

CORTICAL FEEDBACK TO THE THALAMUS IS SELECTIVELY ENHANCED BY NITRIC OXIDE

G. M. ALEXANDER,^{a1} N. C. KURUKULASURIYA,^{a,b1}
J. MU^b AND D. W. GODWIN^{a,b1*}

^aThe Neuroscience Program, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA

^bDepartment of Neurobiology and Anatomy, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA

Abstract—The brain somehow merges visual information with the behavioral context in which it is being processed, a task that is often attributed to the cerebral cortex. We have identified a new role of the gaseous neurotransmitter, nitric oxide (NO), in the early selective enhancement of corticogeniculate communication that may participate in this process at the level of the thalamus. Visual information is dynamically gated through the thalamus by brainstem neurons that release acetylcholine and NO. Using *in vitro* electrophysiology, we characterized NO effects on excitatory postsynaptic potentials and currents (EPSCs) elicited from retinal and cortical pathways in the lateral geniculate nucleus of the ferret. NO selectively and reversibly increased cortically-evoked postsynaptic responses, and this effect was mimicked by cyclic guanosine 3',5'-monophosphate (cGMP). Conversely, NO inhibited retinally-evoked responses independently of cGMP. We demonstrated that these differential effects were specific to postsynaptic *N*-methyl-D-aspartate (NMDA) receptors by studying treatment effects on pharmacologically isolated EPSCs from each pathway. We propose that when brainstem activity is increased during behavioral arousal or rapid eye movement sleep, NO may increase the relative sensitivity of relay neurons to corticogeniculate feedback. The net effect of these changes in synaptic processing may be to selectively suppress peripheral information while unifying data carried by reentrant corticogeniculate loops with the behavioral context in which the visual information is processed. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: nitric oxide, vision, LGN, thalamus, parabrachial brainstem, NMDA.

¹ These authors contributed equally to the manuscript.

*Correspondence to: D. W. Godwin, Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA. Tel: +1-336-716-9437; fax: +1-336-716-4534.

E-mail address: dgodwin@wfbmc.edu (D. W. Godwin).

Abbreviations: ACSF, artificial cerebrospinal fluid; APV, DL-2-amino-5-phosphonovaleric acid; cGMP, cyclic guanosine 3',5'-monophosphate; DNQX, 6,7-dinitroquinoxaline-2,3(1H,4H)-dione; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; GEA 5024, 1,2,3,4-oxatriazolium, 5-amino-3-(3-chloro-2-methylphenyl)-chloride; LGN, lateral geniculate nucleus; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOC-18, 1-hydroxy-2-oxo-3-bis(3-aminoethyl)-1-triazene; PBR, parabrachial brainstem; PPDR, paired pulse depression ratio; PPF, paired pulse facilitation ratio; REM, rapid eye movement; SNAP, S-nitroso-N-acetyl-D,L-penicillamine; SNP, sodium nitroprusside; SOD, superoxide dismutase.

0306-4522/06/\$30.00+0.00 © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2006.06.022

The dorsal lateral geniculate nucleus of the thalamus (LGN) is a prime example of a neuronal circuit that can be redirected according to the behavioral state of the brain. This redirection is exemplified by the fate of visual information during sleep and wakefulness. During sleep, the LGN participates in rhythmic activity that suppresses the flow of visual information through the primary visual pathway (Livingstone and Hubel, 1981; McCormick and Feese, 1990). In the waking brain, the LGN acts as a distribution node that delivers spatial and temporal information to visual cortex, in part through the consolidation of reentrant cortical feedback with the ongoing flow of visual information from the retina to cortex (Sillito et al., 1994; Castelo-Branco et al., 1998). These dual activity modes are enabled by powerful brainstem influences that promote a linear transmission of visual information during the waking hours (Singer, 1977; Marks and Roffwarg, 1989; McCormick and Bal, 1997).

Visual information is borne upon retinogeniculate and corticogeniculate terminals that innervate relay neurons. These terminals release glutamate, which targets rapidly acting ionotropic glutamate receptors (i.e. *N*-methyl-D-aspartate (NMDA) and non-NMDA) as well as more slowly acting metabotropic glutamate receptors (Scharfman et al., 1990; Sillito et al., 1990; McCormick and von Krosigk, 1992; Godwin et al., 1996b; Turner and Salt, 1998; von Krosigk et al., 1999; Alexander and Godwin, 2005). The interaction between retinal and cortical inputs that occurs in relay cells, and the transmission of the result of this interaction to V1 is modulated in part by an ascending projection from the parabrachial brainstem (PBR). PBR terminals contact thalamic relay neurons on proximal dendrites in association with retinogeniculate inputs (Raczkowski and Fitzpatrick, 1989; Erisir et al., 1997) and release both acetylcholine and nitric oxide (NO; Williams et al., 1997). Activation of the cholinergic component of the PBR projection shifts relay cells from "burst" to "tonic" mode of firing (Singer, 1977; Marks and Roffwarg, 1989; McCormick and Bal, 1997). Neuronal nitric oxide synthase (nNOS), the calcium-dependent enzyme that generates NO, is contained within the presynaptic terminal fields of the PBR (Bickford et al., 1993; Carden et al., 2000; McCauley et al., 2003). The nitrenergic signal appears to be coincident in time with the normal activity-dependent cholinergic signal, because higher levels of NO are released during waking and rapid eye movement (REM) sleep compared with slow wave sleep (Williams et al., 1997).

Identifying a function of nitrenergic signaling that is unique from the cholinergic effects of the pathway has been challenging. Several groups have examined the role

of NO in the thalamus in modulating synaptic transmission and intrinsic membrane properties. In a series of *in vivo* studies, Cudeiro et al. (1994a,b, 1996) used local application of NO donors to show that NO enhanced visual transmission in the thalamus and increased the sensitivity of thalamic relay neurons to iontophoretically-applied NMDA. Using *in vitro* approaches, Papé and Mager (1992) showed that NO suppressed burst firing by modulating the mixed cation current, I_h .

The ferret is a model system that expresses nNOS in presynaptic brainstem terminals in the LGN (McCauley et al., 2003; Leamey et al., 2001) and it shares this property with cats and monkeys (Bickford et al., 1993, 2000). In this study, we recorded from LGN relay neurons and characterized NO effects on glutamatergic excitatory postsynaptic potentials and currents (EPSP/Cs) in adult ferrets. Both NO donors and L-arginine, the natural precursor to NO formation by NOS, selectively suppressed EPSPs and EPSCs generated through the retinogeniculate pathway, but enhanced those of corticogeniculate origin. By pharmacologically isolating NMDA and non-NMDA EPSCs, we determined that the NO effects at either synapse were specific to the NMDA-mediated EPSC. The cyclic guanosine 3',5'-monophosphate (cGMP) analog, 8-bromo-cGMP, replicated the effect of L-arginine and the NO donors on corticogeniculate NMDA EPSC enhancement, but did not affect retinogeniculate EPSCs, suggesting different mechanisms of NO influence in the two pathways. Our results reconcile a number of prior studies, and reveal corticogeniculate feedback as an important target of nitrenergic neurotransmission in the thalamus.

EXPERIMENTAL PROCEDURES

Slice preparation

We used previously reported methods for slice recordings from adult ferrets (Alexander and Godwin, 2005; Mu et al., 2003). Male ferrets older than postnatal day 42 were anesthetized with halothane and decapitated in procedures approved by the Wake Forest University Animal Care and Use Committee and in agreement with National Institutes of Health and United States Department of Agriculture guidelines, which included measures to minimize the number of animals used and their suffering. The brain was rapidly removed and immersed in oxygenated (95% O₂:5% CO₂), ice cold sucrose substituted artificial cerebrospinal fluid (ACSF) containing (in mM): 220 sucrose, 12 MgSO₄, 10 glucose, 2 KCl, 1.5 NaH₂PO₄, 26 NaHCO₃, 0.2 CaCl₂ (pH 7.4, osmolarity 290–300 mOsm). A block of tissue containing the LGN was sectioned sagittally on a vibratome (model OTS 5000, Electron Microscopy Sciences, Fort Washington, PA, USA) at 400 μ m, and slices were maintained in oxygenated, warm (34 °C) ACSF containing (in mM): 124 NaCl, 5 KCl, 2 MgSO₄, 2 CaCl₂, 23 NaHCO₃, 3 NaH₂PO₄, 10 glucose (pH 7.4, osmolarity 290–300 mOsm), for at least 1.5 h before being transferred to an interface recording chamber for sharp electrode recordings or a submerged recording chamber for patch recordings (Harvard Apparatus, Holliston, MA, USA). Disadvantages inherent to slice methods include the inability to measure responses to natural visual stimuli, to directly stimulate the PBR pathway to the thalamus, and to know the time of peak drug concentration at the depth in the slice from which we are recording. A disadvantage of attempting to electrically stimulate PBR fibers in slices is that, due to colocalization of acetylcholine and nNOS in PBR terminals, such stimulation would elicit both

nitrenergic and cholinergic responses in addition to other brainstem influences that innervate the thalamus, making the results of such experiments difficult to interpret. However, because the dense network of nNOS-bearing terminals remains in slices, NO formation can be pharmacologically elicited by treatment with exogenous L-arginine. We were interested in the modulatory influence of NO alone on glutamatergic signaling, thus we chose to electrically stimulate glutamatergic pathways while producing NO pharmacologically. The major compensatory advantage of the slice preparation is the ability to identify, isolate and selectively stimulate the retinal and cortical inputs to relay neurons, in a situation where effects of NO on microvascular tone through effects on the vascular endothelium are eliminated. Additionally, slice preparations offer the advantage of total bath substitution and therefore drug concentrations are known. While *in vivo* preparations allow the advantage of identifying the functional effects in the intact system (see Cudeiro et al., 1994a, 1996), our aim in this study was a detailed analysis of isolated synaptic inputs to study the mechanism of NO action.

