Cholinergic modulation of response gain in the primary visual cortex of the macaque

Shogo Soma, Satoshi Shimegi, Hironobu Osaki and Hiromichi Sato

J Neurophysiol 107:283-291, 2012. First published 12 October 2011; doi:10.1152/jn.00330.2011

Journal of Neurophysiology publishes original articles on the function of the nervous system. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. ISSN: 0022-3077, ESSN: 1522-1598. Visit our website at http://www.the-aps.org/.

J Neurophysiol 107: 283–291, 2012.
First published October 12, 2011; doi:10.1152/jn.00330.2011.

Cholinergic modulation of response gain in the primary visual cortex of the macaque

Shogo Soma,[1] Satoshi Shimegi,[1,2] Hironobu Osaki,[2] and Hiromichi Sato[1,2]
Graduate Schools of [1]Frontier Biosciences and [2]Medicine, Osaka University, Toyonaka, Osaka, Japan

Submitted 11 May 2011; accepted in final form 10 October 2011

Soma S, Shimegi S, Osaki H, Sato H. Cholinergic modulation of response gain in the primary visual cortex of the macaque. *J Neurophysiol* 107: 283–291, 2012. First published October 12, 2011; doi:10.1152/jn.00330.2011.—ACh modulates neuronal activity throughout the cerebral cortex, including the primary visual cortex (V1). However, a number of issues regarding this modulation remain unknown, such as the effect and its function and the receptor subtypes involved. To address these issues, we combined extracellular single-unit recordings and microiontophoretic administration of ACh and measured V1 neuronal responses to drifting sinusoidal grating stimuli in anesthetized macaque monkeys. ACh was found to have mostly facilitatory effects on the visual responses, although some cases of suppressive effects were also seen. To assess the functional role of ACh, we further examined how ACh modulates the stimulus contrast-response function, finding that the response gain increased with the facilitatory effect. The response facilitation was completely or strongly blocked by atropine (At), a muscarinic ACh receptor (mAChR) antagonist, in almost all neurons (96% of cells), whereas any residual effect after At administration was fully removed by mecamylamine, a nicotinic AChR (nAChR) antagonist, suggesting a predominant role for mAChRs in this mechanism. Furthermore, we found no laminar distribution bias for the facilitatory modulation, although the relative contribution of mAChRs was smaller in *layer 4C* than in other layers. The suppressive effect was blocked completely by At. These results demonstrate that ACh plays an important role in visual information processing in V1 by controlling the response gain via mAChRs across all cortical layers and via nAChRs, mainly in *layer 4C*.

muscarinic ACh receptors; nicotinic ACh receptors; response gain control; monkey

THE CHOLINERGIC SYSTEM PLAYS important roles in cortical information processing, as its dysfunction can cause psychiatric disorders and neurological disease. For example, a degeneration of cholinergic neurons and axonal terminals and a decrease in ACh receptors (AChRs) have been associated with dementia (Campbell et al. 2001; Gallagher and Colombo 1995; Jones et al. 1992; Newhouse et al. 1988, 2001; Whitehouse et al. 1982).

It is known that cholinergic neurons in the basal forebrain project throughout the cerebral cortex, including the primary visual cortex (V1) (Lehmann et al. 1980; Mesulam and Van Hoesen 1976), where ACh release from axon terminals modulates visual information processing in V1 (Goard and Dan 2009; Müller and Singer 1989; Murphy and Sillito 1991; Roberts et al. 2005; Sato et al. 1987b; Sillito and Kemp 1983; Zinke et al. 2006). For example, microiontophoretic administration of ACh broadens the orientation tuning (Zinke et al. 2006) and decreases the summation area of the classical receptive field (CRF) (Roberts et al. 2005) of V1 neurons in primates. Moreover, lesioning the basal forebrain reduces the magnitude of the visual response of V1 neurons, suggesting that ACh controls the response gain (Sato et al. 1987a).

In macaque V1, two groups of AChRs are expressed: muscarinic AChRs (mAChRs), which are G-protein-coupled receptors (Disney and Aoki 2008; Disney et al. 2006; Mrzljak et al. 1993), and nicotinic AChRs (nAChRs), which are ligand-gated ion channels (Disney et al. 2007; Han et al. 2003). Recently, Disney et al. (2007) examined the effects of nicotine, a nAChR agonist, on visual responses and contrast-response relationships in monkey V1 neurons. They found that activation of nAChRs enhances visual responses and increases the gain of the contrast-response function. In contrast, little is known about whether and how mAChRs modulate visual processing in monkey V1.

We therefore investigated the effects of ACh; atropine (At), a mAChR antagonist; and mecamylamine, a nAChR antagonist, on visual responses and the stimulus contrast-response function in macaque V1. ACh was found mainly to enhance responses across all cortical layers and increase the gain of the contrast-response function without any systematic change in contrast sensitivity. This ACh facilitatory effect was completely or partially blocked with concurrent administration of At in a majority of neurons, suggesting that mAChRs predominantly contribute to the gain control of visual responses in V1. We also found that any residual effect after At administration was fully removed by mecamylamine, which reaffirmed the observation by Disney et al. (2007) that response gain in V1 is controlled by nAChRs as well. ACh suppressed visual responses in a small portion of neurons, and the effect was also completely blocked by At. From these results, we conclude that ACh activation of mAChRs plays an important role in controlling the visual response gain in V1.

MATERIALS AND METHODS

All experimental protocols were approved by the Research Ethics Committee of Osaka University (Japan). All procedures were carried out in accordance with the regulations of the Animal Care Committee of the Osaka University Medical School and National Institutes of Health guidelines for the care of experimental animals. All efforts were made to reduce the number of animals used.

Preparation

Neuronal responses were recorded in area V1 from five anesthetized monkeys (*Macaca fucata*; body wt, 4.9–6.6 kg), as described previously (Sato et al. 1996; Watakabe et al. 2009). The animals were anesthetized with ketamine hydrochloride (Ketalar, Daiichi Sankyo, Tokyo, Japan; 10 mg/kg im), followed by a mixture of isoflurane (Forane, Abbott Japan, Tokyo, Japan; 2–3%) and N_2O:O_2 (2:1). The trachea of each animal was intubated, and a catheter was placed in the femoral vein. Then, the animals were placed in a stereotaxic head holder. Lidocaine was administered at pressure points and around

Address for reprint requests and other correspondence: S. Shimegi, Graduate Schools of Frontier Biosciences and Medicine, Osaka Univ., 1-17 Machikaneyama, Toyonaka, Osaka, Japan (e-mail: shimegi@vision.hss.osaka-u.ac.jp).

