Fast modulation of visual perception by basal forebrain cholinergic neurons

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The basal forebrain provides the primary source of cholinergic input to the cortex, and it has a crucial function in promoting wakefulness and arousal. However, whether rapid changes in basal forebrain neuron spiking in awake animals can dynamically influence sensory perception is unclear. Here we show that basal forebrain cholinergic neurons rapidly regulate cortical activity and visual perception in awake, behaving mice. Optogenetic activation of the cholinergic neurons or their V1 axon terminals improved performance of a visual discrimination task on a trial-by-trial basis. In V1, basal forebrain activation enhanced visual responses and desynchronized neuronal spiking; these changes could partly account for the behavioral improvement. Conversely, optogenetic basal forebrain inactivation decreased behavioral performance, synchronized cortical activity and impaired visual responses, indicating the importance of cholinergic activity in normal visual processing. These results underscore the causal role of basal forebrain cholinergic neurons in fast, bidirectional modulation of cortical processing and sensory perception.

The brain state of an animal is modulated on multiple timescales, from the daily sleep-wake cycle to task-dependent changes in vigilance over seconds or minutes. Different brain states are associated with distinct patterns of ensemble neural activity. For example, during quiet wakefulness the cortical local field potential (LFP) exhibits low-frequency, high-voltage oscillations, whereas during running or active sensing the LFP is desynchronized, characterized by high-frequency, low-voltage activity1-3. These states are controlled by internally generated signals2, but they strongly influence the processing of sensory inputs1-8.

The cholinergic neurons in the basal forebrain constitute an important component of the neuromodulatory system controlling brain states9-11. These neurons are known to be more active during wakefulness and rapid-eye-movement (REM) sleep than during non-REM sleep1-2. During wakefulness, cortical acetylcholine (ACh) concentration also changes in a task-dependent manner on the timescale of seconds13. However, the causal relationship between transient increases in cholinergic activity and behavioral performance remains to be demonstrated. Electrical stimulation of the basal forebrain in anesthetized animals causes desynchronization of cortical activity14,15, a hallmark of wakeful and alert brain states, and it enhances cortical responses to sensory stimuli14-16. However, electrical stimulation also activates numerous noncholinergic neurons in the basal forebrain10,11, making it difficult to discern the contribution of cholinergic neurons. Furthermore, anesthesia precludes behavioral measurements of sensory perception, and the observed neurophysiological effects may not faithfully reflect the function of basal forebrain activation in awake animals.

In this study, we tested the effects of selective manipulation of basal forebrain cholinergic neuron activity on cortical processing and visual perception in awake mice. We found that channelrhodopsin-2 (ChR2)-mediated activation of the cholinergic neurons improved visual discrimination of the animal and enhanced visual coding by cortical neurons, whereas archaerhodopsin (ARCH)- or halorhodopsin (HALO)-mediated inactivation of these neurons caused the opposite effects. Thus, basal forebrain cholinergic neurons potentially have a powerful role in rapid modulation of sensory processing in the awake brain.

RESULTS

We implanted an optic fiber in the basal forebrain of the transgenic mouse expressing ChR2-EYFP under the choline acetyltransferase (ChAT, a specific marker for cholinergic neurons) promoter17 (Fig. 1a,b and Supplementary Fig. 1). Immunohistochemistry confirmed a high degree of colocalization between ChR2-EYFP and ChAT (Fig. 1c; 97% of ChR2-positive (ChR2+) neurons were ChAT+, and 94% of the ChAT+ neurons expressed Chr2). Retrograde tracing with cholera toxin B also confirmed that many cholinergic neurons in the basal forebrain (including those in nucleus basalis, substantia innominata and horizontal limb of the diagonal band of Broca) project their axons to V1 (see Online Methods and Supplementary Fig. 2).

Cholinergic activation rapidly desynchronizes cortical LFP

To measure the effect of activating basal forebrain cholinergic neurons on brain state and visual processing, we used a silicon probe to...
record both LFP and spiking activity from all layers in V1 of awake mice that were head-restrained on a spherical treadmill (see Online Methods, Fig. 1a and Supplementary Movie 1). We found that light activation of basal forebrain cholinergic neurons reliably desynchronized cortical LFP by reducing the power at low frequencies (1–5 Hz), and increasing the power at high frequencies (60–100 Hz) (Fig. 1d; W_{13} = 105, P = 1.2 \times 10^{-5}, Wilcoxon signed-rank test, n = 14 mice). The effect occurred rapidly after laser onset (126 ± 21 ms, mean ± s.e.m.; see Online Methods) and returned to baseline 691 ± 45 ms after laser offset. This is in contrast to electrical stimulation of the basal forebrain, in which a 500-ms pulse induces desynchronization that lasts for 5–20 s (refs. 14, 15). Thus, basal forebrain cholinergic neurons can modulate cortical LFP on a sub–second-to-second timescale.