Recordings

Both sharp electrode current clamp and patch electrode voltage clamp recordings were made in this study. We initially used sharp recordings to prevent possible dialyzation of second messengers through patch recordings. Later experiments required the use of voltage clamp recordings to assess the effects of NO on each component of EPSCs as well as to remove the possible contamination of synaptic responses with other voltage-activated currents. During all recordings, thalamic slices were continuously perfused with warm (34 °C) oxygenated ACSF at a flow rate of 1.5–2 ml/min. Drugs were infused into the ACSF via a calibrated syringe pump which was adjusted based on flow rate to present known drug concentrations to the slice. Drugs applied through the syringe pump required 2–4 min to reach the recording chamber. Recordings were made from either lamina A or A1 of the LGN. Sharp electrodes (70–130 M Ω) were pulled from borosilicate glass (Sutter Instruments, Novato, CA, USA) with a P-87 horizontal puller (Sutter Instruments) and filled with 4 M potassium acetate containing the quaternary lidocaine derivative, QX-314 (50 mM) to block action potentials (von Krosigk et al., 1999), and to allow us to study EPSPs even at relatively depolarized membrane potentials in the range necessary to elicit NMDA currents. Patch pipettes (5–10 M Ω) were pulled from the same glass with a PC-10 vertical puller (Narishige International USA, Inc., East Meadow, NY, USA), and were filled with an internal solution containing (in mM): 100 gluconic acid, 100 CsOH, 10 NaCl, 10 Hepes, 20 TEA-Cl, 1 EGTA, 4 ATP (pH 7.3 with 2 N CsOH, osmolarity 270–290 mOsm). For experiments on the isolated NMDA EPSC, internal pipette solution also contained QX-314 (5 mM) to block voltage sensitive sodium channels. A liquid junction potential of +7 mV was corrected for post hoc. A bipolar stimulating electrode, powered by a stimulus isolator (World Precision Instruments, Sarasota, FL, USA), was positioned in the optic radiations for corticogeniculate stimulation, and in the optic tract for retinogeniculate stimulation. Cellular activity was acquired with an AxoClamp 2B amplifier (Axon Instruments, Union City, CA, USA), digitized with a Digidata 1322 (Axon Instruments), and analyzed using pCLAMP 9.0 software (Axon Instruments). To acquire cells for patch recordings, pipettes were advanced "blind" through tissue in bridge mode until encountering a cell, a >1 G Ω seal was formed, the membrane ruptured to allow whole cell access, and the amplifier was then switched to cSEVC mode (Blanton et al., 1989). For all recordings, clamp gain was greater than 300 nA/mV. Mean membrane resistance was 145.8 \pm 13.3 M Ω .

Single pulses were delivered to the fiber pathways for 100 μ s to record single EPSP/Cs. For paired pulse experiments, dual pulses at 10 Hz frequency were delivered. We chose 10 Hz

because in prior studies we determined this frequency to be near the peak for PPD and PPF effects while still allowing separate decay and measurement of EPSCs (Alexander and Godwin, 2005, 2006; Alexander et al., 2006). Before running a pharmacological experiment, we tested for PPF (cortico-geniculate stimulation) or PPD (retinogeniculate stimulation) for further confirmation of the pathway being stimulated. Before all experiments, synaptic responses were observed for a range of stimulus intensities, and the intensity that produced approximately half maximal response was used in order to allow the EPSP/C to either increase or decrease in amplitude. During the course of experiments, single stimuli were delivered every 20 s while paired stimuli were delivered every 30 s. Baseline responses were measured for 7–10 min to allow the cell time to stabilize, followed by drug delivery for 7–10 min, and subsequent observation for drug effects. During all voltage clamp experiments, a -10 mV step was delivered 300 ms following the synaptic stimulus in order to monitor any changes in resistance that might occur during the course of an experiment. For isolated NMDA experiments, cells were held at -30 mV and stepped to $+40$ mV before delivering a synaptic stimulus. This $+70$ mV step was used to assess changes in resistance. For isolated non-NMDA experiments, cells were held at -75 mV, and for composite EPSC experiments, cells were held at -55 mV. For current clamp recordings, cells were held near -60 mV. Only recordings from putative relay cells are included in our results. We recognized relay cells using electrophysiological criteria, most notably the presence of a prominent low threshold calcium current. This current is normally activated below about -75 mV in relay cells, but because interneurons also possess a strong A-type K^+ current the low threshold calcium current is usually masked in interneurons (McCormick and Papé, 1988).

Analysis

To assess the effect of NO on the magnitude of retinogeniculate and corticogeniculate synaptic responses, we measured either the area under the curve in pre- and post-drug conditions for composite EPSP/Cs, or the amplitude for isolated EPSCs. Area under the curve was measured on baseline subtracted traces between the beginning of the response and where the curve re-crossed baseline. Amplitude measures were taken from baseline subtracted traces. The average of seven values was taken during control conditions, following drug administration and following drug wash-out. Paired *t*-tests were used for comparison of two conditions, and one-way analysis of variance was used for greater than two conditions, followed by a Dunnett's post hoc analysis when necessary. In all bar graphs and throughout text, mean \pm standard error of the mean has been reported.

Pharmacology

For all experiments, GABA_A inhibitory postsynaptic potentials and currents (IPSP/Cs) were blocked with either bicuculline (30 μ M) or picrotoxin (100 μ M). For sharp electrode recordings, the GABA_B antagonist 2-OH saclofen (200 μ M) was included in the ACSF, and for patch recordings, GABA_B inhibitory postsynaptic currents were blocked through the cesium based internal solution. Also included in the ACSF were 6,7-dinitroquinoxaline-2,3-(1H,4H)-dione (DNQX; 30 μ M) for NMDA isolation experiments or DL-2-amino-5-phosphonovaleric acid (APV; 50 μ M) for non-NMDA isolation experiments. All drugs were purchased from Sigma-RBI (St. Louis, MO, USA). NO agents included L-arginine (1 mM; Sigma), L-NAME (1 mM; Sigma), S-nitroso-N-acetyl-D,L-penicillamine (SNAP; 2 mM; Alexis Corporation, San Diego, CA, USA), sodium nitroprusside (SNP; 1.5 mM; Alexis), 1-hydroxy-2-oxo-3,3-bis(3-aminoethyl)-1-triazene (NOC-18; Alexis; 2 mM), 1,2,3,4-oxatriazolium, 5-amino-3-(3-chloro-2-methylphenyl)-chloride (GEA 5024; 200 μ M; Alexis), superoxide dismutase (SOD; 2000 units/ml; Alexis), and 8-bromo-cGMP (1 mM; Sigma). All drugs were made

fresh daily. For SOD experiments, sections were held in SOD containing ACSF for >1 h in the holding chamber and continuously perfused during recordings.

While local drug administration using dropwise application or local perfusion may provide greater temporal resolution of onset and offset of drug effects, in our study we were interested in determining a more precise concentration than is possible with local application. We therefore used full bath substitution to administer drugs through perfusion of drug into the ACSF flow line via a syringe pump, which was adjusted based on the exact flow rate. The ACSF containing drug was delivered for 7–10 min. At a flow rate of 2 ml/min, drug was delivered for 10 min, and at a flow rate of 1.5 ml/min, drug was delivered for 7 min. Allowing time for non-drug ACSF to be fully replaced by ACSF containing drug (~ 3 min), the time at which the known bath-substituted concentration plateau was first achieved was 7–11 min at a flow rate of 1.5 ml/min and 5–13 min at a flow rate of 2 ml/min. Once the peak concentration has been reached in the recording chamber, there is an additional time delay before sampling in which the drug infuses into the slice. For a thick (400 μ M) slice, ~ 5 min may be required for infusion (Nicholson and Hounsgaard, 1983), thus peak concentration may be shifted in the slice by up to 5 min. In order to adopt a consistent sampling time for all experiments, retinogeniculate effects were sampled at 10 min following initiation of drug-containing ACSF. This time point is consistent with onset of glutamate receptor agonist effects in our preparation (Alexander and Godwin, 2005). In preliminary studies at the corticogeniculate synapse, NO- and cGMP-mediated effects were delayed beyond the point of peak drug concentration. As described in the Discussion, this may be due to involvement of second messenger cascades at this synapse. Because of this observed later onset of effect we measured synaptic effects at 15 min following initiation of drug delivery for all corticogeniculate experiments.

RESULTS

Data were collected from 96 LGN relay neurons for this study. Retinogeniculate synaptic responses were studied in 46 of these neurons, and corticogeniculate responses were studied in 50 neurons.

