Synaptically Released and Exogenous ACh Activates Different Nicotinic Receptors to Enhance Evoked Glutamatergic Transmission in the Lateral Geniculate Nucleus

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Guo, Jian-Zhong, Yingbing Liu, Eva M. Sorenson, and Vincent A. Chiappinelli. Synaptically released and exogenous ACh activates different nicotinic receptors to enhance evoked glutamatergic transmission in the lateral geniculate nucleus. J Neurophysiol 94: 2549–2560, 2005. First published June 22, 2005; doi:10.1152/jn.00339.2005 doi:. The effects of activation of nicotinic acetylcholine receptors (nAChRs) on glutamatergic transmission in the ventral lateral geniculate nucleus (LGNv) were examined in chick brain slices. Whole cell recordings showed that monosynaptic postsynaptic currents (PSCs) evoked in LGNv neurons by optic tract stimulation were blocked by glutamate receptor antagonists. Exogenously applied nicotine (0.5 μM), choline (1 mM), or acetylcholine (ACh, 100 μM) markedly increased (>3-fold) these evoked PSCs. Potentiation by ACh was dose-dependent and did not desensitize during a 5-min application. In a second set of experiments, the effect of releasing endogenous ACh by stimulating the lateral portion of the LGNv through a separate conditioning electrode before optic tract stimulation was examined. Conditioning stimulation trains increased PSCs by an average of 5.2-fold, an effect dependent on both the intensity and number of conditioning pulses. This increase in PSC amplitude was most likely caused by released ACh activating α6- and/or α3-containing nAChRs because it was blocked by 100 nM α-conotoxin MII, 100 nM dihydroy-β-erythroidine (DHβE), and 0.1–1.0 μM methyllycaconitine (MLA). In contrast, exogenously applied ACh increased PSC amplitude by activating a pharmacologically different population of nAChRs because this effect was inhibited by 100 nM α-bungarotoxin, 50 nM MLA, and a high concentration (30 μM) of DHβE, indicating that α7- and/or α8-containing receptors were involved. The results are consistent with a model whereby α6- and/or α3-containing nAChRs on retinal ganglion cell nerve terminals are located preferentially at cholinergic synapses, whereas α7- and/or α8-containing receptors are primarily extrasynaptic.

INTRODUCTION

Nicotinic transmission plays a key role during the development of mammalian and avian visual systems. Cholinergic nerve fibers and nicotinic acetylcholine receptors (nAChRs) are present throughout the vertebrate visual nervous system, including the retina, lateral geniculate nucleus (LGN), and certain layers of the visual cortex. Nicotinic receptors mediate the initiation of spontaneous bursts of wave-like activity in the developing retina (Feller et al. 1996; Sernagor et al. 2000). In mice lacking the β2 nAChR subunit, these retinal waves are absent, and the pattern of innervation of the LGN by retinal ganglion cells is abnormal (McLaughlin et al. 2003; Muir-
ously superfused (2–3 ml/min) at room temperature with ACSF, and bicuculline (10 μM) was included in most experiments to eliminate GABAergic currents. All efforts were made to minimize both the suffering and the number of animals used in these experiments, and all experiments conformed to National Institutes of Health guidelines on the ethical use of animals.

**Electrophysiological methods**

Whole cell patch-clamp recordings in brain slices were made from LGNv neurons visualized with Nomarski optics as described in Guo et al. 1998. Patch pipettes were fabricated from borosilicate glass with a two-stage microelectrode puller to produce a tip opening of 1–2 μm with a resistance of 4–8 MΩ. The pipette solution contained (in mM) 150 K-gluconate, 2 MgCl₂, 2 EGTA, 2 Mg-ATP, and 10 HEPES, adjusted to pH 7.3 with 1.0 N KOH. Biocytin (0.3%) was added to the recording solution to facilitate postrecording morphological studies. When examining the voltage dependence of the evoked PSCs, 150 mM Cs-gluconate was used instead of K-gluconate. Electrical stimulation of retinal ganglion cell afferents to the LGNv was accomplished with a concentric bipolar electrode (50-μm tip; FHC Corp.) placed onto the surface of the optic tract about 1 mm ventral to the LGNv (Fig. 1), similar to the approach described by Dye and Karten (1996). Stimulation pulses (5- to 200-μA intensity, 200- to 300-μs duration) were triggered at a frequency of 0.008–0.033 Hz, and evoked PSCs were recorded in LGNv neurons. In experiments examining the effects of endogenously released ACh, a second stimulating electrode was placed onto the surface of the lateral portion of the LGNv (Fig. 1). This electrode was used to provide conditioning stimulation trains. Conditioning stimulation trains (3–8 pulses long, 50- to 200-μs duration at 25 Hz) were triggered at a frequency of 0.008–0.017 Hz and were delivered 0.2–1.0 s before optic tract stimulation. Signals were obtained with an Axopatch-200B amplifier (Axon Instruments, Foster City, CA) in voltage-clamp mode. Data were acquired with Clampex Ver.7.0 (Axon Instruments).

