfeld 2003b). Vibrissa position was measured directly by videography (Fig. 1, *D* and *E*) or indirectly in terms of the differential mystacial electromyogram, denoted ∇ EMG (Fig. 1*F*); the latter measure is amenable to free ranging animals. Each ICMS pulse leads solely to a retraction of the vibrissae in sessile animals, which is mediated by a relatively small and prompt ∇ EMG signal (Fig. 1, *E* and *F*). In contrast, ICMS leads to full whisks in aroused animals, which is mediated by a relatively larger prompt \forall EMG signal and a new, delayed component in the \forall EMG (Fig. 1, *E* and *F*). 3) Last, a standard measure of cortical arousal, which correlates with both the onset of cholinergic activation of neocortex and behavioral activity, is the extent of desynchronization of the electrocorticogram (ECoG) (Buzsaki and Gage 1989; Metherate et al. 1992; Steriade et al. 1990b; Szerb 1967). This is a qualitative measure that correlates with changes in hippocampal rhythmic activity (Green and Arduini 1954; Steriade et al. 1990a). It provides a means to assess the specific activation of vibrissa M1 cortex by stimulation of NBM.

METHODS

Our subjects were 17 female Long Evans rats, 200–300 g in mass. Fourteen of these animals had EMG electrodes implanted in the intrinsic muscles in the mystacial pad, microwire stimulating electrodes implanted in vibrissa M1 cortex and nucleus basalis magnocellularis, and recording electrodes implanted in hippocampus and M1 cortex (Fig. 2A). The overall sequence of experimentation was electrode placement \rightarrow recovery \rightarrow training \rightarrow data collection \rightarrow histology.

The care and all aspects of experimental manipulation of our animals were in strict accord with guidelines from the National Institute of Health (1985) and have been approved by members of the local Institutional Animal Care and Use Committee.

Electrodes

The stimulation electrodes for both M1 cortex and NBM consisted of two teflon coated etched Pt-Ir wires, each with an impedance of 2 M Ω at f = 1 kHz (PI0030.5A10, MicroProbe, Clarksburg, MD). The pair formed a bipolar stimulation electrode, with tips separated by 500 μ m, that was held by adhesive in a 20-gauge stainless steel tube (Small Parts, Miami Lakes, FL) with adhesive (420, Loctite Corp., Rocky Hill, CT). The tip separation is small compared with the lateral extent of the vibrissa area of M1 cortex (Kleinfeld et al. 2002; Neafsey 1990; Sanderson et al. 1984) and NBM. Platinum-iridium was chosen to minimize neural damage from electrochemistry at the site of stimulation (Tehovnik 1996).

The EMG electrodes were 50- μ m-diam teflon-coated tungsten wires (A-M Systems). They were positioned in the mystacial pad to be sensitive primarily to the intrinsic muscles, as described previously (Berg and Kleinfeld 2003a). We report the numerically computed absolute values of the differential measurement between voltages across the two wires in each area, denoted as the ∇ EMG.

The hippocampal recording electrode consisted of a triplet of $50-\mu$ m-diam teflon-coated tungsten wires (A-M Systems, Carlsborg, WA) that was held by adhesive in a 3-mm-long, 25-gauge stainless steel tube (Small Parts). The three tips were separated so they spanned



FIG. 2. Experimental paradigm. A: schematic of location of electrodes overlaid on a midsagittal section of the rat brain (Swanson 1992). The nucleus basalis magnocellularis (NBM), located subcortically, was stimulated, as well as vibrissa primary motor (M1) cortex. Field potentials were recorded from M1 cortex and the ipsi-lateral hippocampus. B: an intracortical microstimulation trial to M1 cortex consisted of 40 regularly timed bursts of electrical stimuli, with an interburst period of 150 ms (*bottom*), that was interrupted halfway through by a 100-ms stimulation to NBM (*top*).

a total axial separation distance of 1 mm, with the deepest electrode positioned in dentate gyrus and the most shallow in CA1. The common electrical reference electrode was a single 50- μ m-diam teflon-coated tungsten wire, with 1 mm of insulation removed near the tip, which was placed in the contralateral occipital lobe. We measured the local field potential (LFP) at each wire and report the numerically computed differential measurement, or current source density (Freeman and Nicholson 1975), across the three wires, denoted as the ∇^2 LFP. It is estimated as ∇^2 LFP $\cong -[V(z + \Delta z, t) - 2V(z, t) + V(z - \Delta z, t)]/\Delta z^2$, where V(z) is the measured field potential in each of the three wires and $\Delta z = 500 \ \mu$ m.

The cortical recording electrode consisted of a pair of $50-\mu$ m-diam teflon-coated tungsten wires (A-M Systems) that were held by adhesive in the same tube as the M1 cortical stimulation electrodes. The tips were separated so they spanned a lateral distance of 1 mm. The recording electrode was adjusted to lie about 1 mm above and to the side of the stimulation electrode, so that it measured from the top of M1 cortex while the stimulating electrode was in layer 5. The reference for the cortical recording leads was the same as for the hippocampal leads. We measured the LFP at each wire and report the numerically computed differential measurement across the pair, similar to the case for the ∇ EMG. This cortical signal is referred to as the electrocorticogram (ECoG).

