

neurones on which they act. The article by Altar and DiStefano¹ inspires these ideas and encourages more function-related views on the mode of action of these very interesting molecules. Only a better understanding of the mode of action of neurotrophic factors will allow us to develop a rational approach to their therapeutic use.

In the same issue, there was also an article by Ibáñez⁷, on the structural biology of neurotrophic factors. It contained very interesting data on the structure of neurotrophic factors and their receptors. It elucidated the functional relationship of these molecules, which from the point of view of a cell biologist was informative. It is, therefore, difficult to understand why the 'Summary and Conclusions' did not tell the reader what the article contained, but discussed the value of neurotrophic factors in health and disease, none of which was relevant to the content of the article. It also referred to their potential therapeutic value, which seemed somewhat irrelevant as neither this nor any other article in this issue of *TINS* contained any evidence that substantiated this point. Such statements, when not supported by reliable evidence, are dangerously misleading, for they

encourage non-experts to believe them. In view of the pursuit to find treatments for intractable neurodegenerative diseases this could lead to serious mishandling of this information and inflict additional suffering on patients, as was seen with the trial of ciliary neurotrophic factor for the treatment of amyotrophic lateral sclerosis⁸.

Gerta Vrbová

Linda Greensmith

Dept of Anatomy and Developmental Biology, University College London, Gower Street, London, UK WC1E 6BT.

Antal Nógrádi

Dept of Ophthalmology, Albert Szent-Györgyi Medical University, PO Box 407, 6701 Szeged, Hungary.

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Reply

I am glad that Drs Vrbová, Greensmith and Nógrádi¹ found my article on the structure–function relationships of neurotrophic factors of interest. It is, however, sad to learn that they are pessimistic about the possible therapeutic potential of neurotrophic molecules, although it explains why they failed to understand how this issue was relevant to the article. I only hope that their frustration is not shared by a larger number of neuroscientists.

Carlos F. Ibáñez

Division of Molecular Neurobiology, Dept of Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden.

Reference

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REVIEW

Sight and insight – on the physiological role of nitric oxide in the visual system

Javier Cudeiro and Casto Rivadulla

Research in the fields of cellular communication and signal transduction in the brain has moved very rapidly in recent years. Nitric oxide (NO) is one of the latest discoveries in the arena of messenger molecules. Current evidence indicates that, in visual system, NO is produced in both postsynaptic and presynaptic structures and acts as a neurotransmitter, albeit of a rather unorthodox type. Under certain conditions it can switch roles to become either a neuronal 'friend' or 'foe'. Nitric oxide is a gas that diffuses through all physiological barriers to act on neighbouring cells across an extensive volume on a specific time scale. It, therefore, has the opportunity to control the processing of vision from the lowest level of retinal transduction to the control of neuronal excitability in the visual cortex.

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VISUAL PROCESSING starts in the retina. Here, the image of the world is broken down through visual filters (the receptive fields of individual neurones). In mammals this visual message then moves to an intermediate station in the thalamus, the dorsal lateral geniculate nucleus (dLGN). This is a laminar structure that receives the ganglion-cell axons in an organized manner depending on the eye from which the image

originated, the cell type and other species-dependent characteristics. Finally, this information is relayed to the primary visual cortex (V1) from which connections are made with many other visual cortical and sub-cortical structures. At every level of this pathway, including the retina, passage of the visual message involves the activation of members of the family of excitatory-amino-acid receptors. However, besides

Javier Cudeiro and Casto Rivadulla are at the E.U. Fisioterapia (Universidade Da Coruña), Centro Universitario de Oza, 15006 A Coruña, Spain.

TABLE 1. Products and targets of neuronal nitric oxide synthase

Nitric oxide-related species	Principal biological target-reactants
N ₂ O	Metals, hydrophobic pockets
NH ₂ OH	Oxidants
NO ⁻ (MNO; SNO)	Thiols, metals, oxygen
NO [•] (MNO; SNO)	Thiols, metals, superoxide, oxygen
NO _x -SNO-MNO (NO ⁺)	Thiols
OONO ⁻	Thiols, metals, tyrosine, methionine
NO ₂ ⁻ -NO ₃ ⁻	-

All of the above nitric oxide (NO)-related species have been identified from purified preparations of neuronal nitric oxide synthase (NOS)⁹⁴. The cellular availability of substrates and cofactors appears to influence the oxidation state of the NOS product. Nitrous oxide (N₂O) and hydroxylamine (NH₂OH) are end-reduction products, while nitrite-nitrate (NO₂⁻-NO₃⁻) are end-oxidation products. The remaining compounds have different biological actions and potential toxicities that reflect their chemistry in different redox milieu⁹⁵⁻⁹⁷. Note that different SNO and MNO species might function as NO[•], NO⁺, or NO⁻ donors. Adapted, with permission, from Ref. 22.

these specific visual signals, there is also modulation of the visual message by a number of non-specific inputs (for example, dopaminergic, cholinergic and noradrenergic), which control the excitability of the neurones and gate the flow of information. Recently, a newly discovered neurotransmitter has emerged rather spectacularly that is involved in the processing of visual information – nitric oxide (NO).

Nitric oxide: ubiquitous neurotransmitter or ‘saint-sinner’?

Since NO was first recognized as a messenger molecule in the brain that mediates the increased cGMP levels that occur on activation of NMDA receptors¹, major efforts have been made to understand the extent of its actions. Nitric oxide is a gas synthesized from L-arginine by the enzyme nitric-oxide synthase (NOS). At least three forms of the enzyme have been characterized: the constitutive endothelial and neuronal types are both Ca²⁺ dependent and the third is a Ca²⁺-independent inducible isoform, which is expressed only in the presence of cytokines. In this review, only data related to neuronal NOS (nNOS) will be considered. Available data from immunocytochemistry, *in situ* hybridization and NADPH-diaphorase histochemistry have given a reasonably comprehensive picture of the anatomical localization of NO-generating cells and their processes throughout the CNS (Ref. 2). The brain contains the highest level of NOS of any tissue so far examined and the broad distribution of the enzyme suggests that NO could be involved in many aspects of CNS function¹.