**Data analysis**

To quantitate evoked glutamatergic synaptic transmission, three to five consecutive responses evoked by optic tract stimulation were obtained before and during exposure to an exogenous nicotinic agonist, either in normal ACSF or in the presence of various nicotinic antagonists. These responses were averaged and plotted with Origin 7.0. A similar approach was taken for conditioning stimulation experiments, except that the conditioning stimulus train took the place of the exogenous nicotinic agonist. Rise time was calculated at 10–90% of the peak amplitude. When comparing only two data sets, a two-tailed t-test was used with P < 0.05 chosen as the level of significance. For nicotinic antagonist experiments, evoked current amplitudes under control conditions were compared with both those in the presence of ACh (or conditioned stimulation) and those determined in the presence of ACh (or conditioned stimulation) + antagonist. Statistical differences between these multiple amplitudes were determined using the two-tailed ANOVA with the Tukey-Kramer multiple comparisons test as a posttest. The level of significance chosen was P < 0.05. Results are expressed as means ± SE.

**Agonist and antagonist application**

Nicotinic antagonist application was done by bath superfusion for ≥15 min before application of agonist. Agonist solutions contained the antagonist at the appropriate concentration whenever the effects of an antagonist were being examined. A separate, gravity-fed U-tube system was used to deliver agonists to LGNv neurons (Alkondon and Albuquerque 1993). The tip of the U-tube had a diameter of 150 μm, and the lower rim of the U-tube tip was placed 2–3 mm from the LGNv neuron being recorded from. The in-flow for all solutions delivered by U-tube into the recording chamber was gravity-fed, whereas the U-tube out-flow from the chamber was regulated by vacuum to provide a steady out-flow suction pressure. This out-flow suction system prevented any leakage of agonist solutions into the bath between agonist applications.

**Visualization of LGNv neurons**

After electrophysiological recordings were completed, the brain slices were placed in 4% paraformaldehyde in 0.1 M phosphate buffer. To visualize LGNv neurons that were filled with biocytin during recording, brain slices were washed three times in 0.1 M PBS and incubated for at least 1 h in streptavidin conjugated to Alexa 568 (1 μg/ml; Molecular Probes) and Nissl green (1:100; Molecular Probes) in 0.1 M PBS. The slices were washed, mounted on slides, and coverslipped using 90% glycerol, 10% PBS, and 4% propyl gallate for the mounting media. An epi-fluorescent microscope equipped with a digital camera connected to a computer running Adobe Photoshop was used to capture low-power images showing the localization of filled neurons within the LGNv. A Bio-Rad 1024 scanning laser confocal microscope equipped with a krypton-argon laser was used to image filled cells in more detail. Z stacks of optical sections were collected for each filled neuron. Each Z stack was converted into a projection image. The projection images making up

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**Figure 1** Placement of stimulating and recording electrodes. Image is a coronal section of the chick brain that has been immunohistochemically stained for choline acetyltransferase using a cobalt-enhanced diaminobenzidine reaction (Sorensen et al. 1989). There is a dense network of cholinergic fibers throughout the ventral lateral geniculate nucleus (LGNv). Stimulating electrodes were positioned with micromanipulators onto the optic tract (optic tract stimulation) and the lateral portion of the LGNv (conditioning stimulation). A patch pipette (recording pipette) was used to record electrical activity in individual LGNv lamina interna neurons. li, lamina interna; ne, neuropil; Rt, nucleus rotundus.
each individual neuron were imported to Adobe Photoshop 7.0, where they were aligned to create a photomontage of each neuron.