surgical incisions for local anesthesia. The rectal temperature and end-tidal CO_2 concentration were adjusted to 37–38°C and 3.5–4.0%, respectively. The ECG, EEG, and heart rate were continuously monitored throughout the experiment. Before recording neuronal activity, the animals were continuously paralyzed with pancuronium bromide (Mioblock, Schering-Plough, Osaka, Japan; 0.02 $mg \cdot kg^{-1} \cdot h^{-1}$ iv) to minimize eye movements and maintained under artificial ventilation. During recording, fentanyl citrate (Fentanest, Daiichi Sankyo; 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ iv) and droperidol (Droleptan, Daiichi Sankyo; 125 $\mu g \cdot kg^{-1} \cdot h^{-1}$ iv) were continuously infused, and the inhalation of isoflurane was stopped. This treatment induced a state of neuroleptanalgesia, which enabled us to record the visual responses of 82 neurons with nearly normal cortical activity [*Experiment A* (*Exp. A*)]. However, it has been reported in an *in vitro* study that droperidol inhibits ion fluxes via transgenetically expressed $GABA_A$ receptors and nAChRs in oocytes (Flood and Coates 2002). Thus droperidol might have affected our results in *Exp. A*. To rule out this possibility, identical experiments in the absence of droperidol were also performed [*Experiment B* (*Exp. B*); $n = 42$]. Because the results for the two conditions had no statistical difference (see RESULTS), we pooled the data obtained from *Exp. A* and *Exp. B* ($n = 124$) for analysis.

Physiological Recordings and Microiontophoresis

A glass microelectrode attached to three barreled drug pipettes was used for extracellular single-unit recordings, whereas the pipettes were used for the microiontophoretic administration of ACh (Nacalai Tesque, Kyoto, Japan; 500 mM, pH 4.5), At (Sigma-Aldrich, St. Louis, MO; 230 mM, pH 5.6), mecamylamine (Sigma-Aldrich; 100 mM, pH 4.5), or Ringer's solution (pH 7.0) (Sato et al. 1996). The tip of the recording electrode protruded 10–30 μm from the tip of the pipettes. The ejecting current was generally between +1 and +60 nA, whereas the retaining current was between −5 and −15 nA. No cell showed changes in spike size or firing frequency during the microiontophoretic administration of Ringer's solution. The recording pipette was filled with 0.5 M sodium acetate containing 4% Pontamine sky blue (Direct Blue 1, Tokyo Kasei, Tokyo, Japan). Dye marks were produced by passing tip-negative direct current at the end of each penetration (100–200 pulses of 8–10 μA for 1 s at 0.5 Hz). This enabled histological verification of recording positions.

Visual Stimulation

When single neuron activity was isolated, we manually assessed minimum response field (MRF) properties (Barlow et al. 1967), including dominant eye, optimal orientation and direction of the stimulus, and MRF size, using a hand-held projector. The retinal eccentricity of the MRF was within 6° of the area centralis. Subsequently, computer-generated visual stimuli were used to determine optimal parameters of the CRF quantitatively. A circular patch of drifting sinusoidal grating was generated by a visual stimulus generator (VSG 2/3; Cambridge Research Systems, Kent, UK) and presented for 1 or 2 s monocularly on a display monitor (CDM-F520, Sony, Tokyo, Japan; mean luminance, 30 cd/m^2; refresh rate, 100 Hz; screen size, 40 × 30 cm^2), placed 57 cm in front of the monkey's eyes.

We then presented a grating stimulus with optimal parameters at varying stimulus contrast to obtain a contrast-response function. The neuronal response was measured while pseudorandomly changing the stimulus contrast, which included 10 contrast levels spanning 0–100%. Background discharge was defined as the spike discharge during the presentation of a blank stimulus with 0% contrast. Each stimulus presentation was interleaved with a blank screen with 0% contrast for 1 s. Each stimulus condition was pesudorandomly repeated five to eight times to construct a poststimulus time histogram (PSTH). Measurements were performed before, during, and after drug administration, which we refer to as the control, drug, and recovery condition, respectively.

Histology

After the recording experiments, the animals were deeply anesthetized with sodium pentobarbital (Nembutal, Dainippon Sumitomo Pharma, Osaka, Japan; 60 mg/kg iv) and perfused transcardially with 0.1 M PBS (pH 7.4), followed by 4.0% paraformaldehyde in 0.1 M PBS. Blocks of the occipital cortex were obtained and immersed in 30% sucrose in PBS for 36–48 h. Sixty micrometer-thick frozen parasagittal sections were sliced on a microtome and kept in PBS. Sections were stained for cytochrome oxidase (Wong-Riley 1979). The laminar locations of the recording sites were then identified under a light microscope. Shrinking in the cortical tissues was corrected for by taking the ratio of the measured dye mark distance and the distance calculated from the micrometer reading (Sato et al. 1996).

Cell Classification

The fundamental (F0) and first harmonic (F1) components of averaged responses were computed from PSTHs that were compiled during each condition (control, drug, and recovery). V1 neurons were classified as "simple" or "complex" based on the F1/F0 ratio (F1/F0 ≥ 1, simple cells; F1/F0 < 1, complex cells; Skottun et al. 1991). The F1 and the F0 components were used as the response magnitude for simple and complex cells, respectively.

Classification of ACh Effects on Contrast-response Tuning Curves

The effects of ACh on the contrast-response curve were categorized according to a previously described nonparametric analysis method (Disney et al. 2007). We calculated the average spike rate (across five to eight trials) at each stimulus contrast and summed them over all nine contrasts tested. This nonparametric analysis is equivalent to measuring the area under the contrast-response curve (response area) and does not depend on fitting a model function to the data. ACh effects were classified as significant facilitation or suppression if the mean of the response area with ACh was 3 SD above or below the mean across the control trials (mean ± 3 SD criteria).

Fitting

To quantify the contrast sensitivity of the recorded neurons, we fitted the contrast-response relationship using the following equation (Naka-Rushton function; Sengpiel et al. 1998): $R = R_{max} (C^n)/(C^n + C_{50}^n) + b$, where R is the neuronal response, C is the contrast of periodic stimuli, and b is the background discharge. R_{max} (maximal response), n (exponent of power function; >0), and C_{50} (contrast for one-half of R_{max}; contrast sensitivity) are free parameters.

Relative Contribution of mAChRs to the ACh Effect

To quantify the relative contribution of mAChRs to the ACh-induced response modulation, we calculated the response area of the contrast-response curves obtained under three drug conditions: control, "ACh only", and "ACh + At". We then subtracted the response area of the control from that of ACh only to estimate the modulatory effect of ACh (ACh effect) and subtracted that of ACh + At from that of ACh only to estimate the magnitude of mAChRs-mediated facilitation (mAChRs effect). The relative contribution of mAChRs was calculated by dividing the mAChRs effect by the ACh effect.

RESULTS

A total of 124 neurons extracellularly recorded from V1 was tested for ACh effects on responses to CRF stimuli with

optimal parameters. Fifty-eight were simple cells, and 66 were complex cells. Since there were no differences in ACh effects between the two types of cells, the data were pooled.

Effects of ACh on Visual Responses and Laminar Distribution

CRF responses in V1 cells were recorded before, during, and after microiontophoretic ACh administration. ACh exhibited either facilitatory or suppressive effects in V1 cells. Figure 1 shows the PSTH of the responses from two *layer 4C* simple cells. Figure 1*A* is an example of a simple cell facilitated by ACh. The firing rate was increased to 144% of control during ACh administration and returned to the preadministration level in the following recovery period. In the other cell (Fig. 1*B*), ACh administration had a suppressive effect, reducing the response magnitude to 7% of the control condition, indicating that the modulatory effects of ACh on the firing rate differ from cell to cell.