However, NO might be a ‘double-edged sword’. In some studies it has been considered to be potentially neurotoxic³. Indeed, in the presence of factors such as oxidative stress, generation of reactive oxygen intermediates and deficient antioxidant systems, NO can induce neural death⁴. The majority of evidence indicates that the participation of NO in neurodegenerative phenomena occurs through a non-enzymatic reaction with the superoxide anion (O₂⁻) to form peroxynitrite (ONOO⁻) (see Table 1), which is a highly reactive molecule and a potent oxidizing agent⁵. Other mechanisms of NO toxicity, by DNA damage⁶ or glutathione depletion⁷ have also been postulated.

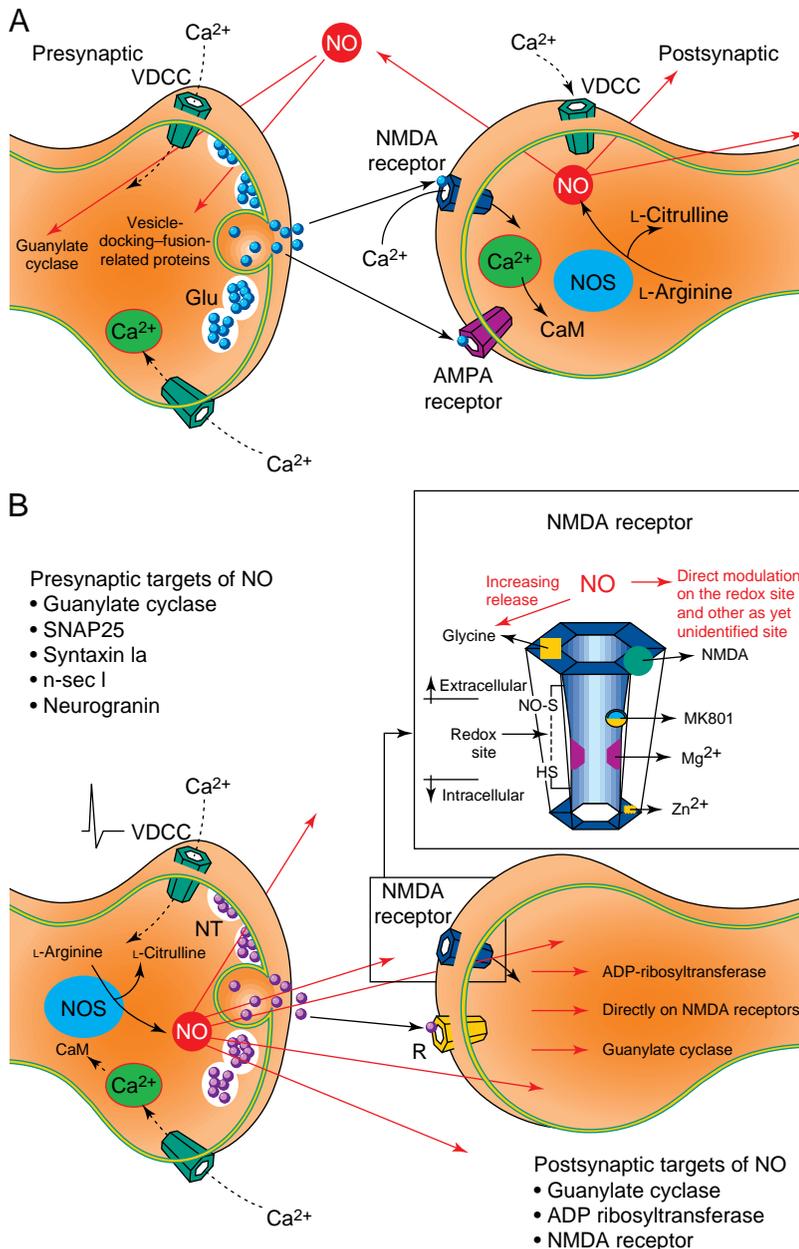


Fig. 1. Presynaptic and postsynaptic locations of nitric-oxide synthase (NOS) and the probable routes of action of nitric oxide (NO). (A) Postsynaptic location of NOS, showing possible presynaptic actions (red arrows). Calcium signal from glutamate-mediated activation of NMDA receptors or voltage-dependent Ca²⁺ channels (VDCCs) binds calmodulin (CaM) and activates NOS, producing NO and citrulline from L-arginine (reviewed in Ref. 1). The free diffusion of NO suggests that this presynaptic activity need not necessarily be restricted to the presynaptic boutons directly involved with this postsynaptic element. (B) Presynaptic location of NOS, showing possible postsynaptic actions. Note the direct actions on the NMDA receptor, which is expanded in the inset box and shows the different modulatory sites. Neurotransmitter (NT) release is triggered by the arrival of an action potential in the presynaptic terminal by the opening of VDCCs. The resulting elevation in the internal Ca²⁺ concentration, is the signal that causes NO production. This has been shown to occur in the dorsal lateral geniculate nucleus of the cat¹⁸⁻²⁰, where NOS is co-localized with ACh within the axons arising from the brainstem²¹. Again, diffusion of NO can induce actions on sites that are remote from the synapse illustrated here. Therefore, the possibility of combined presynaptic and postsynaptic activities from either NOS location cannot be excluded. Abbreviations: Glu, glutamate; SNAP25, 25 kDa synaptosomal-associated protein.