Interestingly, although activating basal forebrain cholinergic neurons caused no significant effect on running speed (Fig. 1e and Supplementary Fig. 3d; W_{13} = 32, P = 0.62, Wilcoxon signed-rank test, n = 14 mice), its effect on LFP depended on the behavioral state of the mouse immediately before laser onset. When the mouse was sitting still (‘no run’), the LFP often exhibited large-amplitude, low-frequency (≤5 Hz) oscillations (Supplementary Fig. 3a) characteristic of quiet wakefulness. Basal forebrain activation caused a strong reduction of the low-frequency activity but no clear change at high frequencies (Supplementary Fig. 3a–c). When the mouse was running, the baseline LFP showed less low-frequency activity, typical of active behavioral states, and basal forebrain activation caused a smaller reduction of low-frequency power but a clear increase at high frequencies. Such an interaction between optogenetic basal forebrain activation and the ongoing behavioral state could be due to additive effects between running- and laser-induced ACh release (for example, increase in high-frequency power may occur only at high ACh concentrations) or to interactions between ACh and other inputs to the cortex that are activated by running.

Enhancement of behavioral performance

Pharmacological enhancement of cholinergic transmission has been shown to increase attentional modulation and increase in high-frequency power may occur only at high ACh concentrations) or to interactions between ACh and other inputs to the cortex that are activated by running.

**Figure 1** Optogenetic activation of basal forebrain cholinergic neurons in awake mice. (a) Schematic illustration of experimental setup. (b) Fluorescence microscopy of basal forebrain cholinergic cells expressing ChR2 and EYFP. Asterisk indicates position of optic fiber; arrowheads indicate the posterolateral and anteromedial borders of basal forebrain (nucleus basalis). (c) ChAT immunohistochemistry in basal forebrain of a ChAT-ChR2-EYFP mouse. (d) Example LFP and running speed traces from three trials showing the effect of basal forebrain stimulation (blue bar). (e) Top, LFP spectra averaged from all experiments. Blue bar, laser stimulation. Power at each frequency was normalized by the baseline from three trials showing the effect of basal forebrain stimulation (blue bar). Middle, time course of desynchronization ratio normalized by baseline. Bottom, average running speed. Gray shading represents ± s.e.m. (For all panels, n = 14 mice, 30 trials per mouse). BF, basal forebrain.
In principle, the laser-induced behavioral improvement could be mediated by increased ACh release in the visual cortex or in higher brain areas such as the prefrontal cortex (PFC). To test the role of the visual cortex, we performed direct optogenetic activation of the cholinergic axons projecting to V1, a widely used approach in optogenetics to isolate the effect of a particular projection (for example, see refs. 13,31–33). Laser stimulation in V1 significantly reduced low-frequency LFP power (Supplementary Fig. 5, \( W(T) = 92, P = 0.01 \), Wilcoxon signed-rank test, \( n = 8 \) mice), similarly to the effect found in the somatosensory cortex. In mice trained to perform visual discrimination, V1 stimulation also significantly improved performance measured by \( d' \) (Fig. 2d,e). \( F_{\text{contrast}(2,10)} = 42.3, P_{\text{contrast}} = 6.5 \times 10^{-8}; F_{\text{interaction}(2,10)} = 2.0, P_{\text{interaction}} = 0.16 \); two-way repeated-measures ANOVA, \( n = 12 \), indicating that optogenetic stimulation within the visual cortex is sufficient to induce a behavioral improvement. Similarly to basal forebrain stimulation, the increase in \( d' \) induced by V1 stimulation was due to a significant increase in hit rate (Fig. 2e, \( F_{\text{contrast}(2,10)} = 54.9, P_{\text{contrast}} = 7.5 \times 10^{-3}; F_{\text{interaction}(2,10)} = 0.76, P_{\text{interaction}} = 0.76 \) with no significant change in false alarm rate (\( F_{\text{contrast}(1,1)} = 0.17, P_{\text{contrast}} = 0.69 \)). Unlike basal forebrain stimulation, however, V1 stimulation improved the performance at 20% and 40% contrasts but not at 100% contrast (Fig. 2d,e). This suggests that the effect of basal forebrain activation may consist of a V1-mediated perceptual improvement at low contrasts and a nonperceptual component through other pathways (for example, basal forebrain projection to the PFC) at high contrasts.

Figure 2 Optogenetic activation of basal forebrain cholinergic neurons or their axons in V1 improved visual discrimination. (a) Schematic illustration of behavioral task. (b) \( d' \) of an example ChAT-CHR2-EYFP mouse in laser-on and laser-off trials for an basal forebrain activation experiment (3 d, 1,048 trials). Error bars, s.e.m. (bootstrap). (c) Population average of \( d' \) (left) and hit and false alarm (FA) rates (right) in laser-on and laser-off trials for basal forebrain activation experiments (\( n = 18 \) mice). Error bars, s.e.m. (d) \( d' \) of an example mouse in laser-on and laser-off trials for a V1 stimulation experiment (3 d, 614 trials). Error bars, s.e.m. (bootstrap). (e) Population average of \( d' \) (left) and hit and false alarm rates (right) in laser-on and laser-off trials for V1 stimulation experiments (\( n = 12 \) mice). Error bars, s.e.m. BF, basal forebrain; FA, false alarm.

Of course, in principle even V1 stimulation could antidromically activate the basal forebrain neurons, which may also project their axon collaterals to other cortical areas. To assess the degree of overlap between the basal forebrain cholinergic neurons projecting to different areas, we performed a dual retrograde tracing experiment by injecting retrobeads of different colors to V1 and other cortical regions. We found very few basal forebrain cholinergic neurons projecting to both V1 and medial PFC or to both V1 and the primary auditory cortex (5% and 6% respectively; Supplementary Fig. 6). However, we found more basal forebrain cells projecting to both V1 and its neighboring higher visual areas (13%; Supplementary Fig. 6), leaving open the possibility that ACh release in higher visual areas could also have a role in the behavioral improvement caused by V1 optogenetic stimulation.