Cells were classified as facilitated and suppressed based on nonparametric analysis (see MATERIALS AND METHODS). Among the observed 124 cells, 67 (54%), 10 (8%), and 47 (38%) cells were categorized as facilitated, suppressed, and no-effect cells, respectively. With the use of histological observations, we reconstructed the laminar position of the recording site for 112 of these cells (Fig. 2), finding that the 62 facilitated cells and 42 no-effect cells were distributed in all cortical layers. The eight suppressed cells were found to distribute across most, but not all layers, although this may be attributable to the small sample size.

Additionally, the inclusion or exclusion of droperidol affected neither the occurrence rate ($P = 0.525$, χ^2 test) nor the laminar distribution of the ACh effects (facilitated, $P = 0.307$; suppressed, $P = 0.187$; no-effect, $P = 0.649$, χ^2 test; data not shown), suggesting that droperidol did not affect our results.

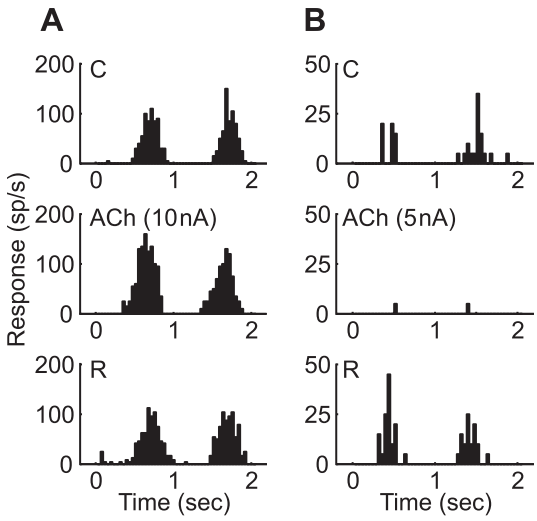

Fig. 1. Facilitatory and suppressive effects of ACh (ACh effects) on the visual responses of primary visual cortex (V1) neurons in *layer 4C*. Each histogram shows a poststimulus time histogram (PSTH) of visual responses to a drifting sinusoidal grating patch with optimal parameters presented for 2 s. Examples of neurons facilitated (*A*) and suppressed (*B*) by ACh. *Top*, *middle*, and *bottom*: PSTHs are visual responses obtained before (C, control), during (ACh), and after (R, recovery) ACh administration (*A*, 10 nA; *B*, 5 nA), respectively. Number of trials, 5; bin width, 40 ms.

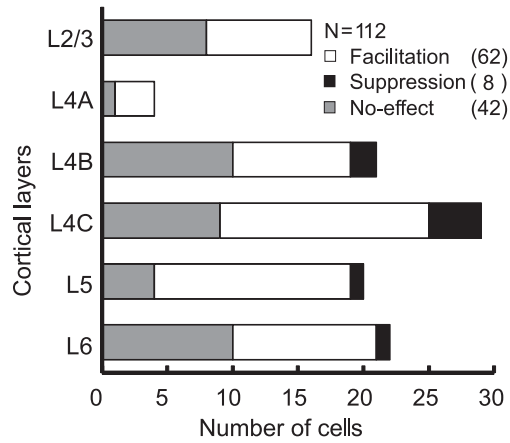

Fig. 2. Layer (L) distribution of ACh effects in V1. V1 neurons ($n = 112$) were reconstructed for laminar location of the recording site on the basis of histological observations. Each column shows the number of cells facilitated (open columns), suppressed (black columns), and unaffected (gray columns) by ACh administration. ACh exerted a facilitatory effect on the visual responses of 62 cells and a suppressive effect on 8 cells. The response facilitation was observed in all layers, whereas the response suppression was distributed across most layers.

In *Exp. B*, regular-spiking (RS; $n = 35$) and fast-spiking (FS; $n = 7$) cells were classified electrophysiologically based on their spike waveforms (Niell and Stryker 2008). These cells were not statistically different in the occurrence rate of the ACh effects ($P = 0.602$, χ^2 test), in which facilitated, suppressed, and no-effect cells were 22 (63%), three (8%), and 10 (29%) in RS cells and four (57%), zero (0%), and three (43%) in FS cells, respectively. Also, there was no statistical difference in the laminar distribution of the response facilitation ($P = 0.059$, χ^2 test). Therefore, we pooled the cells for analysis.

ACh Modulated the Response Gain but not the Contrast Gain in Facilitated Neurons

To clarify the functional role of ACh in visual processing, we examined its effects on the relationship (gain) between the stimulus contrast (input) and response magnitude (output) of a single cell. We tested nine stimulus contrasts of grating stimuli and constructed the contrast-response tuning curves of the visual responses in the presence and absence of ACh.

At least three possible types of gain control in the contrast-response function have been known (Fig. 3): contrast gain control (*A*), response gain control (*B*), and baseline control (*C*) (Sengpiel et al. 1998; Williford and Maunsell 2006). Contrast gain control is characterized by a change in C_{50}; response gain control, by a change in R_{max}; and baseline control, by an increase in background discharge. To examine which type of gain control occurred during ACh administration in the facilitated cells, we fitted Naka-Rushton function to the data. Figure 4 shows a typical example of the ACh facilitatory effect on the contrast-response function. The facilitatory effect was proportional to the stimulus contrast, in which R_{max} increased and the slope of the curve became steeper. In Fig. 4*B*, the two contrast-response functions shown in Fig. 4*A* are normalized to compare the shape of their contrast-response tuning curves. They were almost identical in shape with unchanged C_{50}, indicating that ACh enhanced the response gain but not the contrast gain. To examine this phenomenon at the population level, we compared R_{max} and C_{50} between control and ACh conditions

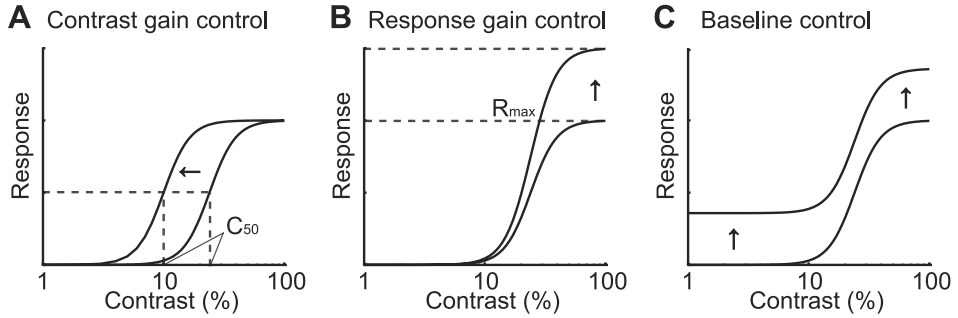

Fig. 3. Three possible types of gain control in the contrast-response function. The contrast-response tuning curve was fitted using the Naka-Rushton function, where R_{max} is the peak (maximal) response, and C_{50} is the contrast value at 1/2 R_{max} (contrast sensitivity). Three kinds of gain control have been known: contrast gain control (*A*), response gain control (*B*), and baseline control (*C*), which are characterized by a change in C_{50}, a change in R_{max}, and increasing background discharge across the whole range of stimulus contrasts, respectively. For example, response facilitation modulates contrast-response function differently according to the type of gain control as depicted by arrows.

