Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11651–11652, December 1992

Commentary

Nitric oxide: Linking space and time in the brain

G. M. Edelman and J. A. Gally

The Neurosciences Institute, 1230 York Avenue, New York, NY 10021

It has been a key tenet of modern brain science that the central nervous system (CNS) processes signals by electrical and chemical transmission. In general, such transmission occurs locally via synapses, neuron to neuron. Images of wires, relays, and unit-to-unit signal transfer are prompted by these observations. The idea that units of brain function might, in fact, be whole groups of cooperatively interactive neurons (1) and that spatial signals involving volumes of neural tissue may be important (2) has drawn the attention of the neuroscience community only recently. One reason for this change in viewpoint is the suggestion that nitric oxide (NO), a diffusible free radical with a short lifetime, might play a key role as a spatial signal controlling cerebral blood flow, changes in synaptic efficacy, and transmitter release.

NO has recently been found to mediate a large number of diverse physiologic functions in many organs (3). In the cardiovascular, genitourinary, gastrointestinal, and renal systems, it acts in a critical manner to relax smooth muscles. In the immune system, it can act to inhibit the cell proliferation that is necessary to mount effective immune responses; at other times, it serves as a toxic agent aimed at pathogens.

A possible role of NO in the vertebrate CNS was first suggested by a report of Garthwaite *et al.* (4) that cerebellar neurons synthesize NO in response to excitatory neurotransmitters. Bredt and Snyder (5) isolated and characterized the neuronal enzyme, NO synthase [now known to be equivalent to NADPH diaphorase (6)], and they investigated the distribution of this enzyme throughout the CNS. It had been known for some time that drugs such as the nitrovasodilators could prevent strokes (but could also cause headaches), as a result of their effects on cerebral circulation.

All of these observations prompt one to wonder whether NO might play a fundamental role in the key aspect of brain function: the processing of neural signals. The possibility that NO is a neurotransmitter has been considered (5), but its chemical properties appear to make it quite unsuitable for such a role, at least as a transmitter is classically defined: a substance mediating a signal from one side of a single synaptic cleft to another. As a hydrophobic diatomic molecule, NO freely diffuses down a concentration gradient across cell membranes and thus cannot be localized within any organelle or bounded tissue compartment. In terms of its diffusion constant and its biological half-life in the brain, NO is very similar to molecular oxygen. Moreover, since NO is a free radical that is rapidly oxidized in the internal environment, it has a half-life within the CNS on the order of a few seconds, and for this reason also it cannot be stored for subsequent release.

The short life and ready diffusibility of NO uniquely qualify it to serve in a novel role in the CNS, that of a spatial signal. In the NO hypothesis, we have suggested three important functions in the CNS for which a spatial signal might be required and have advanced the view that NO is a suitable candidate (7). As a spatial signal, it would act (*i*) to couple local levels of neural activity in the brain to local blood flow, accounting, for example, for changes measured in positron-emission tomography scans; (*ii*) to guide changes of synaptic efficacy within a local volume in such a manner that synapses that are coactive within short time periods are strengthened or stabilized; and (*iii*) to direct the growth of axonal arbors during development such that terminals of axons with correlated temporal patterns of firing are segregated into common anatomical volumes. This third function, which might be critical in the formation of cortical maps, provides a possible mechanism for the experimental observation that, in the brain, axon terminals that "fire together, wire together."

The first two of these proposals have now received experimental support. Northington *et al.* (8), for example, have demonstrated that the introduction of an inhibitor of NO synthase uncouples the increased flow of blood into the somatosensory cortex that normally accompanies stimulation of peripheral nerves. Similarly, a number of laboratories have demonstrated that inhibitors of NO synthesis block long-term potentiation of synaptic strengths induced in hippocampal slices as well as the induction of long-term depression of cerebellar synapses (for review, see ref. 9). The potential significance of these NO-dependent changes in synaptic strengths on overall animal behavior has been suggested by a report that inhibitors of NO synthase partially block spatial learning in rats (10).

That NO release might direct synaptogenesis during development has not yet been subjected to experimental test, but it has been found that a population of neurons containing NO synthase does play a necessary role in cortical map formation (11). Computer simulations have shown that a short-lived spatial signal could give rise to such maps (12). The sparse patchy reciprocal connectivity of the axonal arbors generated in these simulations resembles those found by neuroanatomists as a very general feature of the brain, one that tends to link neighboring cortical neurons with similar response properties into neuronal groups that are reentrantly interconnected with one another (1).

More recently, evidence has been obtained (13–15) to support a fourth physiologic function of NO in the CNS: to facilitate the release of neurotransmitters within local tissue volumes. It has been known for some time that *N*-methyl-D-aspartate (NMDA), a chemical analog of glutamate, the major excitatory neurotransmitter in the brain, would induce the release of neurotransmitters from catecholaminergic nerve endings. This has been offered as evidence for the existence of presynaptic NMDA receptors. An alternative mechanism that might account for this release would be that postsynaptic NMDA receptors exist on neurons that contain NO synthase and that the NO produced from these cells subsequent to Ca^{2+} influx through NMDA receptor channels acts as a spatial signal to facilitate the release of neurotransmitters from surrounding synapses. This is supported by the finding (13) that nitroarginine, a NO synthase inhibitor, or hemoglobin, a NO scavenger, inhibits dopamine release from striatal slices. These experimental results suggest that NO might serve to link the two different sorts of synaptic activity present in the vertebrate cortex and striatum: (*i*) that which is topographically organized within the sensory and motor maps and dependent on thalamocortical or intrinsic cortical neuronal activity and (*ii*) that which is a consequence of activity in the diffuse ascending system arising

from catecholaminergic or serotonergic neurons in the brain stem.