Modulation of V1 neuronal activity

To further investigate the neural mechanisms underlying the improved perception, we recorded the visual responses of neurons in all layers of awake mouse V1 (Fig. 1a). For the grating stimuli (same as those in the behavioral task, at 20%, 40% and 100% contrasts), activation of basal forebrain cholinergic neurons increased V1 firing rates across all contrasts (Fig. 3a–c, \( W_{(100)} = 1,029 \) and \( P < 0.001 \) for all contrasts, Wilcoxon signed-rank test, \( n = 101 \)). Basal forebrain activation also increased the spontaneous firing rate (0% contrast, \( P = 7.4 \times 10^{-4} \)), thus causing a shift in the baseline of the contrast response function.
Figure 4 Effects of basal forebrain activation on V1 neuronal responses to natural movies. (a) Example frames of natural movies. (b) Spike trains of a single unit in response to 30 repeats of a natural movie during control and basal forebrain activation conditions. (c) Spike trains of 15 simultaneously recorded single units in a single trial of natural-movie presentation. Correlation between neurons decreases in basal forebrain activation. (d) Fano factor in response to natural movies for all driven units (open gray circles), basal forebrain on versus control, measured at a bin size of 100 ms. Horizontal and vertical error bars indicate s.e.m., centered around the mean. (e) Population average of single unit versus multi-unit coherence with or without basal forebrain activation centered around the mean. (f) Bin size of 100 ms. Horizontal and vertical error bars indicate s.e.m., centered around the mean. (g) Population average of single unit versus multi-unit coherence with or without basal forebrain activation centered around the mean. (h) Bin size of 100 ms. Horizontal and vertical error bars indicate s.e.m., centered around the mean.

(Fig. 3c), similar to the effect of local application of an ACh agonist in V1 (refs. 10,11,28). We then tested whether these laser-induced increases in V1 responses can improve the discriminability between gratings of different orientations using a receiver operating characteristic (ROC) analysis (see Online Methods). Basal forebrain activation significantly increased the classification performance (Fig. 3d; \( F_{\text{basal forebrain on}}(1,31) = 6.3, P_{\text{basal forebrain on}} = 0.01; F_{\text{contrast}}(2,30) = 29.3, P_{\text{contrast}} = 1.1 \times 10^{-8}; F_{\text{interaction}}(2,30) = 3.7, P_{\text{interaction}} = 0.03; \) two-way repeated-measures ANOVA, \( n = 32 \) cells), further indicating that the laser-induced changes in V1 activity could at least in part explain the observed improvement in behavioral discrimination.

A previous study in anesthetized rats showed that electrical stimulation of basal forebrain can improve V1 coding of natural scenes by increasing the response reliability of individual neurons and decreasing the correlation between neurons15. Although the decreased correlation was shown to depend on muscarinic ACh receptors in the cortex, whether the improved response reliability was caused by cholinergic transmission was unclear. We thus tested the effects of optogenetic basal forebrain activation on V1 responses to natural stimuli in awake mice (Fig. 4a). We found that basal forebrain cholinergic activation caused significant increases in both the firing rate (\( W_{\text{basal forebrain on}} = 4,314, P = 0.002, \) Wilcoxon signed-rank test, \( n = 155 \)) and the trial-to-trial response reliability (Fig. 4b), as quantified by the decrease of Fano factor (Fig. 4d; \( W_{\text{basal forebrain on}} = 1,536, P = 2.2 \times 10^{-5}, \) Wilcoxon signed-rank test, \( n = 61 \)). These effects were not due to changes in eye movements induced by basal forebrain activation (Supplementary Fig. 7). Laser stimulation also caused a marked reduction of the coherence between each neuron and the other simultaneously recorded cells at low frequencies, for the responses to both natural movies (Fig. 4c; \( W_{\text{basal forebrain on}} = 1,696, P = 7.9 \times 10^{-15}, \) Wilcoxon signed-rank test, \( n = 155 \)) and drifting gratings (data not shown, \( W_{\text{drifting gratings}} = 49.4, P_{\text{drifting gratings}} = 2.0 \times 10^{-8}, \) two-way repeated-measures ANOVA, \( n = 40 \)), similar to the effects of ACh application in macaque middle temporal area35.

Local application of ACh receptor antagonists (500 \( \mu \)M atropine and 3 mM mecamylamine) in V1 significantly weakened laser-induced LFP desynchronization (Supplementary Fig. 8a,b; \( W_{\text{basal forebrain on}} = 41, P = 0.03, \) Wilcoxon signed-rank test, \( n = 8 \) mice), increase in neuronal firing rate (Supplementary Fig. 8c; \( W_{\text{basal forebrain on}} = 570, P = 0.007, n = 61 \)) and decrease in the coherence between neurons (Supplementary Fig. 8d; \( W_{\text{coherence}} = 967, P = 5.0 \times 10^{-10}, n = 110 \)), indicating that these effects are mediated by ACh release within V1. Interestingly, ACh receptor antagonists did not block the laser-induced decrease in Fano factor (Supplementary Fig. 8e; \( W_{\text{basal forebrain on}} = 1,011, P = 0.23, n = 58 \), consistent with the previous study with electrical stimulation15. Thus, specific activation of basal forebrain cholinergic neurons is sufficient to increase cortical response reliability, but this effect is probably mediated by basal forebrain projections to other circuits (for example, thalamic reticular nucleus)12,18,36.
Effects of basal forebrain cholinergic inactivation