for all facilitated cells ($n = 67$). Figure 5 shows scatter plots of R_{max} (*A*) and C_{50} (*B*), in which data for ACh were plotted against those for the control. At the population level, ACh significantly increased R_{max} ($P < 0.001$, Wilcoxon signed-rank test) but caused no systematic change in C_{50} ($P = 0.102$, Wilcoxon signed-rank test). Thus ACh increased the firing rate to enhance the response gain (R_{max}) but not the contrast gain.

Next, to know what percentage of cells exhibited baseline control, we examined the ACh effect on the background discharge, which was measured during the presentation of a blank stimulus with 0% contrast. Control and ACh conditions were compared by adopting the mean ± 3 SD criteria for statistical significance (Disney et al. 2007). A significant increase in background discharge was observed in 17 of the 67 facilitated cells.

These 17 cells include cells showing response gain control. To know how many cells showed baseline control only, we subtracted the background discharge from the visual responses and performed nonparametric analysis on the response areas of the subtracted responses. If ACh caused baseline control only, its facilitatory effect should disappear after this subtraction. This occurred in only three of the 17 cells, meaning that the remaining 14 cells were accompanied by response gain control. Therefore, response gain control was observed totally in 64 of 67 facilitated cells, indicating that the predominant ACh effect was on response gain control.

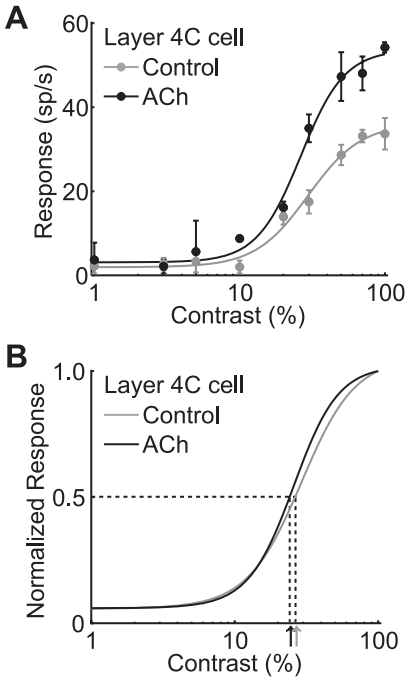

Fig. 4. ACh effect on the contrast-response function of a simple cell. *A*: fits were obtained for contrast-response functions under no drug condition (Control; gray dots and line) and microiontophoretic administration of ACh (black dots and line; ejecting current, 10 nA). R_{max} increased with ACh administration. Error bars = SD. *B*: normalized contrast-response functions. The 2 contrast-response functions in *A* were normalized to each R_{max} to examine the effects of ACh on their shape and C_{50}. The shapes and C_{50} values (arrows) are similar, indicating that the contrast gain is not affected by ACh administration in this facilitated cell.

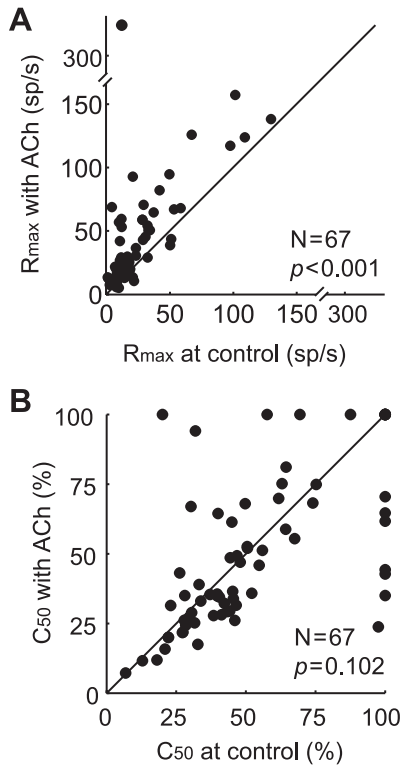

Fig. 5. Population data of R_{max} and C_{50} obtained from facilitated cells under control and ACh conditions. Diagonal lines show 1 to 1 ratios. *A*: most data points fall above the diagonal line, showing that R_{max} values were increased by ACh administration ($P < 0.001$, Wilcoxon signed-rank test). *B*: unlike R_{max}, ACh did not systematically alter C_{50} ($P = 0.102$, Wilcoxon signed-rank test).

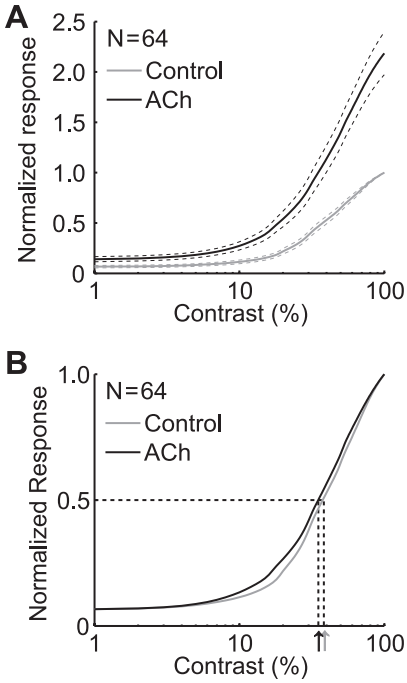

Fig. 6. Population data of the contrast-response functions from cells facilitated by ACh. *A*: population average of contrast-response functions from cells whose visual responses were significantly facilitated by ACh ($n = 64$). Control and ACh data are shown by the gray and black lines, respectively. Dashed lines show SE. *B*: population average of normalized contrast-response functions. Data from the cells used in *A* were normalized to the R_{max} obtained under each condition and averaged. The gray and black arrows show C_{50} of the data obtained from the control and ACh condition, respectively. The shapes of the 2 contrast-response functions and the C_{50} values are similar, suggesting that ACh did not change the contrast gain across the whole population.

For the 64 cells with response gain control, we normalized and averaged their responses to show the contrast-response curves of the population responses. ACh clearly enhanced the response gain, showing a significant increase in R_{max} ($P < 0.01$, Wilcoxon signed-rank test; Fig. 6*A*). To compare the shape of the contrast-response curves, the contrast-response function of the ACh condition in Fig. 6*A* was normalized to R_{max} of the population average (Fig. 6*B*), showing that the shape and C_{50} of the two contrast-response functions are almost identical. Thus population data also reaffirm that ACh plays an important role in enhancing the response gain.