Surgery

Aseptic surgery was performed with the rat anesthetized with ketamine (0.05 mg/g body weight) and xylazine (0.015 mg/g body weight), injected intraperitoneally. The head was held in a stereotaxic frame. The EMG electrodes were implanted first. Then, ~1-mm-diam holes were drilled through the skull, and the dura mater was carefully removed. The combined M1 cortical stimulation and recording array was implanted at the coordinates (A-P, M-L, and D-V = 2.0, 1.5-2.0,and 1.0-1.2 mm, respectively), which is part of the retraction area (Brecht et al. 2004; Haiss and Schwarz 2005), with the two tips offset along the anterior-posterior axis. The medial-lateral coordinates correspond to the nominal depth of layer V in vibrissa M1 cortex. The gross placement was tested and confirmed by ICMS (Asanuma 1989) and, on successful placement, this electrode was permanently cemented (1330 Ortho-Jet, Lang Dental, Wheeling, IL) to screws (00-90-1/8', Small Parts) placed nearby in the skull. The hippocampal recording electrodes were implanted at the coordinates (-3.8, 2.0, 3.0)mm) and secured to a neighboring screw and the reference electrode for recording was implanted at coordinates (-6, 1, 0-1 mm) and secured. Last, the NBM stimulating electrode was implanted at the coordinates (2.1, -1.2, -6.9 mm) and secured to a neighboring screw. The placement was confirmed post hoc by histological analysis. All screws were connected to a common ground with 0.010-in uncoated silver wire. All electrode leads were soldered (Stay Clean Flux, Harris Co., Cincinnati, OH) to miniature 10-pin connectors (2-mm, Samtec, New Albany, IN) in mounts of local design.

Training

The animals were allowed to recover for 5 days after surgery. They were gentled and trained to walk on a raised platform, as previously described (Berg and Kleinfeld 2003a). Data from intact animals was collected on a daily basis for a period of 2 wk beginning \sim 5 days after recovery from surgery.

ICMS

The pair of stimulating electrodes in vibrissa M1 cortex were connected to a bipolar, constant-current stimulation unit (no. 2100, A-M Systems). Stimuli consisted of a train of 35 bursts; the individual bursts consisted of five uniphasic pulses that were 100 μ s in duration

and spaced 2 ms apart (Donoghue and Sanes 1988; Donoghue and Wise 1982; Miyashita et al. 1994; Neafsey et al. 1986; Weiss and Keller 1994). The period between each burst was 150 ms, and a train of 40 bursts was used (Fig. 2B). The magnitude of the current adjusted for a minimal superthreshold value, typically 50–70 μ A, the standard threshold for behavioral responses (Tehovnik 1996). Studies on ICMS in vitro suggest that the primary mode of neuronal activation is through axonal as opposed to somatic activation (Nowak and Bullier 1998, 1996). The spatial extent of activation is set by the bipolar electrode spacing, i.e., 500 μ m, and is presumed to include projection axons in addition to axons to collateral cells.

Visual inspection insured that only the vibrissae, and not the limbs, neck, or other facial structures, moved in response to stimulation. However, fibers of passage to and from nonmotor areas could be activated. The stimulation sequence was repeated 50 times in a recording session: 40 times with NBM stimulation and 10 times without NBM stimulation to serve as control.

NBM stimulation

The NBM was stimulated halfway through each ICMS sequence to assess the impact of stimulation of NBM on vibrissa movement (Fig. 2*B*). The stimulation consisted of 50 uniphasic pulses that were 200 μ s in duration and spaced 2 ms apart. The magnitude of the current was adjusted to a value that would produce cortical activation (Buzsaki et al. 1988; Detari et al. 1999; Sarter and Bruno 2000), as assayed by desynchronization of cortical large-amplitude irregular activity in the sessile animal. This value was typically 100 μ A. In control experiments, only the NBM and not M1 cortex was stimulated.

Data collection

Mystacial EMG activity and cortical LFP activity were impedance buffered, amplified, filtered, sampled with electronics of local design (Fee et al. 1996; Ganguly and Kleinfeld 2004), and then numerically processed as described (Berg and Kleinfeld 2003a; Ganguly and Kleinfeld 2004). Videographs of the motion of the caudal row of vibrissae, acquired at 100 frames/s (MegaPlus ES310, Roper Scientific MASD, San Diego, CA), were obtained when the animal craned from a perch in search of a food tube, as described previously (Berg and Kleinfeld 2003a).

Atropine controls

One set of control experiments involved the systemic subcutaneous administration of atropine sulfate (0.05 mg/kg body weight), which is known to cross the blood-brain barrier (Maalouf et al. 1998) and to exert competitive inhibition of the muscarinic cholinergic receptors. Data were recorded with the ICMS paradigm \sim 20 min after the bolus injection of atropine.