NO differentially modulates retinogeniculate and corticogeniculate EPSPs

Using a variety of pharmacological agents, we found that NO differentially modulates retinogeniculate and corticogeniculate synapses. Application of the NO donor SNAP (2 mM) significantly decreased the area of the retinogeniculate EPSP to $62.1 \pm 8.0\%$ of control ($n=10$, $P<0.01$; Fig. 1A–B). Following washout of SNAP, the EPSP area recovered to $104.7 \pm 13.3\%$ of control ($P>0.05$). We repeated this experiment with the NO donor SNP (1.5 mM) which also reduced the area of the retinogeniculate EPSP ($50.2 \pm 5.4\%$ of control, $n=6$; $P<0.01$).

In contrast to the observations at the retinogeniculate synapse, SNAP (2 mM) significantly increased the area of the corticogeniculate EPSP to $164.7 \pm 22.4\%$ of control ($n=9$, $P<0.01$; Fig. 1C–D). Following washout of SNAP, the EPSP area recovered to $102.4 \pm 10.3\%$ of control ($P>0.05$). In preliminary studies, we also ran this experiment using the NO donor NOC-18 (2 mM) and found an enhancement of the corticogeniculate EPSP to $178.8 \pm 35.9\%$ of control ($n=3$).

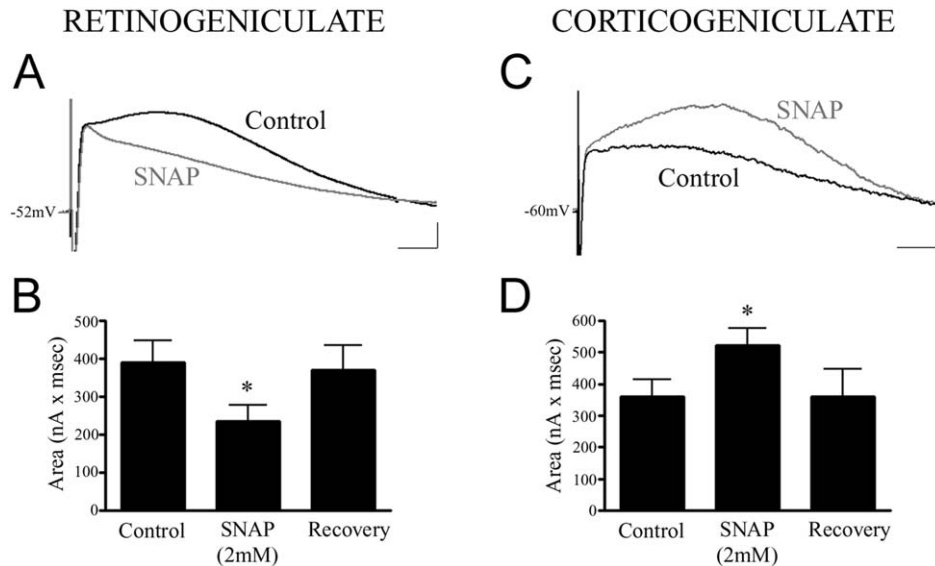


Fig. 1. Different effects of the NO donor SNAP on LGN glutamatergic synaptic transmission. SNAP inhibits the late component of the retinogeniculate EPSP and enhances the late component of the corticogeniculate EPSP. (A, B) Stimulation of the optic tract elicits a bimodal retinogeniculate EPSP. The area of the EPSP was calculated for control, treatment and recovery conditions. SNAP (2 mM) significantly reduced this measure compared with control conditions. (C, D) Stimulation of the optic radiations elicited a bimodal corticogeniculate EPSC. In contrast to the EPSPs elicited from the optic tract, the area measures of EPSPs elicited from optic radiation stimulation were significantly increased by SNAP (2 mM). Scale bars=3 mV, 10 ms in A and C (* $P < 0.01$).

NO effects are not due to peroxynitrites

In some circumstances NO may combine with superoxide to form peroxynitrite, which may have deleterious cellular effects (Katsuki et al., 2001). The nitration of tyrosine residues is another mechanism of protein modification by NO that requires superoxide (Radi, 2004). To control for the possibility that part of our observations may have been due to these mechanisms, we applied SNAP in the presence of SOD (2000 units/ml) which quenches excess superoxide and prevents the conversion of NO to peroxynitrite. SOD was included in the slice holding chamber, and continuously perfused in the ACSF during SNAP treatment. As shown in Fig. 2, in the presence of SOD, the effect of SNAP persisted in both the retinal and cortical pathways ($n=7$). The retinogeniculate EPSP area was reduced by SNAP to $56.8 \pm 8.5\%$ of control in the presence of SOD ($n=3$; $P > 0.05$; not significantly different from SNAP alone) while at corticogeniculate synapses SNAP increased the EPSP area to $167.8 \pm 1.1\%$ of control in the presence of SOD ($n=4$; $P > 0.05$; not significantly different from SNAP alone).

L-Arginine mimics SNAP effects on the retinogeniculate and corticogeniculate EPSP

NO is normally generated in the brain by the conversion of L-arginine to L-citrulline and NO by the action of NOS. Many studies have shown that application of L-arginine is capable of promoting this reaction in brain slices (e.g. O'Dell et al., 1991; Li et al., 2002; Huang et al., 2003), and responses to L-arginine therefore provide an opportunity to assess the effects of NO that are endogenous to the slice. Intracellular concentrations of L-arginine are reported to be

between 0.1 and 4 mM (Moss et al., 2004). In our study, L-arginine (1 mM) significantly reduced the retinogeniculate EPSP area to $46.8 \pm 5.8\%$ of control ($n=6$, $P < 0.01$), an effect that recovered (Fig. 3A). To determine whether the L-arginine effects were specific to the NOS pathway, slices were preincubated in NOS inhibitors and challenged with L-arginine ($n=6$; Fig. 3B). When preincubating in either the NOS inhibitor, L-NAME (1 mM included in ACSF; $n=2$), or

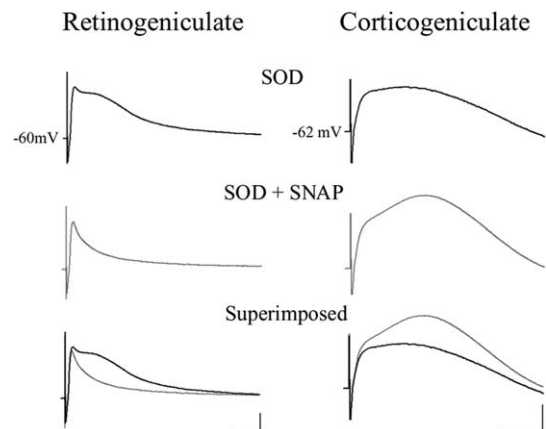


Fig. 2. EPSP modulation by the NO donor SNAP is not due to formation of peroxynitrite. Preadministration of SOD did not block EPSP modulation by SNAP of either the retinogeniculate or corticogeniculate synapses. EPSPs in the left column are of retinal origin, and those on the right are cortical. The top row shows control EPSPs in the presence of SOD. The middle row shows EPSPs following administration SNAP in the presence of SOD, and the bottom row shows the SOD control and SOD+SNAP traces superimposed to illustrate the persistence of the effect of SNAP when administered with SOD. Both scale bars=5 mV, 20 ms.

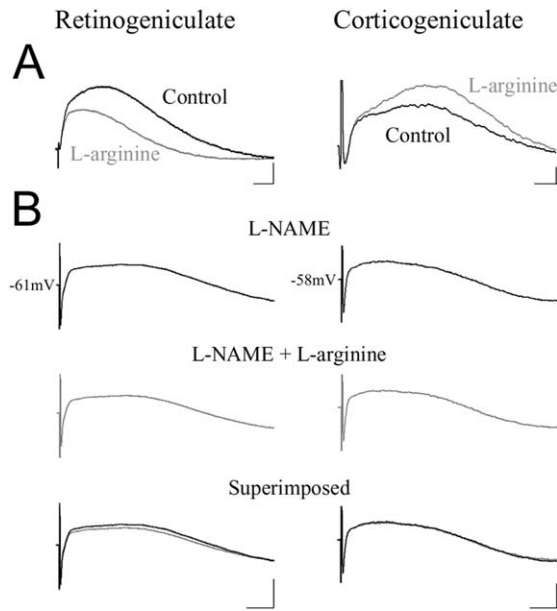


Fig. 3. The effects of SNAP on both the retinogeniculate and corticogeniculate EPSPs are mimicked by the NO precursor, L-arginine, and are blocked when inhibiting the NOS enzyme. Throughout the figure, EPSPs in the left column are of retinal origin, and those on the right are cortical. (A) The retinogeniculate EPSP was attenuated in the presence of L-arginine (1 mM), while the corticogeniculate EPSP was enhanced. Control traces are shown in black and L-arginine traces are shown in gray. (B) The effect of L-arginine on both the retinogeniculate and corticogeniculate EPSPs is blocked by preincubation in the NOS inhibitor L-NAME. Top row shows EPSPs recorded during incubation in L-NAME. L-Arginine treatment is shown in the middle row. The bottom row shows the two traces above superimposed to illustrate the lack of effect of L-arginine. Scale bar=3 mV, 25 ms in A for retinogeniculate and 3 mV, 10 ms for corticogeniculate; B=5 mV, 20 ms for retinogeniculate and 3 mV, 20 ms for corticogeniculate.

the specific nNOS inhibitor, N-propyl-L-arginine (2 mM; $n=4$), L-arginine did not significantly reduce the EPSP amplitude ($P>0.05$). These experiments illustrate that the effect we observed with the donors could be reproduced by the natural precursor for NO, and that the L-arginine effect required activation of the endogenous nNOS enzyme within the thalamic slice.