However, these pathological effects remain, to some extent, controversial and confusing. Some laboratories using brain-slice or primary-tissue-culture models of glutamate neurotoxicity have reported that NO is also involved in these pathologies^{8,9}, while others, employing similar methods, have shown no obvious role^{10,11}. Furthermore, there is also much evidence to suggest that NO might be neuroprotective^{12–14}. Differences between the effects of NO have been attributed, at least in part, to different redox-related species of the NO group and their disparate chemical activities. Neurodestruction has been attributed to peroxynitrite alone and not to NO⁻ (the reduced form), and the neuroprotective properties of NO have been attributed to NO⁺ (the oxidized form), as this species downregulates NMDA-receptor activity by reaction with thiol group(s) in the redox modulatory site of the receptor^{15–17} (Fig. 1).

To complete this brief overview, and yet add another level of complexity, it is important to make clear that, although the best recognized effectors for NO are adenylate cyclase and cGMP, they are not the only ones and many NO-mediated effects are cGMP independent. These pathways have typically been grouped under the broad heading of redox-related NO signals and can be well-regulated post-translational modifications that are part of cellular control mechanisms²². In neurones several enzymes, G proteins, transcription factors, transporters and ion channels are targets for NO (Table 2).

Thus, in brief, NO seems to be an almost-ubiquitous messenger substance in the CNS that can, under certain conditions (such as its excessive production or the absence of regulatory control mechanisms), be toxic to cells, while possibly also being capable of acting as a neuroprotectant. However, under normal, physiological conditions NO seems to act as a neurotransmitter, albeit of a novel and unusual type. Within the more restricted field of sensory neurobiology there currently exists a lesser but no less significant interest in NO. Although NO has been demonstrated at all levels of the sensory CNS and across many modalities, there is a large amount of evidence for its presence and action in the visual system (see Fig. 2). Data exist that show NO has a role in the visual system from retina to cerebral cortex and it seems appropriate that a review of these studies is made.

The retina

In a general scheme of vertebrate retinal physiology, visual excitation in photoreceptors is mediated by the light-triggered hydrolysis of intracellular cGMP and is transmitted via bipolar cells to the output, ganglion-cell layer of the retina. The visual signal is laterally modulated by two major classes of neurones: horizontal cells located in the outer plexiform layer and amacrine cells located in the inner retina. Such modulation is carried out via chemical synapses using a number of different neurotransmitters, and also by electrical coupling⁷⁷. There is now much evidence that demonstrates that NO has a role in the physiological regulation of diverse processes within the retina, from the transduction to the gating of the output signal. These include:

Localization of nitric-oxide synthesis

Nitric-oxide synthase has been reported to have NADPH-diaphorase (NADPH-d) activity^{78,79} and both histochemical detection of NADPH-d activity and immunoreactivity to antibodies raised against NOS are used extensively as methods for identifying nNOS. These

TABLE 2. Neuronal targets of nitric oxide-related species

Targets	Effect	Refs
Guanylate cyclase	LTP, modulation of visual processing at the level of primary visual cortex	
NMDA receptor	Neuroprotection, facilitation of NMDA-mediated responses in dLGN	
SNAP25	Synaptic plasticity and transmission	98
Syntaxin Ia	Synaptic vesicle docking–fusion	99
n-secl	Synaptic vesicle docking–fusion	99
Neurogranin	LTP, neurotransmitter release	100
H ⁺ -ATPase	Vesicular glutamate uptake	101
Cyclic nucleotide-gated channel	Olfactory transduction, visual transduction, modulation of retinal ganglion-cell activity	102
Ca ²⁺ channel (rods)	Retinal photoreceptor function	
Na ⁺ /K ⁺ -ATPase	Ion homeostasis	103
?VAMP		99
ADP ribosyltransferase	LTP, modulation of visual transduction	104

All nitric oxide (NO) targets identified in the CNS to date, with the exception of VAMP, have reactive thiols, that is, they contain cysteines that are potentially subject to general acid–general base modulation. The NMDA receptor and cyclic nucleotide-gated channels are exemplary targets containing the tetra (acid–base) motif. Abbreviations: SNAP25, 25 kDa synaptosomal-associated protein; VAMP, vesicle-associated membrane protein. Adapted, with permission, from Ref. 22.

methods have revealed that horizontal, amacrine and ganglion cells of different mammals^{78,23–28} and non-mammals^{29,30} contain nNOS. Moreover, human retinal tissues have been found to express mRNA for constitutive and inducible NOS (Ref. 31). The presence of nNOS activity in photoreceptors has been a matter of controversy. Several studies using immunocytochemical and NADPH-d histochemical staining failed to localize nNOS activity^{78,80,81}. Nevertheless, other studies, including the most recent, claim that nNOS activity to be present in the inner and outer segments of photoreceptor rods^{25,26,32–35}. Furthermore, using NADPH-d histochemistry to study the cone-dominated retina of the tree shrew it has been possible to reveal several patterns of activity in the cellular subcompartments of the spectral classes of cones, which suggests that NO may be differentially involved in the functioning of different classes of photoreceptors³⁶. Interestingly, there is also evidence that NOS is found in Müller cells of both fish and amphibian species, suggesting yet another route by which NO can modulate retinal function²⁹.