Basal forebrain cholinergic cells have high tonic firing rates in awake animals1–3,12,18. We wondered whether optogenetic inactivation of these cells could impair cortical coding and visual perception. To selectively inactivate basal forebrain cholinergic neurons in mice, we crossed a ChAT-Cre driver line with aloxP-flanked (‘floxed’) ARCH-GFP and a floxed HALO-EYFP reporter line. Immunohistochemistry revealed high specificity and efficiency of ARCH and HALO expression (Fig. 5a; 93% and 96% of ARCH- and HALO-expressing cells were ChAT+ respectively, and 67% and 82% of ChAT+ neurons expressed the opsins). Because the effects of laser in ChAT-ARCH and ChAT-HALO mice were similar, they were combined in this study. Laser-induced inactivation of basal forebrain cholinergic neurons (Supplementary Fig. 1) led to a significant increase in V1 LFP power at low frequencies (1–5 Hz, $t_{(6)} = 2.8, P = 0.03$, paired t-test, $n = 7$) without affecting the power at high frequencies (60–100 Hz, Fig. 5b; $t_{(6)} = 0.89, P = 0.41$), opposite to the effect of ChR2-mediated basal forebrain activation. Low-frequency power began increasing at 430 ± 69 ms (mean ± s.e.m.) after laser onset and returned to baseline at 1.41 ± 0.13 s after laser offset. Basal forebrain cholinergic inactivation also decreased behavioral performance across all contrasts (Fig. 5c,d; $F_{(1,5),LFP} = 9.0, P_{LFP} = 0.03$; $F_{(2,4),contrast} = 11.1, P_{contrast} = 0.003$; $F_{interaction} = 0.16, P_{interaction} = 0.85$, two-way repeated-measures ANOVA; n = 6 mice). This effect significantly different from the control group (wild-type animals receiving blue laser stimulation, $F_{(1,13)} = 4.4, P_{LFP} = 0.04$, two-way ANOVA; n = 8 mice).

At the single-neuron level, basal forebrain inactivation significantly reduced the spontaneous firing rate and the responses to both drifting gratings (Fig. 6a,d; $W_{(40)} > 776$ and $P < 0.01$ for all contrasts, Wilcoxon signed-rank test, n = 47) and natural movies ($W_{(88)} = 3,099, P = 2.1 \times 10^{-6}$, n = 89). The responses to natural movies became less reliable (Fig. 6b,c; $W_{(41)} = 74, P = 2.4 \times 10^{-6}$, Wilcoxon signed-rank test, n = 42), and the low-frequency coherence between neurons increased for the responses to both natural movies (Fig. 6c,f; $W_{(88)} = 3,631, P = 2.7 \times 10^{-11}$, Wilcoxon signed-rank test, n = 89) and drifting gratings ($F_{(1,46)} = 13.0, P_{LFP} = 7.6 \times 10^{-4}$, two-way repeated-measures ANOVA, n = 47). Together, these experiments show that optogenetic manipulation of basal forebrain cholinergic neuron activity can bidirectionally modulate brain state and visual cortical processing.

**DISCUSSION**

Our results show that specific activation of cholinergic neurons in the basal forebrain is sufficient to enhance cortical processing and visual discrimination in awake animals, and this effect occurs rapidly on a sub–second-to-second timescale. Conversely, optogenetic inactivation of the cholinergic neurons impairs cortical responses and behavioral performance, indicating that the activity of these neurons is necessary for normal visual processing. Thus, the basal forebrain cholinergic neurons could have an immediate, powerful role in activating the cortex and improving sensory processing17,38.

Our results strongly suggest that the basal forebrain activation–induced perceptual improvement is mediated partly by the cholinergic projection to V1 (Supplementary Fig. 2). As shown in the ROC analysis, the basal forebrain–induced changes in single neuron responses significantly increased the perceptual limit of the ideal observer (Fig. 3d), and the decrease in inter-neuronal correlation (Fig. 4c,e) should further improve visual coding at the ensemble level19. Both the increase in firing rate and decrease in correlation depended on cholinergic actions within V1, as they were greatly diminished by local application of ACh receptor antagonists (Supplementary Fig. 6). Furthermore, direct optical activation of cholinergic terminals in V1 was sufficient to improve the behavioral performance at low contrasts (Fig. 2d,e), indicating substantial contribution of this pathway. Although in principle stimulation of axon terminals in V1 could cause antidromic activation of the basal forebrain neurons, our dual retrograde tracing experiment showed very little overlap between the basal forebrain cholinergic neurons projecting to V1 and to the PFC (Supplementary Fig. 6). This is consistent with a previous study showing that V1- and PFC-projecting neurons in the basal forebrain are segregated and that visual stimulation can evoke ACh release selectively in the visual cortex but not in the PFC27. Thus, even if the V1 stimulation evoked antidromic spiking of the basal forebrain neurons, it would be unlikely to cause cholinergic modulation of the higher brain areas such as the PFC. Of course, in addition to V1, the behavioral effects observed in the basal forebrain activation experiments (Fig. 2b,c) could also be mediated by cholinergic projections to other brain structures. For example, we found that the increase in response reliability of V1 neurons was not blocked by local application of ACh receptor antagonists. This is consistent with a previous finding that the increased response reliability induced by electrical stimulation of the basal forebrain also occurs in the lateral geniculate nucleus15, suggesting involvement of a basal forebrain projection to...
the thalamus. Furthermore, basal forebrain projections to higher visual cortices and to other brain areas involved in top-down attentional control, especially the PFC, may also contribute to improved behavioral performance.