Materials

Drugs used were obtained from the following sources: acetylcholine chloride, atropine sulfate (atropine), biocuculline (MLA), methyllycaconitine (MLA), and (-)-nicotine bitartrate were purchased from Sigma (St. Louis, MO); dihydro-beta-erythroidine (DHβE), 6,7-dinitroquinoxaline-2,3-dione (DNQX), and AP5 were from RBI/Sigma (Natick, MA); and TTX was from Calbiochem (San Diego, CA). Purified α-conotoxins (α-CTxs) were gifts from Dr. J. M. McIntosh, University of Utah. α-BgTx and κ-BgTx were purified from the crude venom of Bungarus multicinctus as described (Chiappinelli 1983). Atropine, DHβE, and nicotine were prepared in deionized water to make a 10 mM stock solution. Choline and ACh were prepared in deionized water to make a 1 M stock solution. MLA was prepared in dimethyl sulfoxide (DMSO) to make a 10 mM stock solution. Choline chloride (choline), methyllycaconitine (MLA), and (-)-nicotine bitartrate were purchased from Sigma (St. Louis, MO); dihydro-beta-erythroidine (DHβE), 6,7-dinitroquinoxaline-2,3-dione (DNQX), and AP5 were from RBI/Sigma (Natick, MA); and TTX was from Calbiochem (San Diego, CA). Purified α-conotoxins (α-CTxs) were gifts from Dr. J. M. McIntosh, University of Utah. α-BgTx and κ-BgTx were purified from the crude venom of Bungarus multicinctus as described (Chiappinelli 1983). Atropine, DHβE, and nicotine were prepared in deionized water to make a 10 mM stock solution. Choline and ACh were prepared in deionized water to make a 1 M stock solution. MLA was prepared in dimethyl sulfoxide (DMSO) to make a 10 mM stock solution. Choline chloride (choline), methyllycaconitine (MLA), and (-)-nicotine bitartrate were purchased from Sigma (St. Louis, MO); dihydro-beta-erythroidine (DHβE), 6,7-dinitroquinoxaline-2,3-dione (DNQX), and AP5 were from RBI/Sigma (Natick, MA); and TTX was from Calbiochem (San Diego, CA).

RESULTS

Stimulation of optic tract evokes both glutamatergic and GABAergic postsynaptic currents in LGN neurons

The LGNv receives a major sensory input from the optic tract, which consists of the axons of retinal ganglion cells (Guiloff 1991; Gunturkun and Karten 1991; Wu et al. 1999). We placed a stimulating electrode onto the optic tract ventromedial to the LGNv in a coronal brain slice and examined electrically evoked responses using whole cell voltage-clamp recordings from visually identified LGNv lamina interna neurons (Fig. 1). When these LGNv neurons were clamped at −50 mV, single-pulse stimulation of the optic tract evoked biphasic PSCs under control conditions (ACSF with 1 μM atropine; Fig. 2Aa, n = 4).

The short-latency, inward PSCs had a duration of <10 ms, whereas the longer-latency outward PSCs lasted ≤300 ms. The GABA<sub>A</sub> receptor antagonist bicuculline (10 μM) blocked the outward PSCs but had little or no effect on inward PSCs (Fig. 2Ab; n = 3). In contrast, DNQX (20 μM), an antagonist of non-N-methyl-D-aspartate (NMDA) receptors, substantially blocked both inward and outward PSCs (Fig. 2Ad; n = 3). After slices were treated with both DNQX and AP5 (an NMDA receptor antagonist), all evoked responses were completely blocked (Fig. 2Bb; n = 3). These results suggested that the short-latency inward current was glutamatergic and the longer-latency GABAergic response was caused by indirect activation of GABAergic neurons after optic tract stimulation. In the presence of bicuculline (10 μM), the remaining short-latency evoked PSCs reversed between −10 and +10 mV (Fig. 2C; n = 4). The PSCs decayed rapidly at negative holding potentials (−70 to −40 mV) but persisted much longer at positive holding potentials (+50 mV, n = 4, Fig. 2C), indicating that glutamate released from optic tract stimulation was acting on both non-NMDA and NMDA receptors present on LGNv neurons.
neurons. The evoked PSCs recorded in the presence of bicuculline seemed to reflect the activity of a monosynaptic glutamatergic connection. The PSCs had a constant and short latency (<5 ms) and the initial slope of the PSCs was smoothly graded with changing intensity of the stimulus (Fig. 2D; n = 3). Moreover, there was no change in latency or rise time of the PSCs when divalent cation concentrations were increased to 12 mM (Sah and Nicoll 1991) to raise the firing threshold and thereby reduce the likelihood of interneurons participating in the stimulus pathway (Fig. 2E, rise time: 4.4 ± 0.1 ms for control, 4.0 ± 0.4 ms for high divalent cations, \( P > 0.5 \) by t-test, \( n = 3 \)).

**Morphology of electrophysiologically characterized neurons**

The morphology of neurons filled with biocytin during patch recording was examined, and most of these neurons (15 of 20) exhibited many of the characteristics of projection neurons in the lamina interna of the chick LGNv as visualized with Golgi impregnation (Tombol et al. 2004), while the remaining five neurons could not be completely characterized. Cell bodies were located in the lamina interna and processes left the dorsal and ventral aspects of the soma (Fig. 3). The ventrally projecting dendrites reached down into different levels within the neuropil layer of the LGNv such that some were found in the third of the neuropil under the lamina interna (Fig. 3B), whereas others were in the middle of the neuropil (Fig. 3, A and C) or in LGNv neuropil closer to the optic tract (Fig. 3D). Many of these ventral dendrites formed “balls” (Fig. 3, A–C) within the neuropil. The dorsally oriented dendrites appeared to project past the lamina interna to the area between the nucleus rotundus and the LGNv (Fig. 3, B and C). Initial axon segments could be seen in 7 of the 20 neurons. The axons left the cell soma or one of the primary dorsal dendrites and ran medial to the nucleus rotundus (Fig. 3, A, A’, B, B’, and D). Axons that were intact some distance from the cell bodies had collaterals that ran either medial and ventral to the nucleus rotundus or into the lamina interna and neuropil of the LGNv (Fig. 3, A, A’, B, B’, and D).