NO affects the metabolism of cGMP in a variety of cells

(1) Available data show that NO is functionally coupled to a soluble guanylate cyclase and might be able to increase cGMP levels in rod photoreceptors thereby increasing the cGMP-gated conductances³⁷, which affect both response amplitude and response kinetics⁸². Nitric oxide has also been shown to modulate Ca²⁺ channels and transmission of the photoresponse to second-order cells³⁷, and to increase adenosine diphosphate (ADP) ribosylation of a variety of proteins such as transducin, G proteins and other, as yet unidentified, proteins in the outer segment of photoreceptor rods^{39–41}. These alterations of cellular proteins could be a mechanism by which NO modifies the operational mode of enzymes in the visual transduction cascade³⁸. Nitric oxide can also modulate Ca²⁺ and cyclic-nucleotide-gated channels in both rod and cone photoreceptors, which control exocytosis at cone synapses, thereby altering synaptic efficacy^{42,83}.

(2) Work carried out in fish has shown that NO donors, NOS inhibitors and NO-related substances

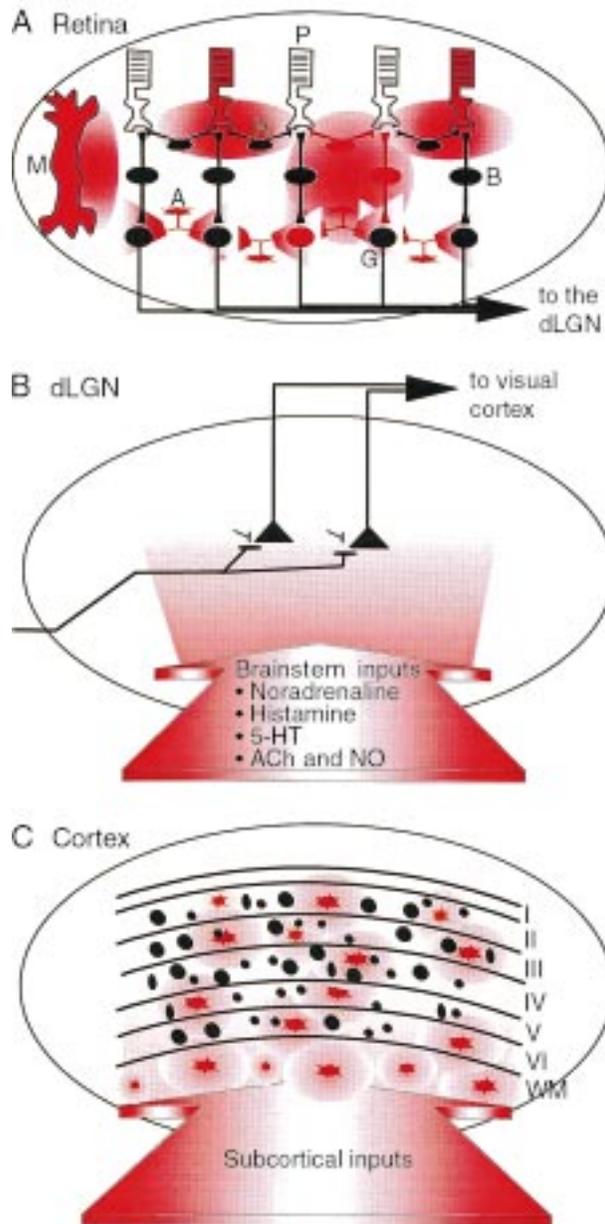


Fig. 2. Distribution of nitric-oxide synthase (NOS) containing cells and NOS positive fibres in the visual system. Summary of the three primary levels of the mammalian visual system. **(A)** Nitric oxide (NO) is produced in photoreceptors (P), horizontal cells (H), amacrine cells (A), Müller cells (M) and ganglion cells (G) (red)^{23–36}. In the retina it regulates phototransduction^{37,38}; modulates photoreceptor output^{37,39–42}, bipolar cells³⁸ and horizontal cells^{43–49}; controls ganglion-cell excitability⁵⁰ and modulates the electroretinogram^{38,51}. The widespread distribution of NOS-positive cells in these areas gives rise to possible modulation of retinal processes at all three levels by NO. The time course of effects at each level is likely to be similar because the half-life of NO *in vitro* has been estimated to be around 6 s (Ref. 52) (although *in vivo* it could be much longer⁵³). These effects on retinal processing include: (1) regulation of phototransduction (altering levels of cGMP and by ADP ribosylation); (2) modulation of output at photoreceptor synapses (by altering Ca²⁺ currents); (3) activation of ON-bipolar cells (by acting on its NO-sensitive GC); (4) decreasing the lateral spread of light responses (by decreasing electrical coupling and responsiveness to glutamate in horizontal cells); and (5) controlling ganglion-cell excitability and thereby the retinal output (by acting on cGMP-gated cation channels). **(B)** In the cat, monkey and human dorsal lateral geniculate nucleus (dLGN) there are no NOS-positive cells^{21,54–57}, but they are found in small mammals such as rats and tree shrews^{58–62}. Massive projection of NOS-positive terminals from the brainstem are shown in red²¹. In the dLGN, nitric oxide modulates NMDA-receptor activation, the gating of visual transmission^{18–20}, the control of oscillatory activity⁶³ and has an important role in development in this region^{64–71}. **(C)** Scattered NOS-producing neurones are found in all layers of the cortex (red), many in the white matter (WM)^{29,55,57,58,62,72–74}. Here NO modulates visual responses via cGMP (Ref. 75) and is involved in the regulation of presynaptic neurotransmitter release⁷⁶.

modify electrical coupling in horizontal cells in such a way that the presence of cGMP, L-arginine or the NO donor, sodium nitroprusside, decreases electrical and dye-tracer coupling between the cells^{43,44}. This modulation of the gap-junctional conductance seems to be produced by activating the cGMP-dependent protein kinase G (PKG) pathway⁴⁵. Nitric oxide might also modify horizontal-cell activity by actions on chemical synapses. For example, NO reduces the responsiveness of glutamate receptors on retinal horizontal cells taken from the hybrid bass retina⁴⁶. Interestingly, while dopamine is known to modify electrical coupling in horizontal cells^{43,47} and NO has been shown to modulate dopamine release in the retina⁴⁸, these actions seem separate, because the action on dopamine release seem to affect only K⁺-mediated release and not basal release⁴⁹. In summary, NO acts to downregulate horizontal-cell activity, which can alter apparent receptive-field size and thus influence the lateral spread of light-responses in the retina, while at the same time protecting horizontal cells from glutamate excitotoxicity.