It is interesting to note that the effects of cholinergic activation we observed—improved task performance, increased spontaneous and visually driven cortical firing rates, improved neuronal response reliability and decreased inter-neuronal correlation at low frequencies—have all been observed in primates during selective visual attention. Given such a strong similarity between the effects of basal forebrain cholinergic activation and visual attention at both the behavioral and neuronal levels, cholinergic transmission is likely to be a key component of the neural mechanism for attentional modulation. In particular, our results are consistent with a recent finding that attentional modulation of neuronal responses and behavioral performance is enhanced by local application of cholinergic agonists in V1 (ref. 21). However, given the axonal branching patterns of individual cholinergic neurons and their volume transmission of ACh in the cortex, it is unclear whether the basal forebrain projections have sufficient spatial specificity to provide the instructive signal for selective attention. Nonetheless, the cholinergic input is likely to have at least a permissive function in attentional modulation.

The basal forebrain cholinergic neurons have long been implicated in crucial brain functions such as arousal, memory and cortical plasticity, spanning timescales ranging from minutes to hours. In crucial brain functions such as arousal, memory and cortical plasticity, spanning timescales ranging from minutes to hours. In crucial brain functions such as arousal, memory and cortical plasticity, spanning timescales ranging from minutes to hours.

### METHODS

Methods and any associated references are available in the online version of the paper.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

L.P. and M.J.G. performed the electrophysiology experiments. L.P., M.J.G. and D.E. performed the behavioral experiments. L.P. and D.E. performed histology. M.X. and A.C.K. performed the behavioral setup. S.-H.L., T.C.H. and L.P. performed the tracing experiments. G.F. provided the ChAT-ChR2-EYFP mice. L.P. and M.J.G. analyzed the data. L.P., M.J.G. and Y.D. conceived and designed the experiments and wrote the paper.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

Animals and surgery. All procedures were approved by the Animal Care and Use Committee at the University of California, Berkeley. Experiments were performed on adult wild-type (C57) and transgenic mice (2–6 months old, 20–45 g, males and females). The animals were housed on a 12:12-h light/dark cycle in cages with up to four other animals before the implants and alone after the implants. To activate basal forebrain cholinergic neurons selectively, we used ChAT-ChR2(H134R)-EYFP mice21 (line 6, Jackson Laboratories, B6.129S6-Chat<tm1(crc)Lowl/J, stock number 014546). To selectively inactivate cholinergic neurons, we crossed ChAT-Cre mice (Jackson Laboratories, B6.129S6-Chat<tm1(crc)Lowl/J, stock number 006410) with either loxp-flanked ARCH-GFP (Jackson Laboratories, 129S-Gt(ROSA)26Sor<tm35.1(CAG-AOP3/GFP)Hze/J, stock number 012735) or loxp-flanked HALO-EYFP (Jackson Laboratories, B6.129S-Gt(ROSA)26Sor<tm35.1(CAG-HP/COP4*EYFP)Hze/J, stock number 014539) reporter mice. For tracing experiments using CTB we used ChAT-tlTomato mice obtained by crossing the ChAT-Cre line with loxp-flanked tlTomato mice (Jackson Laboratories, B6.129S6-Chat<tm1(crc)Lowl/J, stock number 007914). For dual tracing experiments with retrobeads we used wild-type (C57), ChAT-Cre or GAD2-Cre mice (Jackson Laboratories, Gad2tm1(crc/ERT2)Jlh/J, stock number 010702).

For headplate and cannula implant, mice were anesthetized with isoflurane (5% induction and 1.5% maintenance) and placed on a stereotaxic frame (David Kopf Instruments). Temperature was kept at 37 °C throughout the procedure using a heating pad. After asepsis, the skin was incised to expose the skull and the overlying connective tissue was removed. A reference epideral screw was implanted above the left frontal cortex. A half-drilled craniotomy was made to mark the location of the monocular region of the right V1, which was then sealed with a silicone elastomer (Kwik-Cast, World Precision Instruments). We primarily targeted the nucleus basalis, defined as the dorsalmost nucleus in the basal forebrain, lying immediately ventral to the internal capsule and ventromedial to the globus pallidus49 (Supplementary Figs. 1 and 2). However, it is possible that cholinergic neurons in other basal forebrain nuclei at more ventral locations were also activated by the optic fiber when the target was at the edge of the nucleus basalis.