A second set of control experiments was performed with animals in the anesthetized state and involved the topical application of atropine to cortex. Atropine sulfate was mixed in an artificial cerebral spinal fluid solution to a concentration of 150 μ M (Juliano et al. 1990). The rat was anesthetized with ketamine (0.05 mg/g) and xylazine (0.015 mg/g), injected intraperitoneally, and placed in stereotaxic frame. A craniotomy was prepared over vibrissa M1 cortex (Kleinfeld et al. 2002) with nominal coordinates (A-P and M-L = 0-5 and 1-3 mm, respectively). Stimulating electrodes were placed, using stereotaxic coordinates, in M1 cortex and NBM, as in the chronic surgery, except that we used where tungsten electrodes (WE300325A, Micro Probe). The mystacial EMG was recorded and the vibrissa deflection was measured using a magneto-resistive probe and a small rare earth magnet (mass < 1 mg) glued onto the C2 vibrissa as described previously (Berg and Kleinfeld 2003a). The atropine solution was dripped onto the exposed motor cortex and was presumed to reach layer V by diffusion (Juliano et al. 1990). Prior experiments with topical application of ionic solutions (Diamond et al., 1992) or dye solutions (Delaney and Kleinfeld 1996) show that there is negligible flow across the unexposed cortical surface.

State of arousal

The state of arousal was quantified in terms of the spectral components in the hippocampal $\nabla^2 LFP$. The awake and aroused state was equated with 5- to 10-Hz rhythmic activity, i.e., θ -band activity (Green and Arduini 1954; Vanderwolf 1969). The sessile state was equated with large-amplitude irregular activity, generally in the 1- to 5-Hz range or δ -band. In all cases, the power spectra were estimated from 3-s recordings obtained before the onset of intracortical microstimulation. Spectral power was estimated with the multitaper methods of Thompson (Percival and Walden 1993) using five Slepian tapers, for a spectral bandwidth of 1.7 Hz.

Histology

At the end of each recording session, animals were deeply anesthetized and perfused with phosphate buffered saline (PBS; Sigma, St. Louis, MO), followed by 4% (wt/vol) paraformaldehyde in PBS. The brains were removed, postfixed in 4% (wt/vol) paraformaldehyde in PBS for 1 to 7 days, and cryoprotected by equilibration with 30% (wt/vol) sucrose in PBS blocked in the vicinity of both the M1 cortex and NBM electrode tracks, and sectioned at a thickness of 50 μ m on a freezing/sliding microtome. The sections were stored serially in PBS. Sections for immunostaining were chosen to include levels at and around the electrode track as determined from microscopic inspection of wet mounts. To localize the cholinergic neurons in the NBM, sections were incubated for 2 days in solution of a polyclonal antibody that was selective for choline acetyl-transferase (Chemicon, Temecula, CA). A 1:10,000 (vol/vol) dilution of primary antibody was diluted in a diluent, comprised of 10% (vol/vol) goat serum (Vector, Burlingame, CA) and 2% (vol/vol) triton X-100 detergent (Fisher, Pittsburgh, PA) in PBS. Sections were washed in PBS for 1 h and transferred to biotinylated anti-rabbit secondary antibody (Chemicon; 1:2,000 dilution in diluent) for a 2-h incubation. The bound secondary antibody was visualized with the avidin-biotin kit (Vector) followed by a diaminobenzidine substrate kit (Vector) according to the manufacturer's instructions. Sections were mounted onto gelatincoated slides, 1% (wt/vol) in distilled water, and dehydrated through graded alcohols into xylenes for coverslipping with DPX mountant (44581, Fluka, Buchs, Switzerland). Selected sections from each

FIG. 3. Results of NBM stimulation with the initial state of the animal awake and sessile. A: sample trace of typical \bigtriangledown^2 LFP recording from hippocampus, acquired before ICMS onset. Scale bar is 100 μ V. B: power spectrum of the trace from A, calculated as an average over 5 independent estimates of the spectra (1.3-Hz bandwidth). C: sample trace of the mystacial \bigtriangledown EMG (*top*) evoked by ICMS to M1 cortex (*middle*) before and after NBM stimulation (*bottom*). Note straight line that sets the 0 level. Scale bar is 200 μ V. D and E: average (n = 5) ICMS-evoked \lor EMG before and after NBM stimulation, respectively. Scale bar applies to both panels and is 100 μ V. F: \lor EMG (low-pass filtered for clarity) before and after NBM stimulation in the absence of ICMS to M1 cortex. The 3 traces are the trial averaged results (n = 30) for 3 different animals. Scale bar is 200 μ V. G and H: average integrated prompt response (G, *inset*) and delayed response (H, *inset*) following 500 ms after NBM stimulation, normalized and offset by the average integrated response before NBM response. Black columns represent response to NBM stimulation, and gray columns represent control data where NBM was not stimulated. *Significant (P < 0.05) increase in response to NBM stimulation.