(3) Both bipolar and ganglion cells are susceptible to the influence of NO. Guanylate cyclase (GC) of ON-

bipolar cells is of the NO-sensitive type and increased levels of cGMP have been reported after the application of NO donors³⁸. More recently, a cGMP-gated non-selective cation channel, whose conductance is increased by NO donors, has been found in ganglion cells⁵⁰. Thus, because nNOS activity is present within the ganglion-cell layer, it is plausible that a NO–cGMP system exists there that modulates retinal output.

All the studies mentioned so far have been carried out using *in vitro* preparations (mainly dissociated cells). Without doubt these have been useful and have made it possible to investigate problems that are otherwise unexamined by current *in vivo* techniques. However, the disruption of the functional anatomy of the retina can clearly alter response properties of this complex system, which makes the use of different and complementary experimental approaches necessary. Recent studies that recorded the electroretinogram (ERG) in the intact cat eye³⁸ or both ERG and compound action potentials from the optic nerve in the intact rabbit retina⁵¹ during application of NO-related compounds suggest that NO contributes physiologically to retinal processing.

The thalamus

In mammals, the dLGN plays a pivotal role in the transmission of visual information to the cerebral cortex. Unlike the retina, this is a site where both processing and gating of information takes place – thalamic transmission can be modulated by a number of inputs that arise from the brainstem⁸⁴ and are dependent upon the behavioural state of the organism. Moreover, processing and gating in the dLGN are also functions that are intimately associated with, and regulated by, neural functioning in the visual cortex, by virtue of the large descending corticofugal input.

Neuronal NOS activity has been found in the thalamus of variety of species. For example, NADPH-d-positive cells have been seen in the ventral division of the LGN in rats and tree shrews^{58,59}, and NADPH-d reactivity and GABA (Ref. 60) are co-localized in a sub-population of local inhibitory interneurons in the dLGN of the rat⁶¹. Furthermore, NOS activity has also been detected in the visual thalamus by immunocytochemistry⁶². Interestingly, in higher mammals, such as the cat, the monkey and human beings, the dLGN are completely devoid of nNOS-containing neurones, although a dense network of axons and terminals are labelled (Fig. 2B; Refs 54–57). Recent evidence has shown that the cat dLGN contains a unique distribution of nNOS, found exclusively within the cholinergic fibres that originate in the parabrachium²¹. This represents a novel co-localization of neurotransmitters and shows an exclusively presynaptic location for nNOS. It has been demonstrated *in vivo* that NO can potently enhance those visual responses that are due specifically and selectively to NMDA-receptor-mediated excitation in this region^{18–20} (Fig. 3B). Such an action is likely to be postsynaptic in origin and does not involve cGMP (Refs 19,20). This *in vivo* discovery is puzzling because it is contrary to what has been reported by several groups using *in vitro* models, where NO inhibits NMDA-receptor channels by an action on the redox modulatory site¹⁵. One possible explanation is that because, as already outlined, NO can exist in distinct oxidation–reduction states that have different biological actions^{15–17}, it can have opposite effects depending on the local redox milieu. However, there are also reports that NO affects NMDA-mediated activity independently of cGMP and the NMDA-associated redox site⁸⁵. It has even been suggested that NO might modulate NMDA currents by stimulating the release of glycine, the co-factor for NMDA activation⁸⁶ (see Fig. 1). Other experiments in cat dLGN *in vitro* showed that application of NO to thalamocortical neurones had a direct postsynaptic depolarizing effect that was associated with a decrease in input resistance. These small depolarizations appeared to act via the cGMP second-messenger system and relate to the control of oscillatory firing patterns⁶³ (Fig. 3C). This cGMP-mediated action of NO might be involved in the control of different patterns of electrogenic activity during various states of the sleep–wake cycle. However, the demonstrable effects of the block of nNOS *in vivo* do not operate via the cGMP cascade, as the soluble cGMP analogue, 8-bromo-cGMP, has been shown not to affect NMDA-mediated excitation or mimic the effect of NO donors¹⁹.

In summary, in the visual thalamus it can be hypothesized that NO can act wherever brainstem parabrachial terminals arborize, with production regulated by activity levels in these fibres in a Ca²⁺-dependent manner. While the basal release of NO can contribute to control of oscillatory activity, which is dependent on the behavioural status of an organism, it can also facilitate visual transmission from the retina through the dLGN to the cortex, particularly when such transmission invokes the activation of voltage-dependent NMDA receptors. Furthermore, NO might affect simultaneously the functional status of a relatively large neuronal population by local diffusion. Indeed, a theoretical model has predicted that the sphere of influence of a single point source of NO has a diameter