Additionally, we examined the laminar distribution of the 64 cells showing response gain control and found that they were observed across all cortical layers (supragranular *layers 2/3*, seven cells; granular *layer 4*, 27 cells; infragranular *layers 5* and *6*, 26 cells; unknown, four cells).

Receptor Subtypes Mediating the Facilitatory ACh Effect

Our data demonstrate that ACh facilitates visual responses mainly by controlling the response gain across all cortical layers. To identify the responsible receptor subtypes, we examined the effect of At on the facilitatory effect. At blocked the effect in most cells tested (49 of 51 cells), although the degree of the antagonistic effect differed cell to cell. Figure 7 shows two cells with different degrees of antagonistic effects by At. In one, the facilitatory effect was completely antagonized, suggesting that it was mediated by mAChRs only (Fig. 7*A*). In the other, the facilitatory effect was partially antagonized (43%; Fig. 7*B*), suggesting that in this case, the facilitatory effect was also mediated by non-mAChRs.

To quantify the relative contribution of mAChRs to the facilitatory effect, we estimated what proportion was antagonized by At (see MATERIALS AND METHODS). Figure 8*A* shows that the relative contribution of mAChRs varied cell to cell. Thirty of the 51 facilitated cells showed that mAChRs contributed >80%, but in two cells, mAChRs made no contribution. In the remaining 19 cells, the facilitatory effect was partially antagonized by At (21–65%), suggesting partial contribution by mAChRs. We confirmed that this partial effect was not due to insufficient levels of At by noting that the antagonistic effect was unchanged even when increasing the ejecting current.

We also analyzed the laminar distribution of the relative contributions of mAChRs and non-mAChRs on ACh facilitatory effects in V1 (Fig. 8*B*; $n = 47$). An antagonistic effect by At was observed across all cortical layers, which is consistent with the expression of mAChR proteins (Disney and Aoki 2008; Disney et al. 2006). We estimated laminar differences in the relative contribution of non-mAChRs by calculating their mean percentage contribution in each layer. Contributions were 5%, 0%, 31%, 35%, 24%, and 21% in *layers 2/3*, *4A*, *4B*, *4C*, *5*, and *6*, respectively, suggesting that non-mAChRs are most effective in *layer 4C*. Because nAChRs are richly expressed in the thalamocortical-recipient *layer 4C* of V1 in macaque monkeys (Disney et al. 2007; Han et al. 2003), the probable candidate of the non-mAChRs is nAChRs.

To confirm this point directly, we examined the effects of mecamylamine, a specific nAChR antagonist. Figure 9 shows

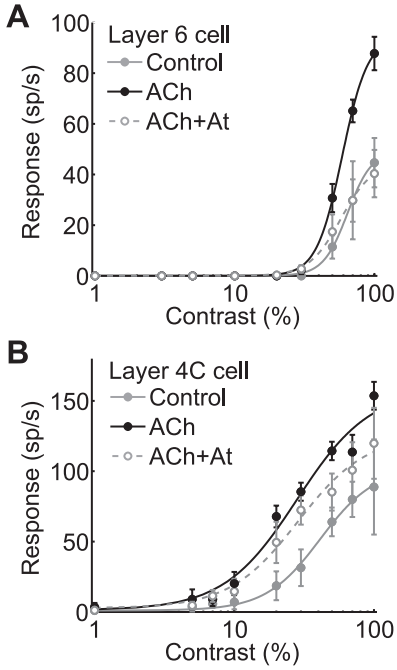

Fig. 7. Atropine (At) completely or partially blocked the facilitatory ACh effect. Fits were obtained for contrast-response functions for 3 drug conditions: control (filled gray circles and solid lines), the administration of ACh (black circles and solid lines; *A*, 50 nA; *B*, 10 nA), and the coadministration of ACh (*A*, 50 nA; *B*, 10 nA) and At (open gray circles and dashed lines; *A*, 60 nA; *B*, 30 nA). The facilitatory effect was blocked completely by At in *A*, suggesting that in this cell, it is mediated exclusively by muscarinic ACh receptors (mAChRs). On the other hand, the response facilitation was only partially blocked in the cell shown in *B*, suggesting that it was mediated by mAChRs and non-mAChRs. Error bars = SD.

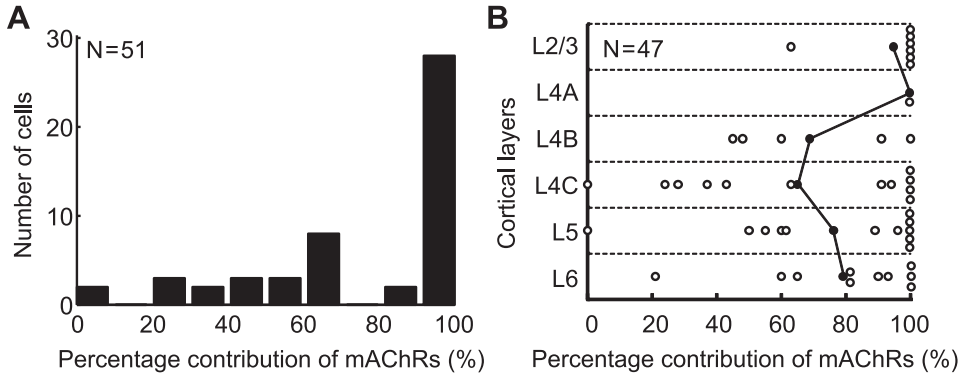

Fig. 8. Relative contribution of mAChRs to the facilitatory ACh effect. We calculated the response area of the contrast-response curves obtained under 3 drug conditions—control, "ACh only", and "ACh + At"—and then quantified the relative contribution of mAChRs to the ACh-induced response facilitation (see MATERIALS AND METHODS). *A*: At antagonized the facilitatory effects in most cases (49 of 51 cells), although the degree differed cell to cell. *B*: the relative contributions of mAChRs in individual cells are plotted as open circles for each cortical layer ($n = 47$). Filled circles show the mean of population data at each layer. The effects of mAChRs were distributed across all cortical layers, and their relative contribution was the smallest in *layer 4C*.

a typical example of a cell tested by two antagonists for mAChRs and nAChRs. The ACh facilitatory effect was partially blocked (60%) by At, and the residual facilitatory effect was completely blocked by mecamylamine, suggesting that response facilitation was mediated by both mAChRs and nAChRs. The same results were observed in all tested cells ($n = 9$) distributed over various layers (*layer 4B*, one cell; *layer 4C*, three cells; *layer 5*, four cells; and *layer 6*, one cell). Taken together with the laminar analysis in Fig. 8, nAChRs seem to contribute to the facilitatory action across almost all cortical layers with some bias toward the thalamocortical-recipient *layer 4C*.

If droperidol inhibits nAChRs, the relative contribution of nAChRs would be underestimated and therefore, should be increased by withdrawal of droperidol. However, there was no significant difference between the two anesthetic conditions in the degree or laminar distribution of the relative contributions of mAChRs and nAChRs (relative contribution of mAChRs, $P = 0.462$, unpaired *t*-test; laminar distribution, $P = 0.906$, χ^2 test; data not shown). Therefore, our finding that the contribution of nAChRs is relatively smaller than that of mAChRs cannot be attributed to an effect of droperidol on nAChRs.