In contrast to the reduction we observed in the retinogeniculate pathway, the L-arginine effect was to enhance the corticogeniculate EPSP. L-Arginine (1 mM) enhanced the composite corticogeniculate EPSP area to $171.1 \pm 27.7\%$ of control ($n=8$, $P<0.01$), which recovered to an area not significantly different from control (Fig. 3A). To determine specificity of this effect to NOS formation of NO, slices were preincubated in the NOS inhibitor, L-NAME, and then challenged with L-arginine (Fig. 3B). The magnitude of the increase in area by L-arginine alone was significantly greater than that produced by L-arginine in the presence of L-NAME ($n=3$, $P<0.05$). These corticogeniculate data are consistent with our findings in the retinogeniculate pathway that the effects of NO could be produced by the natural precursor L-arginine and blocked by a NOS inhibitor.

NO selectively inhibits the NMDA component of the retinogeniculate EPSC

In order to determine the mechanism of NO modulation of retinal and cortical EPSPs and to minimize contaminating voltage dependent currents, we recorded LGN cells under voltage clamp conditions and pharmacologically isolated the NMDA and non-NMDA components of the retinogeniculate and corticogeniculate EPSCs. We have recently reported that the origin of retinogeniculate and corticogeniculate pathways in the ferret LGN can be determined on the basis of responses to paired pulse stimuli (Alexander and Godwin, 2005; see also Granseth et al., 2002). Retinogeniculate EPSCs show a pronounced paired-pulse depression, while corticogeniculate EPSCs exhibit paired-pulse facilitation, and we used this property to physiologically validate the pathway of origin of EPSCs (Alexander and Godwin, 2005, 2006; Alexander et al., 2006).

NMDA-mediated retinogeniculate EPSCs were characterized by their unique pharmacology and voltage-dependency (Fig. 4A–C). In the presence of DNQX, at +60 mV a large outward current was elicited that was blocked by the NMDA receptor antagonist, APV (50 μ M). NMDA-mediated currents exhibited characteristic voltage sensitivity: at membrane potentials negative to -40 mV a minimal response was elicited, while the peak inward current was observed at approximately -10 mV. This current reversed at $+13.7$ mV, and at membrane potentials positive to $+13.7$ mV a linearly increasing outward current was elicited. We studied the outward NMDA EPSC at $+40$ mV, with DNQX (30 μ M) included in the ACSF to block non-NMDA EPSCs. Non-NMDA EPSCs were studied at -75 mV with APV (50 μ M) in the ACSF to block NMDA responses.

To study pharmacologically isolated EPSCs we used the NO donor, GEA 5024, which is more potent and stable at physiological temperature as compared with SNAP (Moilanen et al., 1993; Karup et al., 1994; Kankaanranta et al., 1996; Ma et al., 1999). GEA 5024 (200 μ M) significantly decreased the amplitude of the isolated NMDA current to $55.4 \pm 4.0\%$ of control ($n=5$, $P<0.01$). Following washout of GEA 5024, the EPSC amplitude recovered to $87.6 \pm 7.9\%$ of control ($P>0.05$). In contrast, the amplitude of the isolated non-NMDA EPSC was not significantly changed by GEA 5024 (200 μ M) (EPSC amplitude= $96.4 \pm 3.6\%$ of control; $n=4$, $P>0.05$). Additionally, GEA 5024 had no effect on the paired pulse depression ratio (PPDR) during experiments in which non-NMDA EPSCs were isolated (PPDR= $106.1 \pm 3.8\%$ of control; $n=4$, $P>0.05$), revealing a postsynaptic site of action (Fig. 4D–F). GEA 5024 had no effect on membrane resistance while studying either EPSC component in isolation ($P>0.05$).

L-Arginine replicates the NO inhibition of the retinogeniculate EPSC

L-Arginine (1 mM) significantly reduced the area of the combined NMDA and non-NMDA retinogeniculate EPSC to $62.3 \pm 9.9\%$ of control ($n=6$, $P<0.05$) which recovered

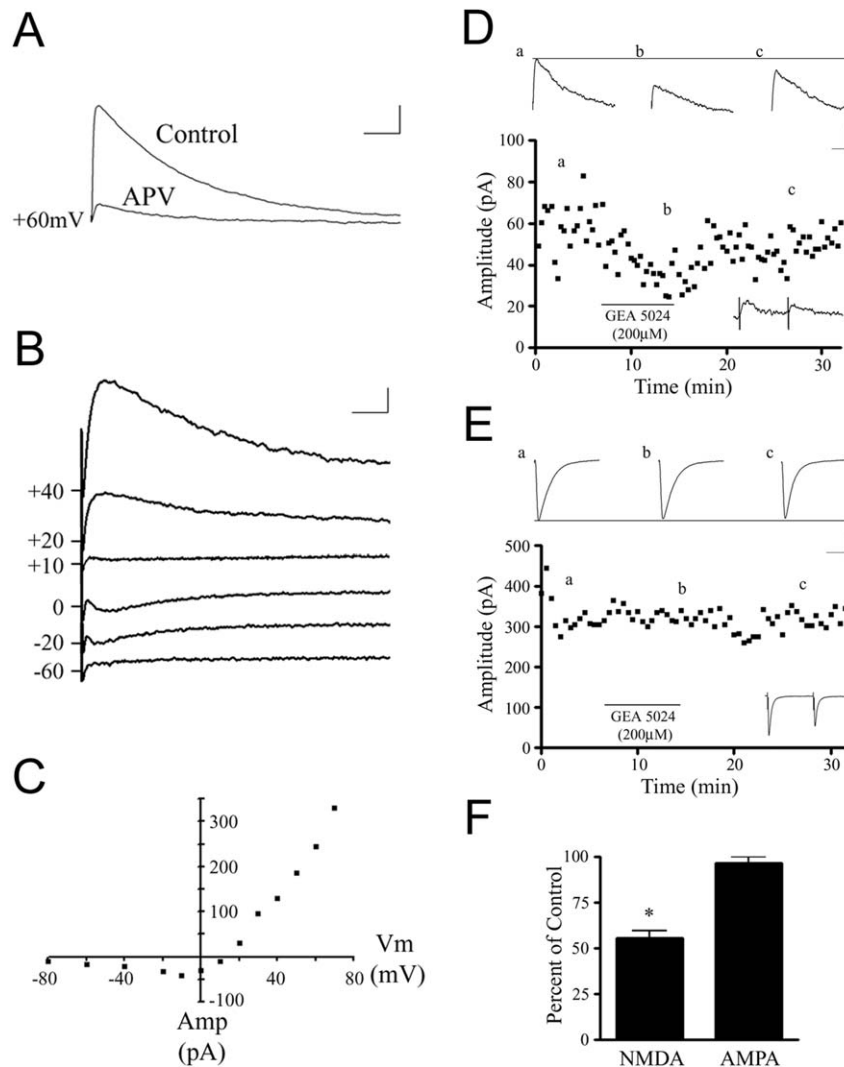


Fig. 4. The NO donor GEA 5024 selectively inhibits the NMDA component of the retinogeniculate EPSC. (A–D) The NMDA component of the EPSC was isolated with DNQX, and the outward current was challenged with the NO donor. (A) At a command potential of +60 mV, the outward current is blocked by the NMDA receptor antagonist, APV. (B, C) A range of command potentials from –80 to +80 mV illustrates the characteristic voltage dependency of the NMDA current which reversed at +13.3 mV. (D) At a holding potential of +40 mV and in the presence of DNQX, optic tract stimulation elicited an outward NMDA current that was reduced in amplitude by the NO donor GEA 5024 (200 μ M). In this and subsequent voltage clamp figures, the inset trace on the graph illustrates a paired pulse depression of EPSCs that is characteristically elicited upon retinogeniculate stimulation (Granseth et al., 2002). Traces labeled with lowercase letters are the averages of five consecutive traces taken from the corresponding regions of the plotted time course: (a) during control, (b) following GEA 5024 administration, and (c) washout. The same convention is followed in subsequent voltage clamp figures. (E) At a holding potential of –75 mV and in the presence of APV, optic tract stimulation elicited an inward, non-NMDA current. GEA 5024 (200 μ M) had no effect on the amplitude of the non-NMDA current. (F) Population data showing the relative effect of GEA 5024 on retinogeniculate EPSCs. GEA 5024 significantly reduced the amplitude of the NMDA EPSC to 55.4% of control amplitude but had no effect on the non-NMDA EPSC (* $P < 0.01$). Scale bar = 150 pA, 50 ms in A; B = 20 pA, 20 ms; D = 20 pA, 50 ms; E = 100 pA, 10 ms.

following washout. No significant change in membrane resistance was associated with this effect (Fig. 5).