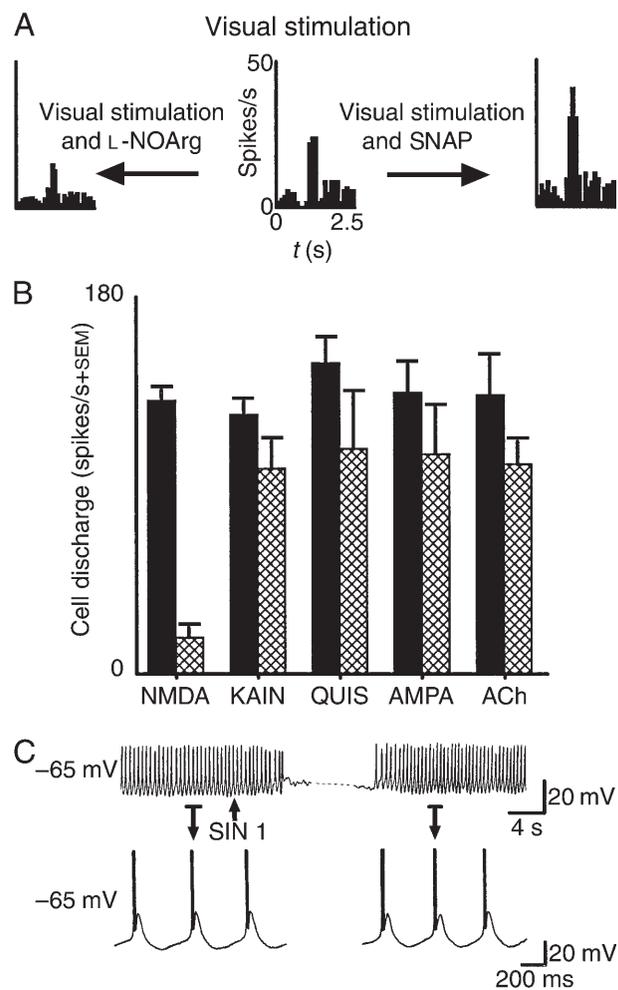


Fig. 3. Summary of the actions of the nitric-oxide system in the feline dorsal lateral geniculate nucleus (dLGN). (A) Control visual responses to small spot of light centred over the receptive field centre (centre). Application of the nitric-oxide (NO) donor, *s*-nitroso-*N*-acetyl-(*D,L*)-penicillamine (SNAP), increases responsiveness (right). Application of *L*-NOArg, a nitric-oxide synthase (NOS) inhibitor, suppresses visual responses (left). Adapted, with permission, from Ref. 20. (B) Histogram showing that application of the NOS antagonist, *L*-NOArg, depresses responses to applied NMDA in a highly selective fashion, compared to similar tests with the other drugs KAIN (kainic acid), QUIS (quisqualic acid), AMPA and ACh. Reproduced, with permission, from Ref. 19. (C) Intracellular recordings of a feline geniculate cell *in vitro*. Example of a neurone that spontaneously generates rhythmic Ca²⁺-mediated burst activity. Generation of NO through SIN 1 reversibly inhibits this activity. Indicated segments are expanded for details. Adapted, with permission, from Ref. 63.

of about 200 μ m, corresponding to a volume in the brain that encloses up to two million synapses¹.

A role for NO in the development of subcortical structures

A role for NO in development has been described in different species. In rats, the superior colliculus is a major target for retinal axons and the refinement of retino-collicular connections takes place during the first few postnatal weeks. Given that nNOS is expressed in the retinorecipient layers of the rat superior colliculus during this period, it has been suggested that NO has a role in synaptic refinement^{64,65}. Similarly, in the chick, the superficial layers of the optic tectum are the main sites for termination of retinal axons. Prior to the innervation by retinal axons, NADPH-d-stained cells are found only in deep layers. When axons innervate this area, NADPH-d-positive cells appear in the superficial layers, progressively increasing in number until the peak period