The suppressive ACh effect was mediated by mAChRs

ACh caused a suppressive effect in a small number of cells (five simple cells and five complex cells) distributed across most layers (Fig. 2; $n = 8$). To identify which receptor subtypes mediate this effect, we investigated the effects of At, finding that it completely antagonized the suppressive effect in all cells tested (nine cells). Figure 10 shows examples of *layers 4B* (Fig. 10*A*) and *4C* (Fig. 10*B*) cells. The suppressive ACh effect on visual responses was completely antagonized by At, suggesting that ACh exerted its response suppression via mAChRs only. To quantify the suppressive effect by ACh and

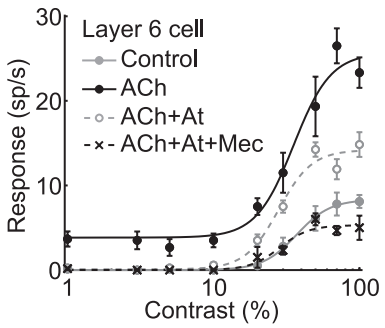

Fig. 9. The facilitatory ACh effect was mediated via mAChRs as well as nicotinic AChR (nAChRs). An example is the *layer 6* cell, showing complete blockade of the ACh facilitatory effect by simultaneous administration of At and mecamylamine (Mec). Administration of ACh (40 nA) alone facilitated visual responses. This ACh effect was partially blocked by At (20 nA), and the residual facilitatory effect was blocked by the additional administration of mecamylamine (20 nA), suggesting that the ACh facilitatory effect was mediated by both mAChRs and nAChRs. Error bars = SD.

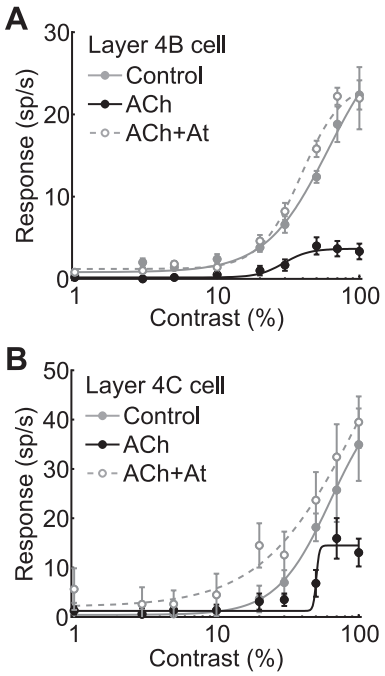

Fig. 10. Example cells showing response suppression by ACh administration. Graphs show data for example cells in *layers 4B* (*A*) and *4C* (*B*). Fits were obtained for contrast-response functions for control (filled gray circles and gray solid lines), the administration of ACh (black circles and black solid lines), and the coadministration of ACh + At (open gray circles and gray dashed lines). *A* and *B*: the ejecting current of ACh or At was 50 nA. In both cells, administration of ACh caused response suppression, which was blocked completely by At. Error bars = SD.

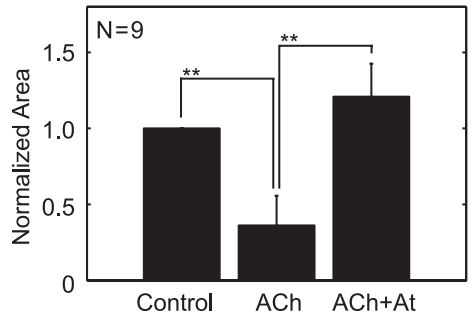

Fig. 11. Suppressive ACh effect was completely mediated by mAChRs. ACh significantly decreased the response area (**$P < 0.01$, Wilcoxon signed-rank test), whereas the administration of At significantly and completely abolished the suppressive effect (**$P < 0.01$, Wilcoxon signed-rank test), suggesting that the suppressive effect was exclusively mediated by mAChRs. Error bar = SD.

the antagonistic effect by At, we normalized the response area of each drug condition (ACh only or ACh + At) by the control-response area and calculated average values for the nine suppressed cells (Fig. 11). We confirmed that ACh significantly reduced the response area ($P < 0.01$, Wilcoxon signed-rank test), and administration of At counteracted this suppressive effect ($P < 0.01$, Wilcoxon signed-rank test). Thus our results suggest that mAChRs are involved in response suppression as well as response facilitation.

DISCUSSION

The main results of the present study can be summarized as follows: *1*) ACh significantly modulated the visual responses in 77 of 124 V1 cells (62%) across all cortical layers, mainly with facilitatory effects (67 of 77 cells); *2*) ACh increased the response gain of the contrast-response relationship in most of these facilitated cells (64 of 67 cells); *3*) the effect on response gain was predominantly mediated by mAChRs, but nAChRs also contributed; *4*) ACh suppressed visual responses in a small population of cells; and *5*) this suppressive modulation was mediated exclusively by mAChRs (nine of nine cells).

Facilitatory ACh Effect in V1

Our data show that the predominant ACh effect in V1 is response facilitation. This finding is consistent with that of previous studies using other primates, including the marmoset (Roberts et al. 2005; Zinke et al. 2006) and the crab-eating monkey (Disney et al. 2007). Similar facilitatory effects by ACh have been seen in the cat (Müller and Singer 1989; Murphy and Sillito 1991; Sato et al. 1987b; Sillito and Kemp 1983) and rat (Goard and Dan 2009). Therefore, ACh seems to enhance neuronal responses ubiquitously in the early visual cortex of various animal species.

We also found that At blocks the ACh facilitatory effect in all cortical layers, albeit at varying degrees (Fig. 8*B*). Consistent with this, mAChR proteins (m1 and m2) have been observed in cells throughout cortical layers in macaque V1 (Disney and Aoki 2008; Disney et al. 2006; Mrzljak et al. 1993), suggesting that m1- and m2-AChRs play important roles in facilitatory modulation. It remains to be clarified, however, how this facilitatory effect is mediated by mAChRs in the neuronal circuitry of V1. Previous *in vitro* studies have reported that ACh exerts modulatory effects directly on pyramidal cells and inhibitory interneurons via m1- and m2-AChRs, respectively (McCormick and Prince 1985, 1986). It is known that activating m1- and m2-AChRs induce different neuronal responses and that enhancing and suppressing the response can be done by stimulating phosphatidylinositol turnover and inhibiting intracellular cyclic adenosine monophosphate production, respectively (Lucas-Meunier et al. 2003). Therefore, mAChRs-mediated response facilitation can be explained by both direct facilitation of the recorded cell and indirect facilitation by suppressing inhibitory interneurons.

To determine the functional role of ACh in visual information processing, we tested ACh effects on the contrast-response function, which describes the input (stimulus contrast) − output (response magnitude) relationship. We found that ACh predominantly acts as a gain controller across all cortical layers and that the response gain control is mainly mediated by mAChRs.