**Activation of nicotinic receptors potentiates evoked glutamatergic PSCs**

We used a U-tube drug application system for agonist applications to facilitate detection of possible fast-desensitizing nicotinic responses. Bicuculline (10 \( \mu M \)) and atropine (1 \( \mu M \)) were present in all solutions throughout the following experiments to block the evoked GABAergic currents as well as any muscarinic responses. U-tube application of ACh (100 \( \mu M \)) produced very little or no direct effect on membrane current but greatly potentiated the evoked glutamatergic responses (3.7 ± 0.7-fold increase vs. control; \( P < 0.002 \) by t-test; \( n = 18 \); Fig. 4A). The time-course for this nicotinic effect on evoked responses was examined. During perfusion for 10 s with ACh (100 \( \mu M \)), the potentiation of evoked responses reached a maximum at 2 s and persisted at that level for the remainder of agonist application (Fig. 4B; \( n = 3 \)). Even after 5-min preexposure to 100 \( \mu M \) ACh, there was little change in the level of potentiation observed (Fig. 4C; \( n = 3 \)). The onset of the response to ACh appeared to be determined primarily by how fast the drug was delivered to the neurons, which depended on the gravity-feed pressure and the distance of the U-tube from the neuron. Because the nicotinic response did not desensitize over 10 s, electrical stimulation of the optic tract was routinely carried out after 4–5 s of agonist application in the following experiments. The potentiation by ACh of glutamatergic-evoked transmission exhibited dose dependence between 0.1 and 10 \( \mu M \) ACh (Fig. 5A; \( n = 3 \)). It is important to note that, because the U-tube was positioned in the flowing bath at a considerable distance (2–3 mm) from the recorded neuron, a significant dilution of the agonist occurred before reaching the neuron. We estimate this dilution to be three- to fourfold, based on a comparison of the maximum inward current generated by glutamate when applied by our U-tube method versus when applied by whole bath perfusion (\( n = 3 \)). U-tube application of other nicotinic agonists, including nicotine (0.5–1.0 \( \mu M \); 4.6 ± 2.3-fold increase; \( P < 0.05 \); \( n = 4 \)) and choline (0.3–1 mM; 3.4 ± 1.7-fold increase; \( P < 0.02 \); \( n = 9 \)), also greatly potentiated evoked glutamatergic responses (Fig. 5B). To determine whether postsynaptic nAChRs played any role in this enhancement, we examined the effect of ACh on the response of LGNv neurons to exogenously applied glutamate in the presence of TTX. As shown in Fig. 5C, U-tube application of glutamate (200 \( \mu M \)) for 5 s produced inward currents of consistent amplitude. A 10-s application of the same concentration of glutamate produced a larger response (4th response in Fig. 5C), indicating that the response to 5 s of glutamate was submaximal. Bath application of ACh (100 \( \mu M \)) did not alter the current produced by exogenously applied glutamate (\( n = 3 \)). The results are consistent with the hypothesis that activation of nicotinic receptors located on glutamatergic retinal ganglion cell terminals was responsible for the observed modulation of evoked transmission in the LGNv.

**Pharmacology of presynaptic nAChRs mediating responses to exogenously applied nicotinic agonists**

Because the LGNv contains at least three different subtypes of nAChRs based on \(^{3}H\)-nicotine–, \(^{125}I\)-\( \alpha \)-Bgt–, and \(^{125}I\)-\( \kappa \)-Bgt–specific binding (Sorenson and Chiappinelli 1992), selective an-
agonists were used to pharmacologically characterize the nAChR subtype(s) mediating the responses to exogenously applied nicotinic agonists. Low concentrations (0.1 and 1.0 μM) of DHβE that are selective for non-α7 nAChRs (Harvey et al. 1996; Pereira et al. 1994; Sabey et al. 1999) did not significantly block the potentiation produced by 100 μM ACh (Fig. 6A; n = 4 at 0.1 μM and n = 6 at 1.0 μM; both P > 0.05 by ANOVA). At a higher concentration of DHβE (30 μM), where most nAChRs should be inhibited, the potentiation was completely blocked (Fig. 6Ad; n = 4; P < 0.01 by ANOVA).
Another the ACh-induced potentiation (Fig. 6). Amplitudes of evoked glutamatergic responses at various times during continuous application of ACh (100 μM) for 10 s through U-tube. Responses were normalized to evoked response measured after 5 s of ACh exposure. Each point and vertical bar represent means ± SE (n = 3) from different LGNv neurons. The results with α7*- and/or α8*-containing (α7*/α8*) nAChRs were tested: MLA, α-Bgtx, and α-CTx ImI. At 10 nM MLA, a concentration reported to completely block mammalian α7* receptors (Mogg et al. 2002; Wooltorton et al. 2003), the ACh-mediated potentiation of glutamatergic transmission was not inhibited (Fig. 6Bc; n = 3; P > 0.05). At 50 nM MLA, the potentiation in response to ACh was completely blocked (Fig. 6Cd; n = 5; P < 0.05). At a concentration of 100 nM, α-Bgtx also completely blocked the ACh-induced potentiation (Fig. 6Dd; n = 6; P < 0.05). Another α7*/α8* antagonist, α-CTx ImI (0.1 μM), however, did not inhibit the nicotinic potentiation of evoked responses (n = 4; P > 0.05, data not shown).