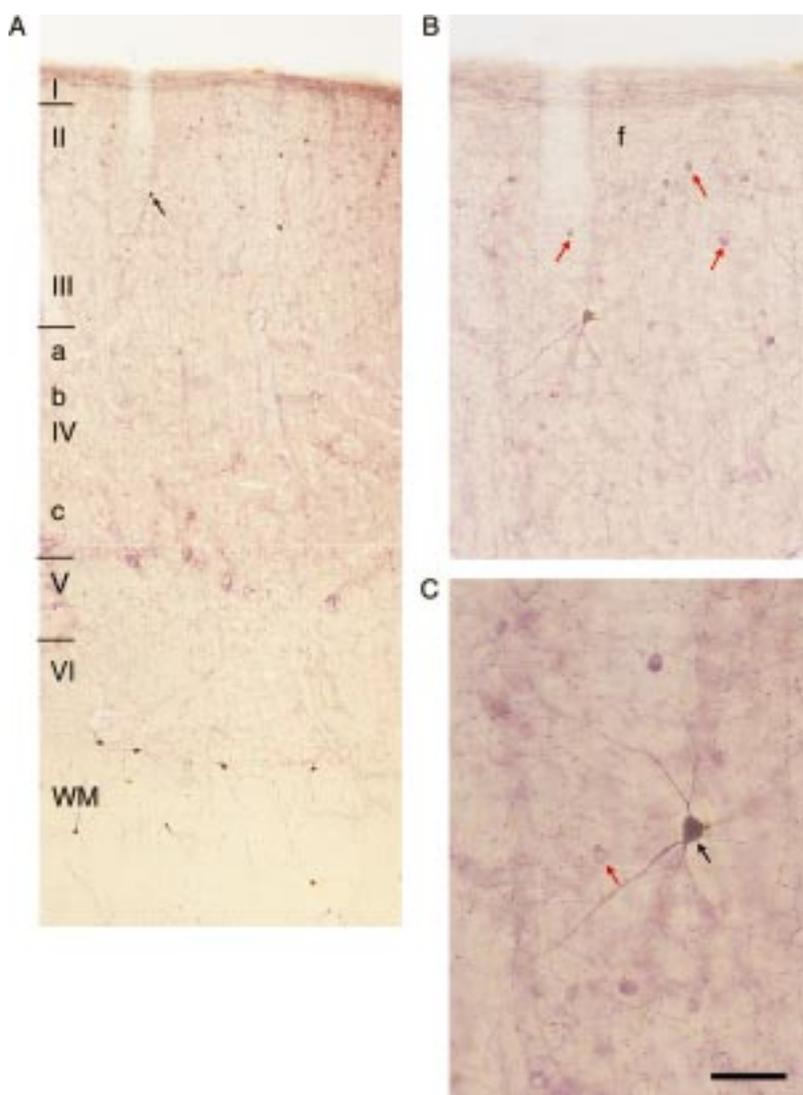


Fig. 4. NADPH-diaphorase staining in macaque primary visual cortex. Photomicrographs of NADPH diaphorase-positive neurones and fibres in the visual cortex (area V1) of a macaque monkey stained according to the protocol of Hope and Vincent⁸⁹. Moderately and darkly stained neurones are localized mainly in layers II, the upper half of layer III, the lower third of layer VI, and in the white matter. (A) Low-power photomicrograph from layer I to the white matter (WM). The black arrow indicates a strongly stained nonpyramidal cell that is also shown at higher magnification in (B) and (C). (B) Photomicrograph from layers I to III showing numerous lightly or moderately stained neurones (red arrows) and the plexus of horizontally oriented fibres (f) located in the upper half of layer I. (C) High-power photomicrograph of the darkly stained cell (black arrow) also indicated in (A) and (B). Note the dense network of fibres running in all directions. The red arrow indicates a lightly labeled neurone. Scale bar, 172 μm in (A), 75 μm in (B) and 35 μm in (C). The photomicrographs were provided by Dr Javier DeFelipe, Cajal Institute, Spain. Cortical neurones synthesizing nitric oxide are currently visualized with NADPH-diaphorase histochemistry or immunocytochemistry for neuronal nitric-oxide synthase (nNOS). These neurones mainly represent a subpopulation of GABAergic non-pyramidal cells (interneurones) that contain the peptides somatostatin and neuropeptide Y, and frequently contain the Ca^{2+} -binding protein calbindin, but not parvalbumin and calretinin. These GABAergic cells also show little or no co-localization with the peptides cholecystokinin, vasoactive intestinal polypeptide, the tachykinins or corticotropin releasing factor⁹⁰.

for remodelling of retinal connections, which coincides with the loss of several transient projections, occurs. This loss is reduced if NO synthesis is inhibited⁶⁶. Although the exact mechanism by which NO mediates this effect is not clearly understood, it has been suggested that coordinated activity in the major inputs, NMDA-receptor activation and NO production could each have a key role⁶⁶. Interestingly, in the tadpole explanted retina, application of NO donors results in the collapse of active growth cones of ganglion-cell axons; such a mechanism

could explain the termination of axonal growth at the tectal level during development⁶⁷.

Similar results have been found in the dLGN of the ferret and the cat where NOS is transiently expressed during the period in which projections from the retina are refined^{68,69}. In the ferret dLGN, retinal information is segregated into ON-OFF sublaminae, a process that requires NMDA-receptor activation, and application of a NOS inhibitor resulted in an overall pattern of sublamination that was clearly reduced when compared with normal animals⁷⁰. In the developing kitten (by contrast with the adult cat), NADPH-d staining of dLGN cells suggested that NO might act in a retrograde fashion and perhaps have a role in the maintenance of associative processes that underlie activity-dependent refinement of retinogeniculate connections⁶⁹. Further indirect results on the putative role of NO on development and plasticity have also been obtained in cats. After monocular lid suturing as kittens, adult cats showed an abnormal presence of NADPH-d-positive cells within the dLGN, which was not seen in normally reared controls, clearly indicating that NOS activity can be induced (or perhaps retained) by visual deprivation⁷¹.

Visual cortex

In the cerebral cortex, NO production could arise from several possible sources (see Fig. 2C): extrinsically from cholinergic fibres that originate in the fore-brain⁸⁷; from cortical blood vessels capable of NO production from endothelial cells of blood-vessel walls⁸⁸ and intrinsically from cells within the cortex that contain nNOS (a subset of the non-spiny cortical cells^{58,78}). Neurones containing nNOS were observed scattered throughout all cortical regions (Fig. 4) from layers II to VI and in the subcortical white matter in several species including the rat^{58,62}, cat⁷², monkey^{55,73} and human beings^{57,74}. Such a diversity of production sites suggests a complex role or roles for NO in cortical visual processing. Examples to support this include: (1) in primate, the distribution of NADPH-d staining is closely aligned with that of cytochrome oxidase and shows a similar laminar and spatial distribution⁷³. This could suggest a role for NOS that is associated with parvocellular, wavelength selective neurones. (2) Recent evidence has shown that NO might be involved in the NMDA-mediated release of noradrenaline and glutamate from rat cortical synaptosomes, thereby suggesting that NO has both direct actions in the visual cortex and actions on modulatory processes⁷⁶. Investigations centred specifically on the visual cortex of the anaesthetized cat have shown that application of compounds that manipulate the NO system alter responsiveness of a substantial proportion of neurones to visual stimuli, either reducing or augmenting visual responses. This regulation of cortical visual processing seems to be mediated via the cGMP second-messenger system⁷⁵ (Fig. 5) and suggests the existence of both upregulation and downregulation of cellular firing in separate subpopulations of cortical cells, which could be related to the level of cholinergic neurone activity in these cells and with changes in the state of arousal of the animal.

Interestingly, in contrast to studies mentioned above that show a role for NO in the development of subcortical visual structures, there is no evidence to date to suggest a similar developmental role for NO in the visual cortex^{91,92}, even though NOS distribution in visual cortex can be altered by manipulation of visual inputs⁵⁵.