We also found that nAChRs contribute to the response gain control of ACh. The nAChRs were estimated to contribute 5%, 0%, 31%, 35%, 24%, and 21% of the response in *layers 2/3*, *4A*, *4B*, *4C*, *5*, and *6*, respectively (Fig. 8*B*). Thus the relative contribution of mAChRs and nAChRs varied, depending on the laminar location of the cell. Consistent with this laminar bias, nAChRs are richly expressed in the thalamocortical-recipient *layer 4C* of macaque V1 (Disney et al. 2007; Han et al. 2003), and the microiontophoretic administration of nicotine increases the response gain via nAChRs in *layer 4C* neurons (Disney et al. 2007). Thus ACh seems to exert response gain control via mAChRs across all cortical layers but also via nAChRs, particularly in the thalamocortical-recipient *layer 4C*.

Suppressive ACh Effect in V1

ACh also exerts a suppressive effect via mAChRs (see Figs. 10 and 11). Since m1- and m2-AChRs are expressed in both excitatory and inhibitory neurons (Disney and Aoki 2008; Disney et al. 2006), at least two possible mechanisms can explain this suppression: direct suppression via m2-AChRs and indirect suppression by activating inhibitory interneurons via m1-AChRs.

Activation of m2-AChRs inhibits adenylate cyclase via pertussis toxin-sensitive G-protein (G_i), which causes the inhibition of voltage-gated Ca^{2+} channels and subsequent suppression of neuronal activity (Lucas-Meunier et al. 2003). Disney et al. (2006) confirmed that a certain population of excitatory neurons expresses m2-AChRs in V1 of the macaque monkey. Therefore, it may be that ACh directly suppresses the visual responses of neurons via m2-AChRs activation.

On the other hand, Müller and Singer (1989) reported that ACh suppresses the visual responses of V1 neurons in the cat and that this suppression is abolished by the local administration of bicuculline, a $GABA_A$ receptor antagonist, implying that the activation of GABAergic interneurons can mediate the ACh-induced response suppression. Consistent with this, in vitro studies have demonstrated that the cholinergic suppressive effect can be abolished by the local administration of a $GABA_A$ receptor antagonist (McCormick and Prince 1986; Müller 1987). It can also be abolished by scopolamine, an mAChR antagonist (McCormick and Prince 1985, 1986), suggesting that the indirect suppressive effect is mediated by mAChRs.

Recently, Disney et al. (2007) reported another type of suppression caused by the microiontophoretic administration

of nicotine. Since activation of nAChRs opens sodium channels to cause response facilitation, nicotine-induced suppression cannot be explained by direct suppression through activation of nAChRs. Therefore, nicotine seems to cause indirect suppression by activating GABAergic interneurons. Taken together with our findings, this suggests at least three possible mechanisms via the two receptor subtypes for suppressive modulation. These mechanisms are not necessarily exclusive of each other, and both receptor subtypes could mediate the suppressive modulation by ACh. However, the actual underlying mechanisms remain unclear because of the small number of suppressed cells observed in both Disney's study (2007) and our own. Further investigations are therefore needed.

Functional roles of Cholinergic Modulation in V1

Because ACh is continuously released in the visual cortex of conscious animals (Jiménez-Capdeville and Dykes 1996), a response gain control involving ACh should operate during normal vision. This is supported by clinical studies (Ballard et al. 1999; Campbell et al. 2001). Dementia with Lewy bodies (DLB) has been recognized as a new form of dementia characterized by the frequent appearance with space- and object-recognition disorders and frequent experiences of visual hallucination. In DLB patients, administration of donepezil hydrochloride, an acetylcholinesterase inhibitor, can dramatically improve these symptoms (Campbell et al. 2001), suggesting that ACh is essential for normal perceptual and cognitive function by the visual system. Additionally, the deprivation of ACh by lesioning the basal forebrain reduces the response magnitude of many V1 neurons in the cat (Sato et al. 1987a). These results argue that a basal level of ACh is essential for normal visual information processing.

In behaving monkeys, ACh contributes to attentional modulation of visual responses in V1, especially via mAChRs (Herrero et al. 2008). Recent studies demonstrated that the attentional modulation is mainly mediated by glutamatergic feedback from higher cortical areas (Gregoriou et al. 2009), but cholinergic inputs are necessary to permit V1 neurons to be responsive to an attentional signal (Deco and Thiele 2011; Harris and Thiele 2011). Thus ACh in V1 seems to be involved in not only response gain control but also a permissive role in behavioral context-dependent response modulation. Interestingly, Thiele et al. (2009) reported that the effect of attentional modulation on V1 neurons of monkeys performing attention tasks is constant across a wide range of stimulus contrast (additive gain modulation), in which attention adds a fixed amount to the neuronal response once the stimulus has sufficient contrast to become visible. Therefore, neuronal responses in V1 seem to be regulated by attention-related additive gain modulation, as well as cholinergic response gain control.

ACh also appears to affect the encoding of stimulus features, as many studies have demonstrated that V1 receptive field properties, such as orientation selectivity, direction selectivity, and length tuning, can be modulated by the microiontophoretic administration of ACh (Müller and Singer 1989; Murphy and Sillito 1991; Roberts et al. 2005; Sato et al. 1987b; Sillito and Kemp 1983; Zinke et al. 2006). Therefore, not only the response gain but also the encoding of stimulus features can be controlled by ACh in normal vision. Thus the present results would provide important information about the functional roles of ACh in normal visual information processing, the neuronal mechanism underlying it, and clinical implications for diseases associated with ACh deficiency.

ACKNOWLEDGMENTS

We express our sincere gratitude to Dr. Ichiro Fujita for useful comments and discussion. We also thank Shin-ichiro Hara, Naofumi Suematsu, Akihiro Kimura, and Dr. Tomoyuki Naito for help with the experimental procedures and Drs. Peter Karagiannis and Quentin S. Fischer for help in improving our English.

GRANTS

This work was supported by grants from Grant-in-Aid for Scientific Research (C) 21500369 to H. Sato and 22500573 to S. Shimegi. H. Osaki was supported by the Japan Society for the Promotion of Science Research Fellowships.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S. Soma, S. Shimegi, and H.S. conception and design of research; S. Soma, S. Shimegi, and H.O. performed experiments; S. Soma and S. Shimegi analyzed data; S. Soma, S. Shimegi, H.O., and H.S. interpreted results of experiments; S. Soma and S. Shimegi prepared figures; S. Soma, S. Shimegi, and H.S. drafted manuscript; S. Soma, S. Shimegi, H.O., and H.S. edited and revised manuscript; S. Soma, S. Shimegi, H.O., and H.S. approved final version of manuscript.

REFERENCES

Ballard C, Holmes C, McKeith I, Neill D, O'Brien J, Cairns N, Lantos P, Perry E, Ince P, Perry R. Psychiatric morbidity in dementia with Lewy bodies: a prospective clinical and neuropathological comparative study with Alzheimer's disease. *Am J Psychiatry* 156: 1039–1045, 1999.