NO selectively enhances the NMDA component of the corticogeniculate EPSC

Fig. 6 shows the effect of GEA 5024 on pharmacologically isolated corticogeniculate EPSCs. GEA 5024 (200 μ M) significantly increased the amplitude of the NMDA current to $195.7 \pm 22.3\%$ of control ($n = 6$, $P < 0.05$). Following washout of GEA 5024, the EPSC amplitude recovered to $94.5 \pm 7.1\%$ of control ($P > 0.05$). In contrast, the amplitude of the isolated

non-NMDA EPSC was not significantly changed by GEA 5024 (200 μ M) (EPSC amplitude = $98.2 \pm 5.8\%$ of control; $n = 6$, $P > 0.05$). Additionally, GEA 5024 had no effect on the paired pulse facilitation ratio (PPFR) during experiments in which non-NMDA EPSCs were isolated (PPFR = $103.8 \pm 4.2\%$ of control; $n = 6$; $P > 0.05$). Paired-pulse ratios have been shown to be a reliable index of presynaptic release dynamics, thus the absence of effect on the PPR confirms the postsynaptic site of action. GEA 5024 had no effect on membrane resistance during these experiments ($P > 0.05$).

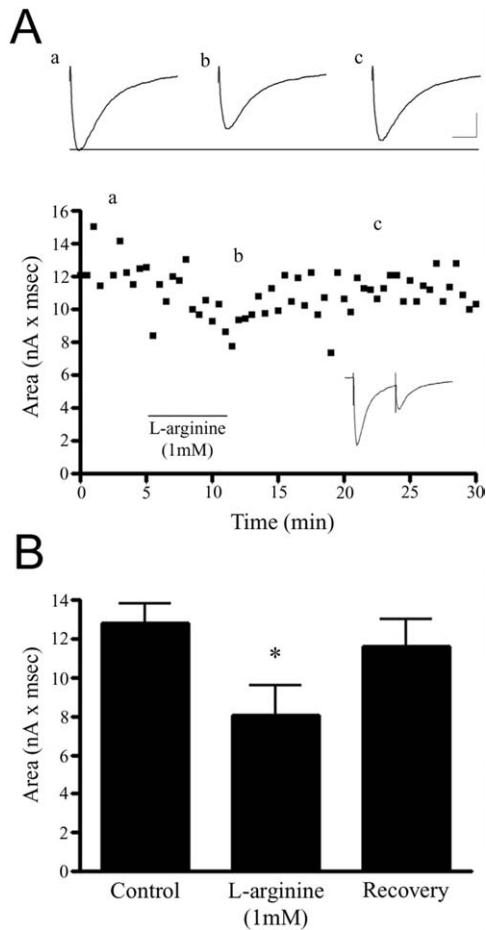


Fig. 5. The NO precursor L-arginine reduced the area of the composite retinogeniculate EPSC, replicating the effect of NO donors. (A) Time course of the effect of L-arginine (1 mM) on the retinogeniculate EPSC. Averages of five traces are shown during control (a), L-arginine (1 mM) exposure (b), and following washout (c). (B) For the population of retinogeniculate stimulated EPSCs, L-arginine (1 mM) significantly reduced the area of the EPSC (* $P < 0.05$ relative to control). Scale bar = 100 pA, 20 ms in A.

L-Arginine treatment enhances the corticogeniculate EPSC

Fig. 7 shows the effect of the NO precursor L-arginine at the corticogeniculate synapse. L-Arginine (1 mM) significantly enhanced the composite corticogeniculate EPSC area ($162.6 \pm 11.8\%$ of control; $n=8$, $P < 0.01$), which recovered following washout. No significant change in membrane resistance was associated with this effect.

We observed a difference in the time to onset and duration of drug effects at the retinogeniculate and corticogeniculate synapses for both NO donors and L-arginine (for example, see Fig. 4D and Fig. 6A). In order to quantify this latency effect we determined the time of the first significant drug effect by calculating the mean and standard deviation of the control data points, then we defined the first significant drug effect as the time at which the data points crossed to a value coinciding with the mean + two times the standard deviation. Significant drug effects were observed in the retinogeniculate pathway at 8.5 ± 0.7 min

after initiation of drug delivery, whereas in the corticogeniculate pathway, significant drug effects were observed at 13.5 ± 1.9 min after drug delivery ($P < 0.01$ retinal versus cortical). Additionally, drug effects at the retinogeniculate synapse lasted for 6.1 ± 1.6 min after the onset of drug effect, whereas at the corticogeniculate synapse drug effects persisted for 14.4 ± 3.7 min ($P < 0.01$) after onset.

This latency difference could not be accounted for by differences in the flow rate of the perfusion system or other

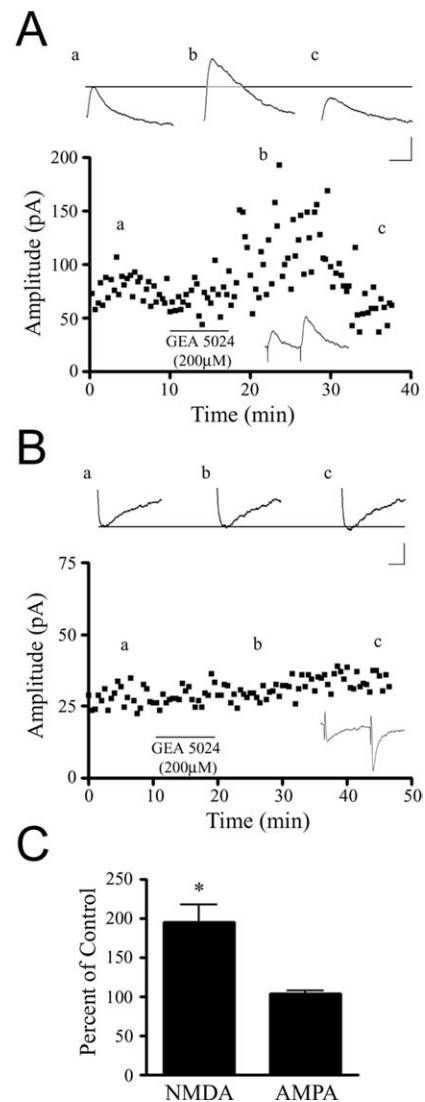


Fig. 6. The NO donor GEA 5024 selectively enhances the NMDA component of the corticogeniculate EPSC. (A) The NMDA current was isolated in the same way as described for retinogeniculate stimulation (Fig. 2). At a holding potential of +40 mV, and in the presence of DNQX, stimulation of the optic radiations elicited an outward NMDA current that was enhanced in amplitude by GEA 5024 (200 μM). (B) At a holding potential of -75 mV and in the presence of APV, optic radiation stimulation elicited an inward non-NMDA current that was not significantly affected by GEA 5024 (200 μM). In both A and B the averages of seven traces are shown during control (a), GEA 5024 (200 μM) exposure (b), and washout (c). (C) GEA 5024 significantly increased the corticogeniculate NMDA EPSC to 195.7% of control while having no effect on the non-NMDA EPSC (* $P < 0.01$). Scale bar = 50 pA, 50 ms in A; B = 15 pA, 20 ms.

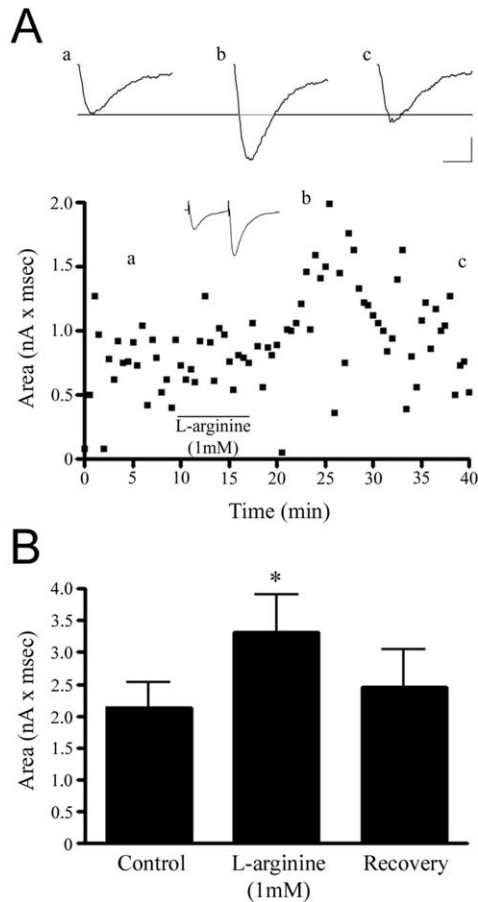


Fig. 7. The NO precursor L-arginine increased the area of the composite corticogeniculate EPSC, replicating the effect of NO donors. (A) Time course of the effect of L-arginine (1 mM) on the corticogeniculate EPSC. Averages of five traces are shown during control (a), L-arginine (1 mM) exposure (b), and following washout (c). (B) For the population of corticogeniculate stimulated EPSCs, L-arginine (1 mM) significantly enhanced the area of the EPSC (* $P < 0.01$ relative to control). Scale bar = 10 pA, 20 ms in A.

aspects of the drug delivery method, which is the same for all experiments. Also, since our delivery methods promote total bath substitution, differences in timing of the effects are unlikely to be due to final concentration differences between the two pathways. Retinal terminals synapse on proximal dendrites and cortical terminals on distal dendrites, but because these relationships occur homogeneously throughout the slice there is no evidence suggesting that two dendritic regions would receive different drug concentrations. These differences in onset and duration times are more consistent with differential mechanisms of drug action at each synapse, particularly since the direction of the treatment effect differs between the pathways.