The next group of antagonists tested were α3*-antagonists. The results with κ-Bgtx (100 nM), which is a potent antagonist of α3β2 and α3α5β4 nAChRs (Conroy and Berg 1995; Luette et al. 1998), were complex. Of six neurons examined, two had a complete blockade of the nicotinic response, two exhibited a partial block, and two were little changed from control (data not shown). α-CTx MII (100 nM), which blocks both α6* and α3β2 nAChRs (Cartier et al. 1996; Champtiaux et al. 2002; Everhart et al. 2004), and α-CTx AuIB (1 μM), a blocker of α3β4* nAChRs (Luo et al. 1998), did not inhibit the nicotinic potentiation of glutamatergic PSCs (n = 3 for each toxin, P > 0.05, data not shown).

Because the LGNv is innervated by a dense network of cholinergic fibers (Fig. 1 in this report; Sorenson et al. 1989), we sought to determine whether endogenous release of ACh from these fibers could enhance evoked glutamatergic transmission in the LGNv. For these experiments, a second stimulating electrode, termed the conditioning electrode, was placed in the lateral portion of the LGNv while recording from LGNv neurons in the central portion of the nucleus. Stimulation of the conditioning electrode evoked small postsynaptic inward currents recorded from LGNv neurons (Fig. 7). These fast inward currents were blocked by 20 μM DNQX, but not by 30 μM DHPβE, indicating that they were likely caused by direct stimulation of glutamatergic fibers running through the LGNv (n = 3). Trains of conditioning stimuli were given before optic tract stimulation. At low conditioning stimulation intensities, there was no change in the glutamatergic response evoked by optic tract stimulation (Fig. 7A). However, when the intensity and/or number of conditioning pulses were increased, there was a marked enhancement in glutamatergic transmission between retinal ganglion terminals and LGNv neurons (5.2 ± 0.6-fold increase vs. control, n = 25, P < 0.0001 by t-test). The timing of the conditioning train was also critical, with the enhance-

![Figure 4](image-url)  
**FIG. 4.** U-tube application of acetylcholine (ACh) potentiates evoked glutamatergic postsynaptic currents (PSCs) without rapid desensitization of effect. A: U-tube application of ACh (100 μM) for 1, 5, or 10 s before electrical stimulation markedly potentiated evoked glutamatergic PSCs. B: amplitudes of evoked glutamatergic responses recorded from LGNv neurons (Fig. 7). These fast inward currents were blocked by 20 μM DNQX, but not by 30 μM DHPβE, indicating that they were likely caused by direct stimulation of glutamatergic fibers running through the LGNv (n = 3). C: potentiation of evoked glutamatergic response by 100 μM ACh persisted after a 5-min exposure to ACh.

![Figure 5](image-url)  
**FIG. 5.** Activation of nicotinic acetylcholine receptors (nAChRs) on glutamatergic terminals by exogenous ACh, nicotine, or choline potentiates evoked glutamatergic PSCs. A: U-tube application of ACh (0.1–10 mM) potentiated evoked glutamatergic PSCs in a dose-dependent manner. B: U-tube application of nicotine (0.5 μM) greatly potentiated evoked glutamatergic PSCs (2nd trace). After washout of nicotine (middle trace), U-tube application of choline (1 mM) markedly potentiated the evoked response. C: U-tube application of glutamate (200 μM) for 10 s increased the inward current (final response in record).
ment disappearing as the time between the conditioning train and optic tract stimulation was extended to between 400 and 900 ms (Fig. 7B; n = 4). Conditioning stimulation trains also markedly enhanced evoked glutamatergic transmission in the presence of high divalent cations (12 mM Ca\(^{2+}\) and 12 mM Mg\(^{2+}\); 2.5 ± 0.6-fold increase, n = 3, P < 0.05; data not shown).