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Optogenetic activation and inactivation. Laser light was delivered to basal forebrain via an optic fiber 200 µm in diameter (Thorlabs) inserted through and protruding 0.5 mm beyond the implanted cannula. For activation of cortical cholinergic terminals we used a 400-µm fiber (Thorlabs) placed ~500 µm above the dura. For ChR2-mediated activation, we used a 473-nm laser (CrystaLaser or Shanghai Laser and Optics Century Co.) at a power of 1–3 mW at the fiber tip for basal forebrain activation and 0.75–2 mW for cortical activation experiments. For ARCH-mediated inactivation, we used a 532-nm laser (Shanghai Laser and Optics Century Co.), 15–30 mW at fiber tip. For HALO-mediated inactivation, we used a 593-nm laser (CrystaLaser), 15–30 mW at the tip. The laser was controlled by TTL pulses generated by either an amplifier (Tucker-Davis Technology) or a stimulus generator (Master 8, A.M.P.L.I.). In behavioral and electrophysiological experiments we used 5-s square laser pulses. Note that in experiments where the optic fiber was targeted at nucleus basalis, some cholinergic neurons in other basal forebrain nuclei could also be activated and thus contribute to the observed effects. Note that some of these cholinergic neurons also project to V1 (Supplementary Fig. 2).

Visual stimulation. Visual stimuli were generated with a GeForce 7300 Graphics card (NVIDIA) in a PC running custom written software. The mice viewed a gamma-corrected 7-inch LCD monitor (Xenarc Technologies, maximal luminance: 250 cd/m2) with a refresh rate of 75 Hz. The monitor was placed 10 cm away from the left eye. All stimuli were presented in a 50° × 50° region centered at the average receptive field location of all simultaneously recorded units.

Sine-wave gratings used in behavioral and electrophysiological experiments (spatial frequency, 0.04 cycles per degree; temporal frequency, 2 Hz) were presented at 20%, 40% or 100% contrast, drifting at either 0° or 90°. For behavior, the drifting grating lasted for 4 s. Laser stimulation lasted for 5 s, from trial start to the end of visual stimulation (Fig. 2a). For electrophysiology, each trial started with 1 s of gray screen followed by 1 s of static grating followed by drifting grating for 4 s. Laser stimulation lasted for 5 s, from the onset of the static grating to stimulus offset. Baseline firing rates were measured using separate trials with a gray screen (0% contrast). Each experiment consisted of 280 trials (40 trials at each orientation and each contrast plus 40 trials at 0% contrast), presented in eight blocks (35 trials per block), with interleaved laser-on and laser-off blocks.

For natural stimuli, we used three 5-s clips selected from the van Hateren natural-movie database22. Each image was repeated for three frames, resulting in an effective frame rate of 25 Hz. Each trial started with 1 s of gray screen, followed by 1 s of the first movie frame, 5 s of movie and 1 s of the last frame. Laser stimulation was concurrent with the movie presentation. Each movie was repeated 60 times in six blocks (three blocks laser on and three blocks laser off).

Electrophysiology. We performed multi-site extracellular recordings from awake, head-fixed mice on a spherical treadmill23. Briefly, an 8-inch Styrofoam ball rested on a metal bowl and was suspended by compressed air to allow the mouse to run with little friction. Running speed was measured with two optical computer mouse plates placed orthogonally at the ball equator using custom-written software in LabVIEW (National Instruments). The mice were habituated to a similar setup for 1–3 d before the recordings. Both spikes and local field potentials (LFP) were recorded using either a Neuralynx Cheetah 27-channel acquisition system (Neuralynx Inc.) or a 32-channel TDT RZ5 (Tucker-Davis Technologies). LFP was band-pass filtered at 1–325 Hz and stored at 30 kHz (Neuralynx) or 700 Hz (TDT). Spikes were filtered at 0.6–6 kHz and stored as raw voltage traces at 30 kHz (Neuralynx) or 25 kHz (TDT). We used a total of 28 ChAT-ChR2-EYFP, five ChAT-ARCH-GFP and two ChAT-HALO-EYFP mice for electrophysiological recordings. We combined the ARCH and HALO data because the results were not statistically different.

Behavior. We trained head-fixed mice on a go/no-go visual discrimination task. The water-restricted mice rested in an acrylic tube located inside a sound-attenuated chamber. Licks were detected by a custom-made infrared lickometer. The animals were typically trained 6 d per week and received all their daily amount of water during the training session (~400 trials, 0.8–1 mL). We monitored their weight daily and made sure it did not drop below 85% of the starting value by giving them supplemental water if necessary. Behavioral experiments were carried out during the light cycle, and the experimenter was not blind to group allocation.
Before the visual discrimination task, the mice underwent a period of response shaping and habituation to the apparatus (3–5 d), followed by a period of inter-
mediate conditioning, in which they viewed only the ‘go’ stimulus (vertically
oriented grating moving rightward) and received a water reward regardless of
licking (2–4 d). Once they licked consistently during the presentation of the go
stimulus, we moved them to the discrimination task with randomly interleaved
go and no-go trials. Trial start was signaled by a 500-ms 5 kHz tone, and the
licking appeared 1 s after tone onset. The duration of grating stimulus was 4 s,
including an initial grace period of 2 s when licking had no consequence followed
by a 2-s response window (Fig. 2a). We found that introducing this grace period
greatly facilitated task learning. In go trials, licking during the response window
resulted in an immediate water reward (~4



µl) from a spout placed close to
the animal’s mouth. In a no-go trial (horizontally oriented grating moving upward),
licking during the response window resulted in an air puff to the cheek
(15–20 p.s.i., 200 ms) and 8-s timeout period. Inter-trial interval was 3 s. Licking
during a go trial was counted as a ‘hit’, and no lick was counted as a ‘miss’. In no-go
trials, licking was counted as a ‘false alarm’ and no lick as a ‘correct rejection’. We
found that performance was variable across days and subjects, in agreement with
previous studies using vision tasks in mice53. Thus, mice were trained on
this task until satisfying the following criteria: for at least three consecutive
days (i) \( d’ \geq 0.5 \) over the first 100 trials of each training session (indicating that
the mouse performed consistently from the beginning of each session), and (ii) \( d’ \geq 1 \)
over the entire training session or condition (i) was fulfilled and the limit of
8 weeks of training was reached. These criteria were chosen to ensure that mice
were performing above chance while avoiding excessive overtraining before the
experimental manipulations.