NO modulation of the corticogeniculate EPSC is mimicked by cGMP

The striking differences we have observed in the effects of NO on the two glutamatergic inputs to the LGN suggest a difference in the targeting mechanism of NO at each type of synapse. Previous *in vitro* studies have impli-

cated cGMP in the mechanism of NO modulation of thalamic function (Papé and Mager, 1992; Shaw et al., 1999). We therefore used the soluble cGMP analog, 8-bromo-cGMP to examine the potential involvement of cGMP in NO modulation of retinogeniculate and corticogeniculate synaptic responses (Fig. 8). Application of 8-bromo-cGMP (1 mM) did not replicate the reduction by NO donors or L-arginine on the retinogeniculate EPSC. There was no significant change in the area of the composite retinogeniculate EPSC ($102.4 \pm 10.4\%$ of control; $n=4$ $P > 0.05$). In contrast, 8-bromo-cGMP (1 mM) replicated the enhancement of the corticogeniculate EPSC produced by NO donors and L-arginine. The area of the composite EPSC was significantly increased to $166.4 \pm 9.3\%$ of control in the presence of 8-bromo-cGMP ($n=4$, $P < 0.01$). The amplitude of the isolated NMDA EPSC in the corticogeniculate pathway was also enhanced by 1 mM 8-bromo-cGMP ($162.9 \pm 16.1\%$ of control; $n=3$; $P < 0.01$). Membrane resistance was not significantly changed in the presence of 8-bromo-cGMP during experiments on either pathway ($P > 0.05$).

DISCUSSION

The results of this study suggest a new role for the ascending cholinergic/nitroergic pathway from the PBR. In addition to the well-described mechanism of cholinergic depolarization of thalamic relay neurons that promotes tonic response mode (Singer, 1977; Marks and Roffwarg, 1989; McCormick, 1989) our data suggest that nitroergic signaling mediates a differential modulation of glutamatergic synaptic responses in thalamic relay neurons. We have found that NO inhibited retinally-evoked NMDA-mediated synaptic responses and enhanced cortically-evoked responses.

Depending on the preparation, the site of action of NO at glutamatergic synapses in has been found to be either pre- or postsynaptic. Others have found a presynaptic reduction in glutamate release by NO to be responsible for inhibition of synaptic responses (Brenowitz and Trussell, 2001). In contrast, our results are most consistent with a postsynaptic route of action of NO on the NMDA receptor. Our current clamp recordings at both the retinogeniculate and corticogeniculate synapses revealed that only the late, APV-sensitive component of the composite EPSP was modulated by NO donors and L-arginine, which would not be reflective of presynaptic release effects that would likely affect both components. Furthermore, we isolated each component of the EPSC in voltage clamp recordings to determine whether each was similarly affected by NO. Pharmacological isolation of the NMDA and non-NMDA EPSCs revealed that only the NMDA EPSC in either pathway was affected by NO, consistent with a postsynaptic mechanism. We also assessed PPRs at each synapse, which served the dual purpose of identifying the pathway of origin of the EPSCs (Alexander and Godwin, 2005) and determining whether NO generation affected presynaptic release dynamics (Alexander and Godwin, 2005; Granseth et al., 2002; Zucker and Regehr, 2002). We did not ob-

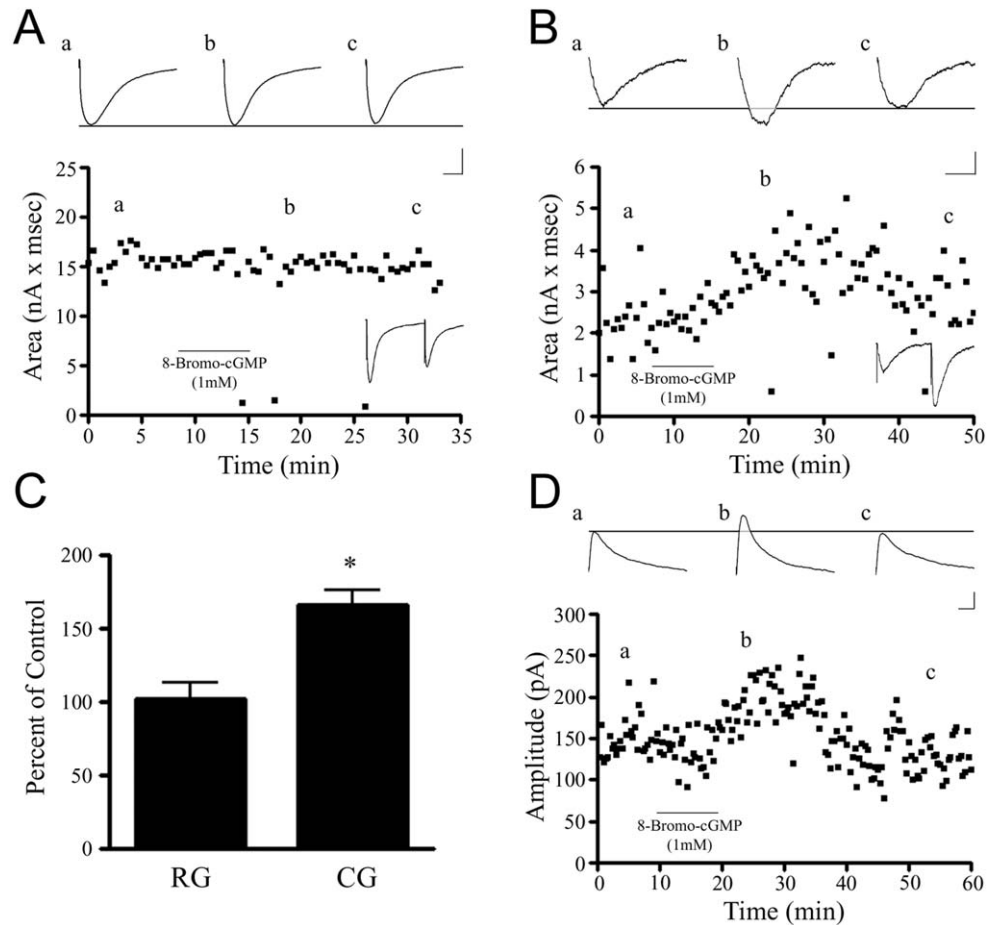


Fig. 8. Cyclic GMP selectively targets corticogeniculate responses. (A) The composite retinogeniculate EPSC is not inhibited by the membrane soluble analog 8-bromo-cGMP (1 mM). (B) The composite corticogeniculate EPSC is enhanced by 8-bromo-cGMP (1 mM) replicating the effect of NO donors and L-arginine. (C) The retinogeniculate EPSC is not changed from control, while the corticogeniculate EPSC was increased to approximately 166.4% of control (* $P < 0.01$). (D) The corticogeniculate isolated NMDA EPSC is enhanced by 8-bromo-cGMP (1 mM). NMDA current was isolated at +40 mV and with inclusion of DNQX (30 μ M) in the ACSF. Scale bar=100 pA, 25 ms in A; B=10 pA, 40 ms; D=50 pA, 40 ms.

serve an effect of L-arginine or NO donors on PPRs, which is consistent with a postsynaptic locus of NO effects.

Our results are consistent with NO promoting a difference in the impact of glutamatergic synapses on LGN relay cells through different mechanisms. While the NMDA EPSC enhancement by NO in the corticogeniculate pathway was found to be mimicked by 8-bromo-cGMP, suggesting NO utilization of the cGMP pathway, the absence of effect (either reduction or enhancement) by the cGMP analog in the retinogeniculate pathway under the same recording conditions suggests an alternative mechanism. The functional consequences of NO interactions with the NMDA receptor are varied in the literature, but there is a suggestion that the effect of NO at a particular synapse depends upon the mode of interaction of NO and its target molecule. Several mechanisms of NO modulation are found in the literature, including tyrosine nitration, s-nitrosylation, and cGMP activation (Choi and Lipton, 2000; Wei et al., 2002; Gow et al., 2004). The finding that the NO reduction of the retinogeniculate NMDA EPSP persisted in the presence of SOD argues against tyrosine nitration,

which is usually dependent upon formation of peroxynitrite (Radi, 2004).

NO can also interact with cysteine residues of the NMDA receptor leading to s-nitrosylation that occurs independently of cGMP formation (Lei et al., 1992; Choi and Lipton, 2000). NO has been shown to inhibit the NMDA receptor through this mechanism (Lei et al., 1992; Lipton et al., 1998). In contrast, NO has been shown to enhance NMDA currents in neurons by cGMP dependent signaling (Wei et al., 2002; Huang et al., 2003). Our results suggest the possibility of two different NMDA targeting strategies in the LGN. The selective sensitivity of the corticogeniculate synapse to cGMP could be due to a difference in the heteromeric NMDA subunit composition at retinogeniculate and corticogeniculate synapses that influence sensitivity to phosphorylation (Hall and Soderling, 1997), or to a difference in the local availability of other components of the second messenger response, such as protein kinase G. The observed differences in the time course of the retinogeniculate and corticogeniculate effects to NO generators may also reflect the involvement of such second

messenger intermediaries in the enhancement of corticogeniculate NMDA currents.