Pharmacology of glutamatergic enhancement after conditioning trains

To determine whether the potentiation of glutamatergic transmission by conditioning stimulation was caused by the release of endogenous ACh, the ability of nicotinic antagonists to block the potentiation was examined. Several nicotinic...
antagonists completely blocked the enhancement of glutamatergic transmission produced by conditioning trains, including 100 nM DHβE (Fig. 8A; n = 10, P < 0.001) and 100 nM α-CTx MII (Fig. 8B; n = 3, P < 0.001). MLA significantly inhibited glutamatergic enhancements produced by conditioning trains at 100 nM (Fig. 8C; 64% inhibition, n = 12, P < 0.05) and completely blocked them at 1 μM (Fig. 8C; n = 4, P < 0.05). No significant inhibition of the conditioning train glutamatergic enhancement was observed in the presence of α-BgTx (100 nM), κ-BgTx (100 nM), α-CTx ImI (0.5 μM), or α-CTx AulB (1 μM) (n = 3–4 for each toxin; P > 0.05 for all).

DISCUSSION

The results show significant nicotinic modulation of evoked glutamatergic transmission in the chick LGNv. Activation of nAChRs with exogenously applied ACh during optic tract stimulation caused an average 3.7-fold potentiation of evoked glutamatergic PSPs recorded in LGNv lamina interna neurons. A similar potentiation was observed with both exogenous nicotine and choline, and this effect appears to be caused primarily by the activation of α7- and/or α8-containing nAChRs located on the glutamatergic nerve terminals of retinal ganglion cells that synapse directly onto LGNv neurons. Electrically stimulated release of endogenous ACh in the LGNv also markedly potentiated glutamatergic transmission (by 5.2-fold), but pharmacologically different nAChRs mediated this nicotinic effect.

Cholinergic fibers and several subtypes of nAChRs are present in high densities throughout the LGNv (Sorenson and Chiappinelli 1992; Sorenson et al. 1989). Some of these receptors are located on glutamatergic and GABAergic nerve terminals, because activation of these presynaptic nAChRs enhances the spontaneous release of glutamate and GABA in the LGNv in the presence of TTX (Guo and Chiappinelli 2002; Guo et al. 1998). These experiments examined the possible modulatory role of nAChRs on evoked excitatory synaptic transmission at LGNv neurons, and it is clear that activation of nAChRs can have a profound effect on glutamatergic signaling between retinal ganglion nerve terminals and LGNv neurons. The nAChRs involved are located presynaptically, because the postsynaptic response to exogenous glutamate was unchanged in the presence of nicotinic agonists.

Exogenously applied ACh potentiated evoked glutamatergic transmission in a dose-dependent manner, and the response was completely blocked by 30 μM DHβE, showing that nAChRs were mediating the response. Choline, a nicotinic agonist with some selectivity for α7-containing nAChRs (Alkondon et al. 1997, 2000), also produced marked potentiation of evoked glutamatergic transmission, and the α7*/α8*-selective antagonist α-BgTx blocked the ACh-induced potentiation. Thus it is very likely that the major nAChRs mediating this enhancement of evoked glutamatergic transmission contain α7 and/or α8 subunits. However, these α-BgTx-sensitive nAChRs differ from classical α7 and α8 receptors in that they are not sensitive to α-CTx ImI (Pereira et al. 1996) and are not blocked at 10 nM MLA (but are blocked at 50 nM). The nicotinic enhancement persisted during a 5-min exposure to ACh applied by U-tube. Previous work in brain slices (Wooltorton et al. 2003) has shown that α7-containing nAChRs are among the least likely of nAChRs to desensitize at lower concentrations of agonist, and because we placed our U-tube 2–3 mm from the recorded neuron, there was a substantial dilution (estimated at 3- to 4-fold) of agonist before it reached the receptor sites. The same persistence of nicotinic agonist effect and α-BgTx sensitivity was previously observed for the

**FIG. 7.** Conditioning stimulus train before optic tract stimulation potentiates evoked glutamatergic PSCs. Aa: conditioning train of 5 pulses (200-μs pulse duration, 25 Hz) was delivered 300 ms before optic tract stimulation. At the lowest stimulus intensity shown (20 μA), evoked response was unchanged from control (none). At 30 μA, marked potentiation of evoked response to optic tract stimulation was observed, and this potentiation reached a maximum at 40 and 50 μA stimulus intensity. Optic tract was stimulated at 11 μA (200 μA). Ab: in the same neuron, number of pulses in the train was varied from 3 to 8 while maintaining stimulus intensity at 50 μA. A 3-pulse train did not alter evoked response, whereas 5- and 8-pulse trains produced marked potentiation of the evoked glutamatergic response. B: in another neuron, the time between the conditioning train and optic tract stimulation was varied. At 70-μA conditioning stimulus intensity, enhancement of glutamatergic-evoked response persisted to ~400 ms after the end of conditioning pulse, but was no longer present after 494 ms. Bb: in the same neuron with a 100-μA conditioning stimulus intensity, enhancement persisted to 700 ms, but was only slightly larger than control after 895 ms.
nAChRs mediating enhanced spontaneous release of glutamate in the LGNv by exogenous nicotinic agonists (Guo et al. 1998). In contrast, presynaptic nAChRs mediating enhanced GABA release in the LGNv exhibited a very different pharmacology, including insensitivity to α-BgTx (Guo and Chiappinelli 2002).