Once the mice reached stable performance defined above (3–8 weeks), we
started adjusting task difficulty by varying the contrast of the gratings. Different
contrasts were presented in different blocks (20 trials per block). Initially we used
six linearly spaced contrast values (0%, 20%, 40%, 60%, 80% and 100%), inter-
leaving every block with a 100% contrast block (2–4 d). This was followed by
a protocol with blocks of 0% (no stimulus), 20%, 40% or 100% contrast, presented
randomly with probability of 1/7, 2/7, 2/7 and 2/7, respectively. We presented
fewer 0% contrast blocks because the performance at chance level within these
blocks often reduced the animals’ motivation to perform the task. The animals
were rewarded randomly in these blocks. After 2–5 d in this protocol, we intro-
duced laser stimulation at random trials with a 0.5 probability, keeping the con-
trast block structure. The laser was a square pulse lasting for 5 s, from trial start to
visual stimulus offset (Fig. 2a). The optic fiber was inserted through the cannula
placed above basal forebrain or positioned directly above V1 daily, and these laser
stimulation experiments lasted for 1–5 d per mouse. We used 18 and 12 ChAT-
ChR2-EYFP mice for basal forebrain activation and V1 activation experiments,
respectively. We excluded another two animals from the basal forebrain activation
group for having mean \( d’ < 0.0 \) across contrasts in laser-off trials. For basal forebrain
inactivation behavioral experiments, we used one ChAT-HALO-EYFP and five
ChAT-ARCH-GFP mice. The control group consisted of eight wild-type mice
(five of which were ChR2-negative littermates of ChAT-ChR2-EYFP mice).

**Histology, immunohistochemistry and retrograde tracing.** To confirm the
location of the cannula for laser stimulation, after each experiment we inserted
a metal wire (200 \( \mu \)m in diameter) through the cannula to mark the location of
the optic fiber. The animal was deeply anesthetized and euthanized by decapita-
tion. The brain was removed from the skull and fixed in 4% (w/v) PFA, followed
by cryoprotection with a 30% (w/v) sucrose solution. The brain was
placed above basal forebrain or positioned directly above V1 daily, and these laser
stimulation experiments lasted for 1–5 d per mouse. We used 18 and 12 ChAT-
ChR2-EYFP animals (12 sections, 63 cells) and two ChAT-ARCH-GFP and
one ChAT-HALO-EYFP animal (17 sections, 182 cells). All cells stained with both
DAPI (nuclear) and anti-ChAT antibody (Alexa 594) were considered cholinergic
cells, and those that were stained with DAPI and were EYFP-positive (or GFP-
positive) were considered ChR2 (or HALO/ARCH)-expressing neurons.

Three ChAT-tdTomato mice were used for single V1 retrograde tracing experi-
ments with CTB. They underwent transcardial perfusion, and their brain samples
were processed as described above. Brains were sliced into 30-\( \mu \)m thick coronal
sections. Images were acquired with a 2×0 water immersion lens in a Zeiss LSM
710 confocal microscope and processed in ImageJ. Only cells with green fluores-
cence levels above background and overlap with DAPI staining were considered
basal forebrain activity, they are presented together.

**Pharmacology.** For pharmacological experiments we bath-applied a cocktail
containing the muscarinic blocker atropine (500 \( \mu \)M, Sigma-Aldrich) and the
nicotinic blocker mecamylamine (3 mM, Sigma-Aldrich) over the V1 craniotomy.
Recordings started 30–45 min after drug application, and recovery experiments
were carried out 90–120 min after drug washout with saline.

**Eye tracking.** We recorded eye movements in the absence of visual stimulation
(gray screen) and during the presentation of natural movies using an infrared
camera (QCam 860ML, Premiertek) placed 2 cm away from the animals’ eye
contralateral to the V1 we recorded from. Images were acquired at a frame rate
of 30 Hz, and tracking was performed offline. Each frame was converted to a
binary image and ellipse fitting was performed to estimate the center of the pupil
position using custom-written software in Matlab.

**Data analysis. Spike sorting.** Spikes were detected offline with custom-written
software. We grouped nearby channels of the silicon probe into groups of three or
four and performed semiautomatic spike sorting using Klusters54. Spike clusters
were considered single units if their auto-correlograms had a 2-ms refractory
period and their cross-correlograms with other clusters did not have sharp peaks
within 2 ms of 0 lag. We excluded cells with average firing rates <0.5 Hz. To
estimate the cell type we recorded from we computed spike width and peak-to-
trough ratio on all average waveforms. Cells fell into two clusters corresponding
to putative fast spiking interneurons (~20%) and putative regular spiking cells
(~80%) (data not shown). Because there were no systematic differences between
the two putative classes regarding the effects of optogenetic manipulation of basal
forebrain activity, they are presented together.