The pathway-specific differences we have observed in NO modulation of glutamatergic signaling in the LGN joins other recently described differences between retinogeniculate and corticogeniculate pathways. Corticogeniculate synapses use a postsynaptic metabotropic glutamate receptor (mGluR1), while retinogeniculate synapses do not (Godwin et al., 1996a; Vidnyanszky et al., 1996; Turner and Salt, 1998). More recently, we have functionally localized mGluR2 to presynaptic corticothalamic terminals, but not retinogeniculate terminals (Alexander and Godwin, 2005, 2006). The differential effect of NO at the two synapses is unlikely to involve mGluRs. MGLuR1 activation affects cation conductances (McCormick and von Krosigk, 1992; Golshani et al., 1998) and agonists applied to LGN cells have not revealed direct effects on NMDA responses (Godwin et al., 1996b; Salt, 2002; Salt and Binns, 2000). MGLuR2 effects are presynaptic in nature, and a mechanism involving NO stimulation of these receptors is unlikely because mGluR2s reduce glutamate release from presynaptic terminals (Alexander and Godwin, 2005, 2006), which would not be consistent with the type of postsynaptic NMDA-specific enhancement we have observed in the present study.

The results presented here serve to reconcile prior literature on NO effects in the thalamus (Cudeiro et al., 1994a,b, 1996; Do et al., 1994; Shaw et al., 1999). Cudeiro et al. (1996) interrupted NO generation *in vivo* by local iontophoretic application of inert NOS substrates which led to reduced spiking of relay cells and reduced responses to iontophoretically-applied NMDA, while NO donors enhanced responses to iontophoretically-applied NMDA as well as spontaneous activity. Consistent with our study, little effect was found on non-NMDA receptor-mediated transmission. While the conclusion from these studies was that NO generation has an enabling role for retinogeniculate spike transfer (Cudeiro et al., 1994a, 1996), others have found no specific potentiation of retinogeniculate synaptic responses using 8-bromo-cGMP (Shaw et al., 1999), even though sensory transmission was found to be enhanced. Our data resolve these seemingly disparate findings by revealing the corticogeniculate pathway as a target of enhanced synaptic responsiveness in the presence of NO.

Rethinking the relationship between the brainstem and the thalamus

We report here a striking difference in how NO affects postsynaptic thalamic relay neuron responses to retinal and cortical inputs. Our results suggest a reconsideration of thalamic function to include the idea that the relative influence of retinal and cortical inputs on LGN cells may be dynamically determined by NO and modulated with behavioral state changes by the PBR. This idea extends the classical view of the functional role of the PBR, in which the depolarization by cholinergic inputs to the thalamus elicits increased tonic signal transmission through the thalamus (Eysel et al., 1986; McCormick and Prince, 1987; Francesconi et al., 1988; McCormick and Papé, 1988; McCormick, 1989; McCormick and

Bal, 1997) in a way that does not preferentially favor one pathway over another. Our results suggest a specific function for nitrgergic signaling that is unique from cholinergic influences in the same pathway. In addition to changes in overall relay cell excitability with behavioral state changes, LGN relay cells may change their responsiveness to retinal and cortical inputs in the presence of nitrgergic activation during periods of wakefulness and REM sleep.

The consequences of this alteration in the balance of synaptic transmission during NO release are unknown, but the known features of corticogeniculate feedback suggest the possibility of significant non-linear effects on visual signal processing. The LGN is the first site in the visual system to receive peripheral inputs from retinal ganglion cells as well as cortical feedback, an arrangement that comprises an important node at which the cortex may regulate its own input. Murphy and Sillito (1996) found that a single layer VI cell may innervate an ensemble of LGN cells, which is supported by physiological studies showing that cortex may control the degree of synchronous firing between LGN units within ensembles exhibiting similar response properties (Sillito et al., 1994). One possible *in vivo* function of enhanced responses to corticogeniculate inputs seen during the generation of NO is increased persistence and temporal summation of corticogeniculate EPSPs, which may serve to enhance the synchronization of groups of LGN cells that binds common visual features.

The NO-mediated persistence of corticogeniculate inputs may broaden the timing window during which corticogeniculate EPSPs integrate with retinogeniculate EPSPs that have been simultaneously sharpened by NO. The advantage of sharpening the retinal EPSPs is to promote the encoding of rapidly changing retinal signals by diminishing nonlinear distortions due to NMDA summation (Futai et al., 2001). This suggests that enhancing cortical feedback may promote a form of coincidence detection in which temporally precise retinal signals are matched with persistent feedback cortical signals. Those retinal signals that match the cortical receptive field position reported by positive layer VI feedback, within a certain timing window, would be amplified (Koch, 1987).

CONCLUSION

We have demonstrated a surprising new role for NO in thalamic physiology that is consistent with previous findings, but is unique with respect to the dual mechanisms of nitrgergic signaling we have found to be embodied in the same nucleus. While the ascending influences from the brainstem and the descending influences from cortex are often treated separately in theory and experiments, the NO effects we have observed may provide an early opportunity for processed information from the cortex to be communicated back to the thalamus via extensive feedback connections, where brainstem nitrgergic influences may confer the salience of this feedback in tandem with behavioral state changes of the reticular activating system.

Acknowledgments—This work is supported by EY11695, AA13246 and NS046222. The authors thank Steven Frank and Justin Rawley for aid in data collection and analysis, and Ashok Hegde, PhD, for reading the manuscript.

REFERENCES

- Alexander GM, Fisher TL, Godwin DW (2006) Differential response dynamics of corticothalamic glutamatergic synapses in the lateral geniculate nucleus and thalamic reticular nucleus. *Neuroscience* 137:367–372.
- Alexander GM, Godwin DW (2005) Presynaptic inhibition of corticothalamic feedback by metabotropic glutamate receptors. *J Neurophysiol* 94:163–175.
- Alexander GM, Godwin DW (2006) Unique presynaptic and postsynaptic roles of group II metabotropic glutamate receptors in the modulation of thalamic network activity. *Neuroscience* 141:501–513.
- Bickford ME, Gunluk AE, Guido W, Sherman SM (1993) Evidence that cholinergic axons from the parabrachial region of the brainstem are the exclusive source of nitric oxide in the lateral geniculate nucleus of the cat. *J Comp Neurol* 334:410–430.
- Bickford ME, Ramcharan E, Godwin DW, Erisir A, Gnad J, Sherman SM (2000) Neurotransmitters contained in the subcortical extraretinal inputs to the monkey lateral geniculate nucleus. *J Comp Neurol* 424:701–717.
- Blanton MG, Lo Turco JJ, Kriegstein A (1989) Whole cell recording from neurons in slices of reptilian and mammalian cortex. *J Neurosci Methods* 30:203–210.
- Brenowitz S, Trussell LO (2001) Minimizing synaptic depression by control of release probability. *J Neurosci* 21:1857–1867.
- Carden WB, Datskovskaia A, Guido W, Godwin DW, Bickford ME (2000) Development of the cholinergic, nitrenergic, and GABAergic innervation of the cat dorsal lateral geniculate nucleus. *J Comp Neurol* 418:65–80.
- Castelo-Branco M, Neuenschwander S, Singer W (1998) Synchronization of visual responses between the cortex, lateral geniculate nucleus, and retina in the anesthetized cat. *J Neurosci* 18:6395–6410.
- Choi YB, Lipton SA (2000) Redox modulation of the NMDA receptor. *Cell Mol Life Sci* 57:1535–1541.
- Cudeiro J, Grieve KL, Rivadulla C, Rodriguez R, Martinez-Conde S, Acuña C (1994a) The role of nitric oxide in the transformation of visual information within the dorsal lateral geniculate nucleus of the cat. *Neuropharmacology* 33:1413–1418.
- Cudeiro J, Rivadulla C, Rodriguez R, Martinez-Conde S, Acuna C, Alonso JM (1994b) Modulatory influence of putative inhibitors of nitric oxide synthesis on visual processing in the cat lateral geniculate nucleus. *J Neurophysiol* 71:146–149.
- Cudeiro J, Rivadulla C, Rodriguez R, Martinez-Conde S, Martinez L, Grieve KL, Acuña C (1996) Further observations on the role of nitric oxide in the feline lateral geniculate nucleus. *Eur J Neurosci* 8:144–152.
- Do KQ, Binns KE, Salt TE (1994) Release of nitric oxide precursor, arginine, from the thalamus upon sensory afferent stimulation, and its effects on thalamic neurons in vivo. *Neuroscience* 60:581–586.
- Erisir A, Van Horn SC, Bickford ME, Sherman SM (1997) Immunocytochemistry and distribution of parabrachial terminals in the lateral geniculate nucleus of the cat: A comparison with corticogeniculate terminals. *J Comp Neurol* 370:233–254.
- Eysel UT, Papé HC, van Schayck T (1986) Excitatory and differential disinhibitory actions of acetylcholine in the lateral geniculate nucleus of the cat. *J Physiol (Lond)* 370:233–254.
- Francesconi W, Muller CM, Singer W (1988) Cholinergic mechanisms in the reticular control on transmission in the cat lateral geniculate nucleus. *J Neurophysiol* 59:1690–1718.
- Futai K, Okada M, Matsuyama K, Takahashi T (2001) High-fidelity transmission acquired via a developmental decrease in NMDA receptor expression at an auditory synapse. *J Neurosci* 21:3342–3349.
- Godwin DW, Van Horn SC, Erisir A, Sesma M, Romano C, Sherman SM (1996a) Ultrastructural localization suggests that retinal and cortical inputs access different metabotropic glutamate receptors in the lateral geniculate nucleus. *J Neurosci* 16:8181–8192.
- Godwin DW, Vaughan JW, Sherman SM (1996b) Metabotropic glutamate receptors switch visual response mode of lateral geniculate nucleus cells from burst to tonic. *J Neurophysiol* 76:1800–1816.
- Golshani P, Warren RA, Jones EG (1998) Progression of change in NMDA, non-NMDA, and metabotropic glutamate receptor function at the developing corticothalamic synapse. *J Neurophysiol* 80:143–154.
- Gow AJ, Farkouh CR, Munson DA, Posencheg MA, Ischiropoulos H (2004) Biological significance of nitric oxide-mediated protein modification. *Am J Physiol Lung Cell Mol Physiol* 287:L262–L268.
- Granseth B, Ahlstrand E, Lindstrom S (2002) Paired pulse facilitation of corticogeniculate EPSCs in the dorsal lateral geniculate nucleus investigated in vitro. *J Physiol (Lond)* 544:477–486.
- Hall RA, Soderling TR (1997) Differential surface expression and phosphorylation of the N-methyl-D-aspartate receptor subunits NR1 and NR2 in cultured hippocampal neurons. *J Biol Chem* 272:4135–4140.
- Huang CC, Chan SH, Hsu KS (2003) cGMP/protein kinase G-dependent potentiation of glutamatergic transmission induced by nitric oxide in immature rat rostral ventrolateral medulla neurons in vitro. *Mol Pharmacol* 64:521–532.
- Kankaanranta H, Rydell E, Petersson AS, Holm P, Moilanen E, Corell T, Karup G, Vuorinen P, Pedersen SB, Wennmalm A, Metsä-Ketelä T (1996) Nitric oxide-donating properties of mesoionic 3-aryl substituted oxatriazole-5-imine derivatives. *Br J Pharmacol* 117:401–406.
- Karup G, Preikschat H, Wilhelmsen ES, Pedersen SB, Marcinkiewicz E, Cieslik K, Gryglewski RJ (1994) Mesoionic oxatriazole derivatives: a new group of NO-donors. *Pol J Pharmacol* 46:541–542.
- Katsuki H, Tomita M, Takenaka C, Shirakawa H, Shimazu S, Ibi M, Kume T, Kaneko S, Akaike A (2001) Superoxide dismutase activity in organotypic midbrain-striatum co-cultures is associated with resistance of dopaminergic neurons to excitotoxicity. *J Neurochem* 76:1336–1345.
- Koch C (1987) The action of the corticofugal pathway on sensory thalamic nuclei: a hypothesis. *Neuroscience* 23:399–406.
- Leamey CA, Ho-Pao CL, Sur M (2001) Disruption of retinogeniculate pattern formation by inhibition of soluble guanylyl cyclase. *J Neurosci* 21:3871–3880.
- Lei SZ, Pan ZH, Aggarwal SK, Chen HS, Hartman J, Sucher NJ, Lipton SA (1992) Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. *Neuron* 8:1087–1099.
- Li DP, Chen SR, Pan HL (2002) Nitric oxide inhibits spinally projecting paraventricular neurons through potentiation of presynaptic GABA release. *J Neurophysiol* 88:2664–2674.
- Lipton SA, Rayudu PV, Choi YB, Sucher NJ, Chen HS (1998) Redox modulation of the NMDA receptor by NO-related species. *Prog Brain Res* 118:73–82.
- Livingstone MS, Hubel DH (1981) Effects of sleep and arousal on the processing of visual information in the cat. *Nature* 291:554–561.
- Ma HT, Favre CJ, Patterson RL, Stone MR, Gill DL (1999) Ca²⁺ entry activated by S-nitrosylation. *J Biol Chem* 274:35318–35324.
- Marks GA, Roffwarg HP (1989) The cholinergic influence upon rat dorsal lateral geniculate nucleus is dependent on state of arousal. *Brain Res* 494:294–306.
- McCauley AK, Carden WB, Godwin DW (2003) Brain nitric oxide synthase expression in the developing ferret lateral geniculate nucleus: analysis of time course, localization, and synaptic contacts. *J Comp Neurol* 462:342–354.
- McCormick DA (1989) Cholinergic and noradrenergic modulation of thalamocortical processing. *Trends Neurosci* 12:215–221.