The α6 and β3 nAChR subunits are not widely distributed in neuronal tissue, but both are expressed in the retina of chick and mice (Champetiaux et al. 2002; Vailati et al. 1999, 2000). In the chick, two nAChR subtypes containing β3 subunits have been immunopurified and pharmacologically characterized, one of which also contains at least one α6 subunit (Vailati et al. 2000). These nAChR subtypes do not appear to be major subtypes involved in the present enhancement of glutamatergic evoked responses by exogenous agonists in the LGNv, because they are not sensitive to blockade by α-BgTx. Furthermore, immunoprecipitation experiments using anti-β3 antibodies failed to precipitate any α-BgTx–binding nAChRs in chick brain homogenates (Vailati et al. 2000), indicating that the receptors mediating enhanced glutamate release by exogenous agonists in this study are unlikely to contain β3 subunits.

The somata of chick retinal ganglion neurons express both α8 and α7 subunits (Keyser et al. 1993). At least three subtypes of α-BgT–sensitive nAChRs are present in the chick retina, including α8*, α7α8*, and α7*, and there is biochemical evidence that these receptors contain additional subunits (Gotti et al. 1997). After unilateral retinal lesions in the chick, there is a decrease in the intensity (from intense to moderate) of neuropil staining for α8-like immunoreactivity in the contralateral LGNv, whereas α7-like immunoreactivity remains unchanged at intense (Brito et al. 1994), suggesting that nAChRs containing the α8 subunit are present on retinal ganglion terminals in the LGNv. β2-like immunoreactivity decreased even more substantially after retinal lesion, indicating that some nAChRs located on retinal ganglion terminals are likely to contain this subunit. Chick (and rat) α7 and β2 subunits can combine to form functional receptors (Girod et al. 1999; Khiroug et al. 2002), so that combinations of the β2 subunit with either α7 or α8 (or both) might occur in the retinal ganglion terminals within the LGNv. Because the heteromeric α7β2* receptor desensitizes significantly slower than the homomeric α7 receptor (Khiroug et al. 2002), such a combination could contribute to the prolonged nature of the nicotinic response seen in this study in the LGNv. Slowly desensitizing α7* receptors have also been described in rat intracardiac ganglia (Cuevas and Berg 1998). The α7* responses observed here are also distinguished by a faster recovery during washout of α-BgT than is observed for mammalian α7* receptors, with substantial recovery of responses observed after 10–15 min of washing out α-BgT.
Electrical stimulation in the lateral LGNv (conditioning stimulation) produced marked enhancement of optic tract-evoked glutamatergic transmission in LGNv neurons. The potentiation was dependent on the intensity and number of conditioning pulses. A single conditioning train at higher intensity could elicit potentiation of optic tract-LGNv neuron-evoked glutamatergic transmission for >500 ms. The glutamatergic enhancement after conditioning stimulation was nicotinic in nature, because it was completely blocked by 100 nM α-CTx MII and 100 nM DHβE. The conditioning response was most likely caused by the release of endogenous ACh from cholinergic fibers after electrical stimulation through the conditioning electrode. The nAChRs mediating this response to endogenously released ACh were clearly distinct from the receptors mediating the enhancement because of exogenously applied nicotinic agonists. The blockade by low concentrations of α-CTx MII and DHβE, the lack of sensitivity to α-BgtTx, and the requirement for relatively high concentrations of MLA to produce a complete block (1.0 μM) all make it unlikely that α7* or α8* receptors are the major nAChR subtypes mediating the conditioning response. Instead, the response to endogenously released ACh seems to be mediated primarily by presynaptic α6- and/or α3-containing nAChRs based on pharmacological properties (Mogg et al. 2002; Salminen et al. 2004). Immunoimmobilized chick α6* receptors exhibited the following K₅ against 3H-epibatidine binding: α-CTx MII, 66 nM; MLA, 1.35 μM, DHβE, 2.8 μM (Vailati et al. 1999). In comparison, the concentrations required to completely block the functional response to conditioning stimulation in this study were as follows: α-CTx MII, 100 nM; MLA, 1.0 μM, DHβE, 0.1 μM. It is known that the α6-containing receptors in retinal ganglion cell bodies are heterogeneous, and many also contain α3 and/or β3 subunits (Vailati et al. 1999, 2000, 2003). In both chick and rodent, these α6* receptors migrate down the optic nerve and tract and are present in the optic lobe and LGNv (Champtiaux et al. 2002; Vailati et al. 1999). Thus the most parsimonious explanation for the enhancement of glutamatergic transmission observed in this study after conditioned stimulation is that ACh released from cholinergic fibers activates α6* and/or α3* (α6*/α3*) nAChRs located on retinal ganglion nerve terminals, causing enhanced glutamate release. It is not yet clear why exogenous nicotinic agonists enhance glutamatergic transmission in the LGN primarily by activating α7*/α8* receptors, whereas endogenous ACh acts mainly on α6*/α3* receptors. One possibility is that the α6*/α3* receptors are located preferentially at cholinergic synapses, whereas the α7*/α8* receptors are primarily extrasynaptic. Exogenous ACh would readily activate all extrasynaptic α7*/α8* receptors but would have more limited access to the likely smaller number of synaptic α6*/α3* receptors, whereas endogenously released ACh would have ready access to α6*/α3* receptors but would not be released nearby α7*/α8* receptors. Such segregation of nAChR subtypes at cholinergic synapses has been described in chick ciliary ganglia neurons, where α3* nAChRs are preferentially located at synapses, whereas α7* receptors are excluded from the synapses but are present in extrasynaptic locations (Shoop et al. 1999). The α3 subunit shares substantial sequence homology with the α6 subunit (Champtiaux et al. 2002), and it is has been shown to be critical in targeting nAChRs to synapses in the ciliary ganglion (Temp-burni et al. 2000).