Concluding remarks

One of the most intriguing features of NO, considering the simplicity of the molecule, is that it is involved in so many different regulatory functions and has many other effects. At low concentrations, it can work as a neuromodulator or a retrograde messenger in the CNS; at relatively high concentrations it can be toxic. It is tempting to speculate that NO could have multiple roles in separate regions and circuits, each role related to local physiological functions and not necessarily part of the more general role that NO has in neurotoxicity or neuroprotection. These represent alterations of normal homeostatic function of the CNS and its regulatory mechanisms. It is important to note the significance of *in vivo* studies and to understand the need for studies of the physiology of whole systems.

Nitric oxide, the gas, the common air pollutant, the suspected carcinogen and the destroyer of ozone could be the archetypal example used to illustrate the concept of 'parasympaptic' information transmission in the brain, a domain of versatility and plasticity, as formulated by Schmitt⁹³ '...new ways of conceptualization of information-transactional chemical processes as applied to basic concepts of neurobiology', in this case to the concept of vision.

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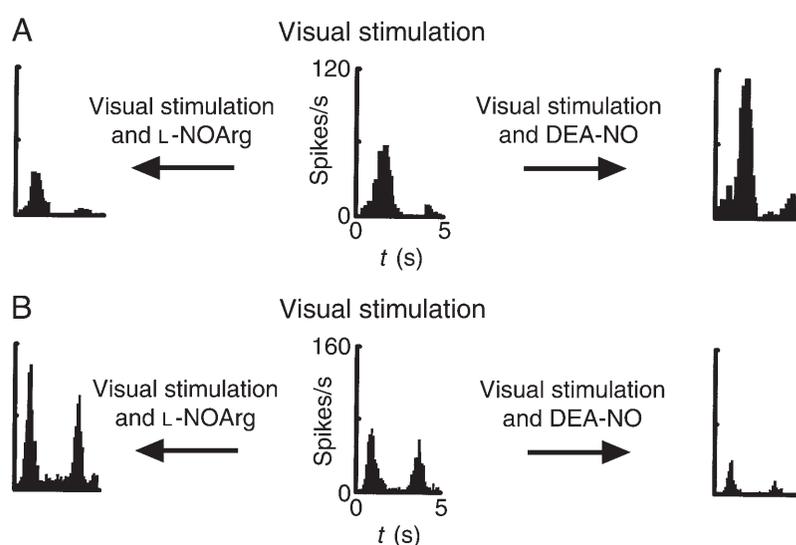


Fig. 5. Peri-stimulus time histograms illustrating the range of activities of nitric oxide (NO) and related compounds in cat visual cortex. (A) Control visual response of a directionally selective complex cell (centre). Application of DEA-NO, a NO donor, increases the response to the stimulus (right). Application of L-NOArg, a NOS inhibitor, decreases the same response (left). (B) Control visual responses of a non-directionally selective complex cell (centre). DEA-NO causes almost complete response suppression (right). L-NOArg application greatly increases responses in both directions equally (left). Drugs were applied by iontophoresis and responses averaged over a number of stimulus trials. Cells were driven monocularly through the dominant eye and the stimulus orientation selected from a quantitatively derived orientation tuning curve. Adapted, with permission, from Ref. 75.

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The TINS Lecture

Understanding the roles of *Otx1* and *Otx2* in the control of brain morphogenesis

Dario Acampora and Antonio Simeone

At the 1998 Forum for European Neuroscience, held in Berlin, the plenary lecture by Antonio Simeone was sponsored by TINS. The following article is adapted from this lecture.

The murine homologs of the *orthodenticle* (*otd*) gene of *Drosophila*, *Otx1* and *Otx2*, have an important role in brain morphogenesis. Analysis of *Otx1* and *Otx2* null mice reveals that *Otx1* is required primarily for corticogenesis and sense-organ development, while *Otx2* is necessary for specification and maintenance of anterior neural plate as well as for proper gastrulation. Cross-phylum recoveries of *Otx1* abnormalities by *Drosophila otd*, and vice versa, indicate that genetic functions required in mammalian-brain development evolved in a primitive ancestor of flies and mice. Knock-in mouse models in which *Otx2* was replaced with *Otx1*, and vice versa, provide evidence that the existence of *Otx1*^{-/-} and *Otx2*^{-/-} divergent phenotypes largely reflects differences in expression patterns rather than in the biochemical activity of OTX1 and OTX2. In evolutionary terms, some of these findings lead us to hypothesize a fascinating and crucial role for *Otx* genes that contributes to the genetic program required for the specification of the development of the vertebrate head.

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Dario Acampora and Antonio Simeone are at the International Institute of Genetics and Biophysics, CNR, 80125 Naples, Italy.

IN VERTEBRATES, a remarkable amount of data has been collected in recent years about the role of gene candidates for the control of developmental programs that underlie brain morphogenesis. Most of these genes are the vertebrate homologs of *Drosophila* genes that code for signal molecules or transcription factors^{1,2}. Among these, the *orthodenticle* group includes *orthodenticle* (*otd*) of *Drosophila* and the vertebrate *Otx1* and *Otx2* genes, which contain a bicoid-like homeodomain³.

In *Drosophila*, *otd*, the homeobox-containing gene, *empty spiracles* (*ems*) and the zinc-finger gene, *button-*

head (*btd*), show a partially overlapping expression pattern that includes adjacent head segments. Mutations in each of these three genes cause heavy defects in the anterior head segments where they are transcribed, which suggests that they might act as gap genes along the cephalic segments⁴⁻⁶.

In mouse embryos, the expression patterns of two cognates for *otd* (*Otx1* and *Otx2*) and two for *ems* (*Emx1* and *Emx2*)⁷⁻⁹ have shown a remarkable similarity with the counterpart genes in *Drosophila*. This suggests that they might be part of a general control system