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animal were photographed under bright-field illumination at low magnification.

RESULTS

Data were obtained from awake animals, prepared with EMG recoding electrodes in the intrinsic muscles, ICMS stimulation electrodes in M1 cortex, stimulation electrodes in NBM, and ECoG recording electrodes in M1 cortex (Fig. 2A). Our experimental paradigm was to apply rhythmic ICMS to vibrissa M1 cortex at a frequency of 7 Hz, which has been shown to evoke fictive rhythmic whisking (Berg and Kleinfeld 2003b; Haiss and Schwarz 2005). Halfway through the stimulation of M1 cortex, we transiently activated NBM. This allowed us to assay the effect of NBM activity on cortical control of whisking (Fig. 2*B*). The overall state of arousal of the animal before the onset of stimulation was assessed by the relative power in the hippocampal theta band for a 3-s period before the onset of ICMS to vibrissa M1 cortex. Trials were sorted according to sessile versus aroused states (Fig. 1*C*).

We consider first the case of trials for which the animal was in the awake/sessile state (Fig. 3, A and B). A typical evoked response consisted solely of a prompt response in the mystacial \forall EMG (Fig. 3, C and D). The corresponding vibrissa movement was retraction, in agreement with past studies (Berg and Kleinfeld 2003b). However, brief activation of NBM was observed to quantitatively and qualitatively change the ICMSevoked ∇ EMG: the prompt response was increased in amplitude and a delayed component appeared (Fig. 3, C and E). This delayed component represented an active forward sweep of the vibrissa (cf. Fig. 1, E and F). The stimulus- and trial-averaged difference in vibrissa response to ICMS before and after activation of the NBM highlights the impact of NBM activation on motor output (Fig. 3, D and E). Last, brief activation of NBM in the absence of ICMS to M1 cortex often led to a slight shift in the set-point of the vibrissa position, as shown for three animals in Fig. 3F, and may further lead to head turning or other orienting behavior. Brief activation of NBM in the absence of ICMS never led to whisking (n = 3 animals), in accord with early studies (Vanderwolf 1968).

As a means to quantify the vibrissa response across trials and animals, we consider metrics that are based on the integrated \forall EMG response. The prompt response for a given ICMS is defined as the integrated value of the \forall EMG within the interval from 10–30 ms after the onset of the ICMS (Fig. 3*G*, *inset*)

$$\int_{\text{prompt}} dt \,\,\nabla \text{EMG} \equiv \int_{10 \,\,\text{ms}}^{30 \,\,\text{ms}} dt [\nabla \text{EMG}(t) - \min_{t} \{\nabla \text{EMG}(t)\}]$$

where $\min_{t} \{\nabla \text{EMG}(t)\}$ is a constant that represents the level of background activity, e.g., the set-point of the vibrissae, and is uncorrelated with ICMS-evoked whisking. The delayed response is defined as the integrated value of the ∇EMG from 30 to 150 ms after the onset of the ICMS (Fig 3*H*, *inset*)

$$\int_{\text{delayed}} dt \,\,\nabla \text{EMG} \equiv \int_{30 \,\,\text{ms}}^{150 \,\,\text{ms}} dt [\nabla \text{EMG}(t) - \min_{t} \{\nabla \text{EMG}(t)\}]$$

The above metric provides a value for the ICMS-evoked response to an individual stimulus. To compute the average

change in the integrated \forall EMG that is induced by NBM activation, we average this metric over individual stimuli and trials for each animal

< Change in ICMS-evoked \forall EMG >

$$=\frac{1}{N}\sum_{N \text{ trials}}\frac{\frac{1}{5}\sum_{ICMS \ 2I-25}\int_{prompt}dt \ \nabla EMG - \frac{1}{20}\sum_{ICMS \ I-20}\int_{prompt}dt \ \nabla EMG}{\frac{1}{20}\sum_{ICMS \ I-20}\int_{prompt}dt \ \nabla EMG} \times 100\%$$

for the prompt response, with an equivalent expression for the delayed response. The number of trials, N, varied between 20 and 40 per animal, and we used trials obtained over the course of a single day. In terms of this average, we observed that activation of NBM led to a large increase in the amplitude of the prompt ∇ EMG response in seven of seven animals (Fig. 3G, black bars) and to the appearance of a significant delayed response in the ∇ EMG in six of seven animals (Fig. 3*H*). The significance of this increase was judged by two criteria. First, the increase was required to exceed a standard (P < 0.05) confidence level above zero, i.e., no change. Second, the increase was required to exceed the standard confidence level above the value for changes in the ICMS-evoked response that were related to the brief pause in ICMS pulses that occurs during the activation of NBM (interval between pulses 20 and 21; Fig. 2B). In terms of these criteria, activation of the NMB led to a significant change in the prompt component of the ∇ EMG response in 100% of the animals the sessile state (cf. black vs. gray bars; Fig. 3G) and a significant change in the delayed component of the ∇EMG response in 86% of these animals (Fig. 3H).