Barlow HB, Blakemore C, Pettigrew JD. The neural mechanism of binocular depth discrimination. *J Physiol* 193: 327–342, 1967.

Campbell S, Stephens S, Ballard C. Dementia with Lewy bodies: clinical features and treatment. *Drugs Aging* 18: 397–407, 2001.

Deco G, Thiele A. Cholinergic control of cortical network interactions enables feedback-mediated attentional modulation. *Eur J Neurosci* 34: 146–157, 2011.

Disney AA, Aoki C. Muscarinic acetylcholine receptors in macaque V1 are most frequently expressed by parvalbumin-immunoreactive neurons. *J Comp Neurol* 507: 1748–1762, 2008.

Disney AA, Aoki C, Hawken MJ. Gain modulation by nicotine in macaque V1. *Neuron* 56: 701–713, 2007.

Disney AA, Domakonda KV, Aoki C. Differential expression of muscarinic acetylcholine receptors across excitatory and inhibitory cells in visual cortical areas V1 and V2 of the macaque monkey. *J Comp Neurol* 499: 49–63, 2006.

Flood P, Coates MK. Droperidol inhibits GABA and neuronal nicotinic receptor activation. *Anesthesiology* 96: 987–993, 2002.

Gallagher M, Colombo PJ. Ageing: the cholinergic hypothesis of cognitive decline. *Curr Opin Neurobiol* 5: 161–168, 1995.

Goard M, Dan Y. Basal forebrain activation enhances cortical coding of natural scenes. *Nat Neurosci* 12: 1444–1449, 2009.

Gregoriou GG, Gotts SJ, Zhou H, Desimone R. High-frequency, long-range coupling between prefrontal and visual cortex during attention. *Science* 324: 1207–1210, 2009.

Han ZY, Zoli M, Cardona A, Bourgeois JP, Changeux JP, Le Novere N. Localization of [3H]nicotine, [3H]cytisine, [3H]epibatidine, and [125I]alpha-bungarotoxin binding sites in the brain of *Macaca mulatta*. *J Comp Neurol* 461: 49–60, 2003.

Harris KD, Thiele A. Cortical state and attention. *Nat Rev Neurosci* 12: 509–523, 2011.

Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A. Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature* 454: 1110–1114, 2008.

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

Jones GM, Sahakian BJ, Levy R, Warburton DM, Gray JA. Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. *Psychopharmacology (Berl)* 108: 485–494, 1992.

Lehmann J, Nagy JI, Atmadia S, Fibiger HC. The nucleus basalis magnocellularis: the origin of a cholinergic projection to the neocortex of the rat. *Neuroscience* 5: 1161–1174, 1980.

Lucas-Meunier E, Fossier P, Baux G, Amar M. Cholinergic modulation of the cortical neuronal network. *Pflügers Arch* 446: 17–29, 2003.

McCormick DA, Prince DA. Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. *J Physiol* 375: 169–194, 1986.

McCormick DA, Prince DA. Two types of muscarinic response to acetylcholine in mammalian cortical neurons. *Proc Natl Acad Sci USA* 82: 6344–6348, 1985.

Mesulam MM, Van Hoesen GW. Acetylcholinesterase-rich projections from the basal forebrain of the rhesus monkey to neocortex. *Brain Res* 109: 152–157, 1976.

Mrzljak L, Levey AI, Goldman-Rakic PS. Association of m1 and m2 muscarinic receptor proteins with asymmetric synapses in the primate cerebral cortex: morphological evidence for cholinergic modulation of excitatory neurotransmission. *Proc Natl Acad Sci USA* 90: 5194–5198, 1993.

Müller CM. Differential effects of acetylcholine in the chicken auditory neostriatum and hyperstriatum ventrale—studies in vivo and in vitro. *J Comp Physiol A* 161: 857–866, 1987.

Müller CM, Singer W. Acetylcholine-induced inhibition in the cat visual cortex is mediated by a GABAergic mechanism. *Brain Res* 487: 335–342, 1989.

Murphy PC, Sillito AM. Cholinergic enhancement of direction selectivity in the visual cortex of the cat. *Neuroscience* 40: 13–20, 1991.

Newhouse PA, Potter A, Kelton M, Corwin J. Nicotinic treatment of Alzheimer's disease. *Biol Psychiatry* 49: 268–278, 2001.

Newhouse PA, Sunderland T, Tariot PN, Blumhardt CL, Weingartner H, Mellow A, Murphy DL. Intravenous nicotine in Alzheimer's disease: a pilot study. *Psychopharmacology (Berl)* 95: 171–175, 1988.

Niell CM, Stryker MP. Highly selective receptive fields in mouse visual cortex. *J Neurosci* 28: 7520–7536, 2008.

Roberts MJ, Zinke W, Guo K, Robertson R, McDonald JS, Thiele A. Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex. *J Neurophysiol* 93: 2062–2072, 2005.

Sato H, Hata Y, Hagihara K, Tsumoto T. Effects of cholinergic depletion on neuron activities in the cat visual cortex. *J Neurophysiol* 58: 781–794, 1987a.

Sato H, Hata Y, Masui H, Tsumoto T. A functional role of cholinergic innervation to neurons in the cat visual cortex. *J Neurophysiol* 58: 765–780, 1987b.

Sato H, Katsuyama N, Tamura H, Hata Y, Tsumoto T. Mechanisms underlying orientation selectivity of neurons in the primary visual cortex of the macaque. *J Physiol* 494: 757–771, 1996.

Sengpiel F, Baddeley RJ, Freeman TC, Harrad R, Blakemore C. Different mechanisms underlie three inhibitory phenomena in cat area 17. *Vision Res* 38: 2067–2080, 1998.

Sillito AM, Kemp JA. Cholinergic modulation of the functional organization of the cat visual cortex. *Brain Res* 289: 143–155, 1983.

Skottun BC, De Valois RL, Grosof DH, Movshon JA, Albrecht DG, Bonds AB. Classifying simple and complex cells on the basis of response modulation. *Vision Res* 31: 1079–1086, 1991.

Thiele A, Pooresmaeili A, Delicato LS, Herrero JL, Roelfsema PR. Additive effects of attention and stimulus contrast in primary visual cortex. *Cereb Cortex* 19: 2970–2981, 2009.

Watakabe A, Komatsu Y, Sadakane O, Shimegi S, Takahata T, Higo N, Tochitani S, Hashikawa T, Naito T, Osaki H, Sakamoto H, Okamoto M, Ishikawa A, Hara S, Akasaki T, Sato H, Yamamori T. Enriched expression of serotonin 1B and 2A receptor genes in macaque visual cortex and their bidirectional modulatory effects on neuronal responses. *Cereb Cortex* 19: 1915–1928, 2009.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* 215: 1237–1239, 1982.

Williford T, Maunsell JH. Effects of spatial attention on contrast response functions in macaque area V4. *J Neurophysiol* 96: 40–54, 2006.

Wong-Riley M. Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. *Brain Res* 171: 11–28, 1979.

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