Nicotinic modulation of glutamatergic transmission in the LGNv may play an important physiological role. The LGNv communicates between the thalamofugal and tectofugal visual pathways, the two primary and parallel visual pathways in birds as well as reptiles and mammals (Shimizu and Karten 1993). In addition to the optic tract fibers, the LGNv receives afferents from the visual wulst, the homolog of the visual cortex and part of the thalamofugal system, and fibers from the optic tectum, the homolog of the superior colliculus and part of the tectofugal system (Crossland and Uchwat 1979; Ehrlich et al. 1989). The LGNv has been reported to send projections to the optic tectum and the area pretectalis and lesioning studies support a role for the LGNv in communicating between the thalamofugal and tectofugal pathways (Hodos et al. 1982; Reiner et al. 1982). Our electrophysiological recordings were from projection neurons in the lamina interna, and these neurons appear to function as color-opponent units in the bird (Maturana and Varela 1982). In our reconstructions of these neurons, the main axon seen traveling medial to the nu. rotundus is likely on its way to the area pretectalis. The main axons also had collaterals, some of which were localized to the area between the nu. rotundus and the LGNv, the nucleus marginalis tractus optici. These axon collaterals may be involved in modulating the activity of the optic tectum through neuropeptide Y-expressing marginalis tractus optici neurons that also send efferents to the optic tectum (Gunturkun and Karten 1991). The dendrites of the visualized neurons descend into the neuropil where optic tract fibers terminate (Crossland and Uchwat 1979). The interconnections of the LGNv with the optic fibers and the thalamofugal and tectofugal visual systems suggest that nicotinic modulation of the LGNv may have important effects on the regulation of the visual processing system as a whole.

In humans, a functional MRI (fMRI) study has shown that nicotine can markedly enhance activation in human brain areas involved in visual attention, including the thalamus and posterior cortex, during a rapid visual information-processing task that tests both vigilance and working memory (Lawrence et al. 2002). Consistent with this neuronal activation of visual centers by nicotine in humans, we found that nicotine can markedly enhance excitatory transmission through the chick LGNv at a concentration that can be attained after cigarette smoking in humans (500 nM; Henningfield et al. 1993). In another fMRI study in humans, attention to a task was shown to substantially enhance neural responses to attended stimuli in the thalamic LGN, leading the authors to propose that the LGN acts as a “gatekeeper” in regulating attentional gain control (O’Conner et al. 2002). Our finding that endogenously released ACh acts on nicotinic receptors to significantly increase excitatory transmission through the chick LGNv provides a mechanism whereby cholinergic fibers and nicotinic receptors could serve to modulate attentional signaling through the LGNv. Nicotinic receptors have now been shown to play functional roles at three separate levels of the visual nervous system. In the visual cortex, fast evoked nicotinic transmission occurs before and during innervation by thalamic afferents (Roerig et al. 1997). In the retina, activation of nAChRs initiates spontaneous bursts of wave-like activity during development, and in β2-null mice, the normal pattern of innervation of the LGN by retinal ganglion cells is disrupted (Rossi et al. 2001). This study shows for the first time that activation of presynaptic nigral cholinergic neurons.
nACHRs either by endogenously released ACh or exogenously applied nicotine, choline, or ACh markedly potentiates neurotransmission at glutamatergic synapses formed by retinal ganglion cell terminals and LGNv neurons.

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