We now turn to the case of trials for which the animal was initially in an awake/aroused state (Fig. 4, A and B). As expected, and unlike the case of sessile animals, the vibrissa response to ICMS now showed a small but significant delayed component (cf. Figs. 4C and 3D). Interestingly, stimulation of NBM led to an increase in the amplitude of both the prompt and delayed components of the ∇EMG (cf. Fig. 4, C and D). In terms of the above criteria for significance, activation of the NBM led to a significant change in the prompt component of the ∇ EMG response in 86% of the animals in the aroused state (cf. black vs. gray bars; Fig. 4*E*) and a significant change in the delayed component of the ∇ EMG response in 100% of these animals (Fig. 4F). We conclude that, across multiple behavioral states, activation of NBM leads to enhanced control of both the amplitude (increased prompt response; Figs. 3F and 4F) and motion sequence (onset of delayed response; Figs. 3G and 4F) of whisking by vibrissa M1 cortex.

We now consider a series of control experiments aimed to delimit the specificity of NBM activation in facilitating ICMSevoked vibrissa motor output. We consider first the case of an awake but sessile animal for which the ECoG shows large, irregular activity (Fig. 5A). Electrical stimulation of NBM results in a transient suppression of this activity and the onset of fast activity with lower amplitude (Fig. 5A). Similar behavior occurs for the hippocampal ∇^2 LFP. These data are consistent with the classic, desynchronizing action of NBM (Metherate et al. 1992; Vanderwolf 1990) and the transient nature of



FIG. 4. Results of NBM stimulation with the initial state of the animal awake and aroused. A: sample trace of typical ∇^2 LFP recording from hippocampus, acquired before ICMS onset. Scale bar is 100 μ V. B: power spectrum of the trace from A, calculated as average over 5 independent estimates of the spectra (1.3-Hz bandwidth), where the units are arbitrary but the same as in Fig. 2B. C and D: average (n =5) ICMS-evoked ⊽EMG before and after NBM stimulation, respectively. Scale bar applies to both panels and is $100 \ \mu V$. E and F: average integrated prompt response and delayed response after NBM stimulation, normalized, and offset by the average integrated response before NBM response. Black columns represent response to NBM stimulation, and gray columns represent control data where NBM was not stimulated. *Significant (P < 0.05) increase in response to NBM stimulation.

< Change in ICMS-evoked \forall EMG > _{per trial}



this action (Buzsaki and Gage 1989; Vanderwolf 1968). The transient, 1- to 3-s duration for NBM-induced desynchronization of the ECoG (Fig. 5A) defines a temporal window that the enhancement of the prompt and delayed components of the ICMS-evoked \forall EMG is expected to follow.

To compare the evolution of the ICMS-evoked \forall EMG with the time-course of cortical desynchronization, we defined a measure of the average change in the ICMS-evoked vibrissa \forall EMG on a per trial basis



We observed that for both the prompt (Fig. 5*B*) and the delayed (Fig. 5*C*) components of the vibrissa \forall EMG, the increase in

FIG. 5. Time-limited effect of NBM stimulation. A: hippocampal \bigtriangledown^2 LFP (*top*) and electrocorticogram (ECoG; *bottom*) from ipsilateral M1 cortex in a representative animal in the sessile state. Both the hippocampal activity and ECoG consist of large amplitude irregular activity that is eliminated after NBM stimulation (70- μ A maximum current), indicated by a vertical black line. Hippocampus \bigtriangledown^2 LFP shows *θ*-activity after stimulus. Change in activity over 3 s, equal to the extent of 20 ICMSs delivered to M1 cortex. Vertical scale bars are 500 μ V. *B* and *C*: integrated prompt response (*B*) and delayed response (*C*) after NBM stimulation as a function of ICMS number (Fig. 1*C*), normalized and offset by the average integrated response to NBM stimulation, and gray traces represent response to NBM stimulated.

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FIG. 6. Physiological and histological controls for NBM stimulation. A and B: sample traces from 2 different animals, with effective stimulation (A) and ineffective stimulation (B). Traces show hippocampal $\nabla^2 LFP$ activity (top) and the M1 ECoG (bottom) for 2 extreme states: sessile (left columns; NBM stimulation is indicated by vertical lines) and aroused (right columns). In the animal used for the data in A, stimulation through the NBM electrode induced desynchronization in the ECoG and θ -rhythm in the hippocampus (Effective). In the animal used for the data in B, stimulation through the NBM electrode had no effect (Ineffective). Vertical scale bars for all panels are 200 μ V. C and D: average (n = 5) ICMS-evoked ∇ EMG before and after stimulation through the NBM electrode, respectively. Scale bars in both panels are 50 μV . E: localization of ChAT immunoreactive neuronal cell bodies at the level of the NBM. Scale bars are 500 µm. The drawings are a composite of the electrode tracks from 14 animals: 8 with effective (solid symbols) and 6 with ineffective (open symbols) NBM electrodes. T, electrode tract; V, 3rd ventricle, which is centered at the midline.

the average ICMS-evoked motor response decayed to baseline values with the \sim 1-s time-course seen for the decay of cortical desynchronization. Last, the concurrence in recovery from the effects of NBM stimulation was observed across all animals (n = 7).