**Spectral analyses.** Time-frequency decomposition of LFP was done with multi-
taper analysis55 using the Chronux toolbox (http://www.chronux.org/).
LFP was down sampled to 200 Hz before decomposition. We used five tapers
(time-bandwidth product of three) and a 1-s sliding window with 50-ms steps.
Spectra were averaged across channels. The spectra shown in Figures 1 and 5
and Supplementary Figures 3, 4 and 6 were obtained from 30 trials of 5-s square
laser pulses (inter-trial interval of 30 s) in which the mice viewed a gray screen.
For these analyses, each frequency band was normalized to the average power
in the 5-s baseline period preceding basal forebrain stimulation.

We computed the desynchronization ratio as the average power at high frequencies
(60–100 Hz) divided by the average power at low frequencies (1–5 Hz).

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To estimate the latency of effect onset and offset we calculated the variance of the raw LFP traces for each trial and recording channel over a 250-ms sliding window with 5-ms steps. Onset latency was defined as the first time point at which the average variance was beyond 2 × s.e.m from the mean of the 5-s baseline period preceding laser onset. Offset latency was defined as the first time point after which variance returned to baseline levels for at least 2.5 s. Latency values for each animal were averaged across channels and trials.

For the analysis in Supplementary Figure 3, we decomposed the LFP as described above and averaged the power at each frequency over the 5-s windows before and during each trial of laser stimulation. The power in each frequency band was z-scored, and for each 5-s window we averaged the z-scored power between 1 and 5 Hz (low frequency) and between 60 and 100 Hz (high frequency).

Single unit versus multi-unit coherence was also computed with multi-taper analysis using the same parameters as for LFP. Multi-unit activity was defined as the summed activity of all simultaneously recorded single units except the single unit under comparison. Spiking activity was binned at 1 kHz for all 5 s of natural-movie stimulation and for the 4 s of drifting gratings and the average spiking rate was subtracted. Coherence for each single unit versus multi-unit pair was averaged across trials. Statistical significance of coherence results was calculated on the average across frequencies <5 Hz. Baseline coherence levels were estimated by performing five random trial shuffles for each pair and averaging them. Using an additional correction for differences in firing rate between laser-on and laser-off conditions by randomly deleting spikes from the condition with the higher firing rate until the firing rates matched yielded very similar results (data not shown).

Single unit responses. For each single unit we computed peri-stimulus time histograms (PSTHs) binned at 10 Hz. For the responses to gratings, we selected units that were significantly driven by either the horizontal or vertical grating (response evoked by 100% contrast grating significantly greater than spontaneous firing rate, measured without basal forebrain stimulation, as assessed by Wilcoxon signed-rank test at \( P < 0.05 \)). Using this criterion, 101/153 and 47/77 neurons were selected for further analysis in basal forebrain activation and inactivation experiments, respectively. For the analysis of response reliability (Fano factor) to natural movies, we selected only cells that were driven by the movies, defined as those that had significantly higher within-movie Pearson correlation coefficients than between-movie correlations\(^{15}\).

To determine whether cholinergic activation increases the discriminability of neural responses to different orientations (Fig. 3d), we calculated the average neurometric function\(^{56}\) under each condition (basal forebrain on, control). For each neuron, the mean spike rate for each single-trial response was used to create response distributions for preferred and nonpreferred orientations. The receiver operating characteristic (ROC) was compiled for the distributions using a range of 100 criterion values spanning from the minimum to maximum response. The discriminability of the responses was then calculated by integrating the area under the ROC curve\(^{56}\). This analysis was performed separately for each contrast (20%, 40% and 100%) to generate a neurometric function for each condition. Because visual cortical neurons exhibit a wide range of orientation preferences, the majority of recorded neurons did not discriminate well between the two orientations used in the experiments. Thus, in generating an average neurometric function, it was necessary to exclude nondiscriminating neurons to reduce variability. For the data shown in Figure 3d, we excluded neurons that discriminated with less than 70% accuracy (across contrasts and conditions). The results were similar for discrimination thresholds anywhere in the range 55–85% (discrimination thresholds above 85% included too few neurons for robust statistical testing).

Fano factor for the responses to natural movies was calculated as the time average of variance (spike count)/mean (spike count) using a bin size of 100 ms. Using other bin sizes or Pearson correlation coefficients of between-trial responses as a measure of response reliability yielded qualitatively similar results (data not shown).

**Behavioral data.** For the analysis of behavioral data, hit rate was defined as number of hits/(number of hits + number of misses) and false alarm rate as number of false alarms/(number of false alarms + number of correct rejections). Behavioral performance was summarized as \( d' = Z(\text{hit rate}) - Z(\text{false alarm rate}), \) where \( Z \) is the inverse of the cumulative Gaussian distribution. Individual behavioral sessions were truncated for analysis at the last trial in which the mouse licked.

**General statistics.** Data sets were tested for normality using the Lilliefors modification of the Kolmogorov–Smirnov test and then compared with the appropriate tests (t or rank tests, two sided unless stated otherwise). Groups being compared had similar variance. Statistical significance of experiments with factorial design (i.e., involving different contrasts and control/laser conditions) was assessed using two-way repeated-measures ANOVA. Unless otherwise stated, data are presented as means ± s.e.m. The experiments were not randomized; no statistical method was used to predetermine sample size.