- McCormick DA, Feeser HR (1990) Functional implications of burst firing and single spike activity in lateral geniculate relay neurons. *Neuroscience* 39:103–113.
- McCormick DA, Papé HC (1988) Acetylcholine inhibits identified interneurons in the cat lateral geniculate nucleus. *Nature* 334:246–248.
- McCormick DA, Prince DA (1987) Actions of acetylcholine in the guinea-pig and cat medial and lateral geniculate nuclei, in vitro. *J Physiol (Lond)* 392:147–165.
- McCormick DA, Bal T (1997) Sleep and arousal: thalamocortical mechanisms. *Annu Rev Neurosci* 20:185–215.
- McCormick DA, von Krosigk M (1992) Corticothalamic activation modulates thalamic firing through glutamate “metabotropic” receptors. *Proc Natl Acad Sci U S A* 89:2774–2778.
- Moilanen E, Vuorinen P, Kankaanranta H, Metsa-Ketela T, Vapaatalo H (1993) Inhibition by nitric oxide-donors of human polymorphonuclear leucocyte functions. *Br J Pharmacol* 109:852–858.
- Moss MB, Brunini TMC, Soares de Moura R, Novales Malagris LE, Roberts NB, Ellory JC, Mann GE, Mendes Ribeiro AC (2004) Diminished L-arginine bioavailability in hypertension. *Clin Sci* 107:391–397.
- Mu J, Carmen WB, Kurukulasuriya NC, Alexander GM, Godwin DW (2003) Ethanol influences on native T-type calcium current in thalamic sleep circuitry. *J Pharmacol Exp Ther* 307:197–204.
- Murphy PC, Sillito AM (1996) Functional morphology of the feedback pathway from area 17 of the cat visual cortex to the lateral geniculate nucleus. *J Neurosci* 16:1180–1192.
- Nicholson C, Hounsgaard J (1983) Diffusion in the slice microenvironment and implications for physiological studies. *Fed Proc* 42:2865–2868.
- O'Dell TJ, Hawkins RD, Kandel ER, Arancio O (1991) Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. *Proc Natl Acad Sci U S A* 88:11285–11289.
- Papé HC, Mager R (1992) Nitric oxide controls oscillatory activity in thalamocortical neurons. *Neuron* 9:441–448.
- Raczkowski D, Fitzpatrick D (1989) Organization of cholinergic synapses in the cat's dorsal lateral geniculate and perigeniculate nuclei. *J Comp Neurol* 288:676–690.
- Radi R (2004) Nitric oxide, oxidants, and protein tyrosine nitration. *Proc Natl Acad Sci U S A* 101:4003–4008.
- Salt TE (2002) Glutamate receptor functions in sensory relay in the thalamus. *Philos Trans R Soc Lond B Biol Sci* 357:1759–1766.
- Salt TE, Binns KE (2000) Contributions of mGlu1 and mGlu5 receptors to interactions with N-methyl-D-aspartate receptor-mediated responses and nociceptive sensory responses of rat thalamic neurons. *Neuroscience* 100:375–380.
- Scharfman HE, Lu SM, Guido W, Adams PR, Sherman SM (1990) N-methyl-D-aspartate receptors contribute to excitatory postsynaptic potentials of cat lateral geniculate neurons recorded in thalamic slices. *Proc Natl Acad Sci U S A* 87:4548–4552.
- Shaw PJ, Charles SL, Salt TE (1999) Actions of 8-bromo-cyclic-GMP on neurons in the rat thalamus in vivo and in vitro. *Brain Res* 833:272–277.
- Sillito AM, Jones HE, Gerstein GL, West DC (1994) Feature-linked synchronization of thalamic relay cell firing induced by feedback from the visual cortex. *Nature* 369:479–482.
- Sillito AM, Murphy PC, Salt TE, Moody CI (1990) Dependence of retinogeniculate transmission in cat on NMDA receptors. *J Neurophysiol* 63:347–355.
- Singer W (1977) Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. *Physiol Rev* 57:386–420.
- Turner JP, Salt TE (1998) Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurones in vitro. *J Physiol (Lond)* 510:829–843.
- Vidnyanszky Z, Gorcs TJ, Negyessy L, Borostyankio Z, Knopfel T, Hamori J (1996) Immunocytochemical visualization of the mGluR1a metabotropic glutamate receptor at synapses of corticothalamic terminals originating from area 17 of the rat. *Eur J Neurosci* 8:1061–1071.
- von Krosigk M, Monckton JE, Reiner PB, McCormick DA (1999) Dynamic properties of corticothalamic excitatory postsynaptic potentials and thalamic reticular inhibitory postsynaptic potentials in thalamocortical neurons of the guinea-pig dorsal lateral geniculate nucleus. *Neuroscience* 91:7–20.
- Wei JY, Jin X, Cohen ED, Daw NW, Barnstable CJ (2002) cGMP-induced presynaptic depression and postsynaptic facilitation at glutamatergic synapses in visual cortex. *Brain Res* 927:42–54.
- Williams JA, Vincent SR, Reiner PB (1997) Nitric oxide production in rat thalamus changes with behavioral state, local depolarization, and brainstem stimulation. *J Neurosci* 17:420–427.
- Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. *Annu Rev Physiol* 64:355–405.

(Accepted 5 June 2006)