A second control exploited a population of animals in which the electrodes implanted as a means to activate NBM failed to do so. For these rats, electrical stimulation did not elicit desynchronized cortical activity or a change in the hippocampal ∇^2 LFP (cf. Fig. 6, A and B). Concomitant with the failure of these electrodes to induce cortical desynchronization was the failure to change the ICMS-evoked vibrissa response (cf. Fig. 6, C and D). We treated this population of animals as surgical controls for the placement of stimulating electrodes relative to cholinergic neurons. After the end of recording, the rats were perfused and brain slices were stained with antibodies to choline acetyl transferase, an enzyme in the acetylcholine synthetic pathway. Large cholinergic somas were observed in close vicinity of the vestige of the electrode tips in animals with effective NBM stimulation (Fig. 6E, solid symbols). Such cells were more distal in animals with ineffective NBM stimulation (Fig. 6*E*, open symbols). A map of successful (n = 8)and unsuccessful (n = 6) stimulating locations indicates that successful sites were either close to or directly overlapped with the territory of cholinergic somata in the nucleus basalis (Paxinos and Watson 1986).

A final group of control experiments used the administration of atropine sulfate, which is known to exert competitive inhibition on muscarinic cholinergic receptors and to cross the blood-brain barrier (Maalouf et al. 1998) in an attempt to reversibly block the effect of cholinergic activation of cortex by NBM. We considered first the case of a systemic block in the awake but sessile animal. Before the administration of atropine, electrical stimulation of NBM results in a suppression of the ECoG activity and the onset of fast activity with lower amplitude (Fig. 7A). Similar behavior occurs for the hippocampal ∇^2 LFP (Fig. 7A). After systemic administration of atropine, desynchronization was no longer observed to follow NBM stimulation, even with multiple stimuli (Fig. 7B). To assess the block of NBM activation on the ability of M1 cortex to control vibrissae motion, we applied our ICMS paradigm before and after the administration of atropine. Before atropine administration, the ICMS-evoked VEMG response showed the expected increase in both prompt and delayed components (cf. Figs. 7C with 3D and 4C). After systemic administration of atropine, there was a much weaker or negligible change in the ICMS-evoked \forall EMG in response to NBM stimulation (Fig. 7D). The atropine block was reversed when animals were



FIG. 7. Control experiment with systemic atropine. A: hippocampal ∇^2 LFP activity (top) and M1 ECoG activity (bottom) during the sessile state. Vertical lines represents NBM stimulation (70 μ A). Stimulation is able to change activity in M1 cortex and hippocampus. Vertical scale bars are 400 μ V. B: after systemic administration of atropine, several pulses of stimulation of NBM at higher current (90 μ A) are not enough to cause similar change in activity. C and D: average (n = 5) ICMS-evoked VEMG before and after NBM stimulation just before (C) and 30 min after (D) the systemic administration of atropine with the animals in the aroused state. Vertical scale bar for both panels is 50 μ V. E and F: average integrated prompt response (E) and delayed response (F) after NBM stimulation, normalized and offset by average integrated response before the NBM response. Black columns represent response before administration of atropine, and gray columns represent data taken 30 min after administration of systemic atropine. *Significant (P < 0.05) decrease in response after atropine administration. Note that the control data for the change in the absence of NBM stimulation, for the case of no atropine, is shown in Fig. 3, E and F (gray bars).

tested on the following day. In toto, atropine administration was performed for six animals. Of these, 100% showed a significant decrement of NBM-evoked changes in the prompt component (Fig. 7*E*), and 67% showed a significant decrement for the delayed component (Fig. 7*F*).

Systemic application of atropine sulfate could have its main effect outside of vibrissa M1 cortex. As a means to localize the cholinergic block to M1 cortex, we used an acute preparation of anesthetized animals for which we could apply atropine topically through a craniotomy above vibrissa M1 cortex (Juliano et al. 1990). The NBM stimulating electrode was implanted, as in the case for chronic animals, but the animals were held in a stereotaxic and the stimulating electrode for vibrissa M1 cortex was held by a micromanipulator. With this set-up, ICMS-evoked movement of vibrissa position was measured directly with a magnetic probe rather than EMG electrodes (Fig. 8A, inset). This probe was fairly linear for deflections with a magnitude $<5^{\circ}$ and would underestimate larger deflections (Fig. 8A). A sample trace of the ICMS-evoked response before and after NBM stimulation shows a clear enhancement of the prompt signal by NBM stimulation, either

on the stimulus-by-stimulus response (Fig. 8*B*) or in the stimulus-triggered average of the first five responses (Fig. 8*C*). Thus the anesthetized preparation captures one of two features of the EMG, i.e., the prompt but not the delayed response, which are augmented by NBM stimulation. This enhancement was eliminated within 10 min after the topical application of 150 μ M atropine (Fig. 8, *B* and *C*). As in the case of awake animals (Fig. 5, *B* and *C*), the effect of NBM activation on the ICMS-evoked response waned after 1 s (Fig. 8*D*). Last, as a compilation across three control experiments with anesthetized animals, the average NBM enhancement exceeded 100% and was completely blocked by the topical application of atropine (Fig. 8*E*).

DISCUSSION

We have shown, in awake rats, that the activation of the NBM enhances the ability of motor cortex to initiate a sequential motor task. Our assay made use of ICMS of vibrissa M1 cortex (Fig. 3, D-G), which leads to full whisking motion for animals in the aroused state, but only weak retraction for



FIG. 8. Control experiment with topical application of atropine to M1 cortex. A: calibration curve for the magnetic probe used to measure the angular deflection of vibrissa C2. A small rare earth magnet (mass < 1 mg) attached 1 cm from the base of the vibrissa. Voltage output from the probe is shown on ordinate and the angular deflection on the abscissa. It has a linear range for deflections of approximately $\pm 6^{\circ}$. B: ICMS-evoked deflection of vibrissa C2 before and after the NBM was stimulated under normal conditions (top) and 10 min after topical application of 150 μ M atropine sulfate to M1 cortex (bottom). NBM stimulation is indicated by a black mark. C: stimulus triggered average deflection before (gray line) and after (black line) NBM stimulation from the data in B. D: average change in deflection after NBM stimulation as a function of ICMS number (Fig. 1C), normalized and offset by the average deflection before NBM response. Black curves represent the response to NBM stimulation; dark gray curves represent control data where NBM was stimulated in the presence of topical atropine. E: change in deflection, averaged over the ICMS numbers 21-25 and averaged over 40 trials, for the 3 conditions in D.

animals in the sessile state (Berg and Kleinfeld 2003b). The magnitude of both prompt and delayed electromyogram (\forall EMG) components, generators of retraction and protraction, respectively (Fig. 1, *E* and *F*), were increased by activation of NBM (cf. Fig. 3, *D* with *E* and Fig. 4, *C* with *D*). The effect is particularly dramatic for animals that were initially in the sessile state (cf. Fig. 3, *D* and *E*), where the delayed component rises from the baseline and results in a conversion from an ICMS-triggered retraction to a full sequential motion of retraction followed by protraction (Fig. 3*C*). These data define a proof-of-principle for the involvement of NBM in the execution, i.e., the nonlearning phase, of a motor task.

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A signature of cortical arousal that is strongly correlated with NBM activation is the desynchronization of the ECoG (Green and Arduini 1954; Steriade et al. 1990a). This desynchronization diminished over a 1- to 3-s period of time after the offset of NBM stimulation (Fig. 5A). Of interest, we observed that the NBM-induced facilitation of whisking also diminishes over the same period of time (Fig. 5, *B* and *C*). This suggests that enhanced control of whisking that follows activation of NBM stems from the same neuronal mechanisms that produce widespread desynchonization of the ECoG. Furthermore, a cortical locus for the effect of NBM stimulation (Johnston et al. 1981; Rye et al. 1984) is supported by the lack of innervation of projections from the NBM to hindbrain regions (Hattox et al. 2002), which includes the vibrissa motoneurons in the facial motor nucleus (Klein and Rhoades 1985).

The systemic administration of atropine sulfate is a commonly used procedure to block cholinergic activation of sensory areas in cortex (Maalouf et al. 1998), such as occurs after NBM stimulation in awake and sessile animals. We observed in the presence of atropine that NBM stimulation fails to produce cortical desynchronization, completely abolishes the facilitation of the ICMS-induced prompt \forall EMG response, i.e., retraction of the vibrissa, and largely abolishes the ICMS-induced delayed \forall EMG response, i.e., protraction of the vibrissa (Fig. 7). Nucleus basalis has both cholinergic projections to cortical pyramidal cells (Gritti et al. 1997) and GABAergic inputs to stellate cells (Freund and Meskenaite 1992). This systemic atropine control experiment highlights a predominant role for the cholinergic component of nucleus basalis in the cortical control of normal whisking. The small remaining effect of NBM stimulation in the presence of systemic atropine may result from the GABAergic disinhibition of cortical inhibitory interneurons.

To further circumscribe the subset of cortical projections from NBM necessary for the facilitation of the ICMS-induced vibrissa response, an additional control experiment made use of anesthetized rats as a means to localize the atropine block directly to vibrissae M1 cortex (Fig. 8). In anesthetized animals, activation of NBM substantially facilitates the prompt (retraction) vibrissa response (Fig. 8, B and C). We observed that topical application of atropine to vibrissa M1 cortex blocks facilitation of the prompt response by NBM stimulation (Fig. 8, B, C, and E). This result shows that specific cholinergic activation of vibrissa motor cortex is necessary for the facilitation of whisking output. We suggest that the effect of NBM stimulation consists mostly of a cholinergic modulation of putative pyramidal cells in motor cortex (Buzsaki et al. 1988; Metherate and Ashe 1993). The nature of the cortical signal for the control of sequential protraction and retraction in whisking, as well as whether the locus of the control resides in M1 cortex and/or in downstream subcortical circuits, remains to be determined.

It remains an open issue as to whether NBM plays an obligatory role during normal motor function. Particularly specific means to lesion cholinergic NBM cells involve local injections of either immunotoxin saporin-192, which is specific for basal forebrain cholinergic cells or AMPA-type glutamate receptor excitotoxins (Everitt and Robbins 1997). The data of Gharbawie and Whishaw (2003) and Connor et al. (2003) show that lesioned animals can complete a forelimb-based retrieval task with normal efficiency. However, the forelimb movements involved in the task are abnormal in animals with lesions (Gharbawie and Whishaw 2003). Other studies find deficits in beam-walking (Galani et al. 2002), walking on an inclined screen (Waite et al. 1995), and swimming (Berger-Sweeney et al. 1994; Dornan et al. 1997) after bilateral lesions and the occurrence of circling after unilateral lesions (Gharbawie and Whishaw 2003). The studies of Santos-Benitez et al. (1995) and Richardson and DeLong (1990) show that NBM is active during motor movement. However, there is an equal increase in NBM activity during movement or the absence of movement in the choice phase of a Go/No-Go task (Richardson and DeLong 1990). The increase during the choice phase may be explained by the presence of reward, which itself correlates with heightened activity in NBM (Richardson and DeLong 1991).

The molecular basis of cholinergic activation divides into rapid effects, which dominate for the first $\sim 1-10$ ms after the onset of acetylcholine release, and prolonged effects, which have delayed onset but persist from ~ 100 ms to 10 s after acetylcholine release (McCormick 1993). On short time scales, acetylcholine activates ionotropic nicotinic receptors and leads to an augmentation of glutamine-based excitatory transmission, a suppression of GABA-mediated inhibitory transmission (Rovira et al. 1983), and possibly direct excitation of GABAergic interneurons (Alkondon et al. 1997; Jones and Yakel 1997). The consequence of these actions on network dynamics is presently unresolved. On long time scales, of particular relevance to this study, acetylcholine binding to metabotropic muscarinic receptors leads to the activation of currents through G protein pathways. Multiple K⁺-currents are suppressed. These include the M-type currents (Adams et al. 1982), which are defined by the KCNQ2/3 family of channels (Shapiro et al. 2000; Wang et al. 1998), the functionally similar currents defined by the *ether-a-go-go* receptors (Stansfeld et al. 1996), the small-conductance calcium activated potassium current (Madison et al. 1987), and the A-type voltage-gated potassium current (Nakajima et al. 1986). The suppression of K⁺-currents is consistent with a heightened likelihood of neuronal activation. However, the K⁺ inward rectifier is enhanced (Kofuji et al. 1995), the noninactivating Na⁺-current is suppressed (Mittmann and Alzheimer 1998), and the high voltage-gated calcium currents, which are essential to synaptic transmission, are suppressed (Haley et al. 2000). All of these latter effects are consistent with a decrease in network activity. One interpretation of the net reduction in both inhibitory and excitatory currents is that activation of muscarinic receptors leaves the average transmembrane potential roughly constant, or even slightly elevated, but sharply decreases the variance of the transmembrane potential. This is consistent with observations on the effect of stimulation of nucleus basalis on the intracellular potential of cortical neurons (Metherate and Ashe 1993). We conjecture that a decrease in variance, coupled with enhanced neuronal excitability (Hasselmo and McGaughy 2004), may provide the means for the sequential neuronal processing to emerge as a network phenomena.

Complementary neuromodulatory systems may subsume the role of acetylcholine in the face of cholinergic depletion or blockade. In particular, serotonin has been implicated in this role. It is established that depletion of cholinergic input leads to synchrony of cortex (Buzsaki et al. 1988; Dringenberg and Vanderwolf 1998; Steriade et al. 1990b). However, cortical desynchrony still occurs during movement even in the presence of cholinergic block (Dringenberg and Vanderwolf 1998). Blocking both cholinergic and serotonergic modulation leads to a lack of synchronization in the ECoG regardless of motor activity as well as severe deficits in motor execution (Dringenberg and Vanderwolf 1998; Gharbawie and Whishaw 2003).

In summary, this study provides evidence that the cholinergic center of the basal forebrain has an essential modulatory role on the output from motor cortex and that it is able to significantly facilitate sequential motor output patterns. To the extent that the representation of an animal's sensory world in S1 cortex is enhanced by NBM activation, it is not surprising that the representation of an animal's motor world in M1 cortex is also enhanced by NBM activation. As a practical issue, the state of NBM activity should be considered in the use of a neuroprosthetic device whose control is based on the pattern of electrical activity in motor cortex. We hypothesize that the precision of these patterns may gain relevance and accuracy for describing limb motion when enhanced by activation of NBM.

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