In the three-dimensional network of the brain, classical neurotransmitters signal in two dimensions (if we consider time), from neuron to neuron at precise sites. NO acts four-dimensionally in space and time over volumes containing many synapses, regardless of its precise cellular site of origin. Techniques able to demonstrate or quantitate the exact sites of NO synthesis have only recently become available (16). The location of NO synthase in tissue slices has been studied by immunologic and enzymatic histochemical methods, and the enzyme has been traced to the cytoplasm (cell soma, dendrites, and axons) of a population of nonpyramidal local circuit neurons throughout the brain (6). Although these make up only 1–2% of the total neuronal population in the telencephalon, they are broadly dispersed and project a meshwork of processes throughout the grey matter. Roughly speaking, therefore, since there are approximately a billion synapses in every cubic millimeter of neuropil, 10 million of these might be expected to involve these cells that stain heavily for NO synthase. This density of sources for a spatial signal would not be inconsistent with the expectations of the NO hypothesis, and, indeed, it is possible that these cells have no physiologic function other than to serve as a source of NO. The only NO synthase-positive neurons whose physiologic function has been clearly delineated are the sensory neurons in the olfactory epithelium (6). The dendrites of these remarkably short-lived cells possess receptors for aromatic chemicals on cilia that project into the nasal cavity, whereas their axons grow into in the olfactory bulb. There, the terminal axonal arbors of these cells form a large number of distinct structures called glomeruli. Evidence suggests that neurons sharing a common odorant receptor terminate in a common glomerulus and that neurons with unlike receptors terminate in different glomeruli. Since it appears that the odorant specificity of these cells arises by a more-or-less random process and there is no spatial clustering of cells with similar specificities in the epithelium, the mechanism ensuring that developing axons find and arborize in the appropriate glomeruli has been a subject of some discussion (17). We suggest that the developmental function proposed for NO may apply in this case also. In this instance, however, the NO acting to stabilize coactive synapses together within the same tissue volume would be made presynaptically. This is in accord with observations that axons of these sensory neurons form glomeruli if allowed to grow into a variety of different cortical targets or even if postsynaptic elements are altogether absent (18).

A search for other molecules carrying out spatial signaling in the brain is likely to prove fruitful. Hydrogen peroxide and hydroxyl radicals, for example, might plausibly be formed in sites of high synaptic activity, diffuse rapidly though tissues volumes, be potent modulators of target enzymes, and have short lifetimes. Zhang and Snyder (19) have suggested carbon monoxide (CO) as a diffusible signal sharing most of these properties, but given the great stability of the molecule in the body, it appears ill suited to perform the functions we have suggested for NO. The use of heme oxygenase to form CO as a chemical signal would be remarkably expensive and inefficient in biochemical terms (biochemically equivalent to burning down the barn to roast a pig) and it would raise particularly challenging issues concerning the supply of the substrate, heme, and the removal of the by-product, bilirubin, itself a neurotoxin.

From a mechanistic point of view, the most important question to be answered concerns the molecular target of NO in cells. So far, we know that it binds to one form of guanylate cyclase, activating it to produce cyclic-GMP. It also induces ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase (19–21). More targets are likely to emerge, but so far a coherent causal account of NO action eludes us. Of course, NO, like CO, could serve to mediate toxic effects, but toxicity has not yet been shown to be a function of NO in the CNS. Indeed, any toxicity of NO may, instead, merely be an unavoidable side effect to be tolerated or compensated for in order to take advantage of its other biological properties.

Can we think of a dramatic way of revealing the importance of NO function in the nervous system? One technique that might either surprise or disappoint would be to generate transgenic mice in which the gene for neuronal NO synthase is deleted. It is fortunate for this enterprise that the enzyme located in nerve cells is encoded by a gene distinctly separate from those that give rise to NO synthase in nonneuronal tissue. Whatever the outcome, the place of NO in brain space and brain time seems assured.

1. Edelman, G. M. (1987) *Neural Darwinism* (Basic, New York).
2. Fuxe, K. & Agnati, L. F. (1991) *Volume Transmission in the Brain* (Raven, New York).
3. Moncada, S., Palmer, R. M. & Higgs, E. A. (1991) *Pharmacol. Rev.* **43**, 109–142.
4. Garthwaite, J., Charles, S. L. & Chess-Williams, R. (1988) *Nature (London)* **336**, 385–388.
5. Bredt, D. S. & Snyder, S. H. (1992) *Neuron* **8**, 1–20.
6. Vincent, S. R. & Hope, B. T. (1992) *Trends Neurosci.* **15**, 108–113.
7. Gally, J. A., Montague, P. R., Reeke, G. N. & Edelman, G. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3547–3551.
8. Northington, F. J., Matherene, G. P. & Berne, R. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6649–6652.
9. Bashir, Z. I. & Collingridge, G. L. (1992) *Curr. Op. Neurobiol.* **3**, 328–335.
10. Chapman, P. F., Atkins, C. M., Allen, M. T. & Steinmetz, J. E. (1992) *Neuroreport* **3**, 567–570.
11. Ghosh, A. & Shatz, C. J. (1992) *Science* **255**, 1441–1443.
12. Montague, P. R., Gally, J. A. & Edelman, G. M. (1991) *Cereb. Cortex* **1**, 199–220.
13. Hanbauer, I., Wink, D., Osawa, Y., Edelman, G. M. & Gally, J. A. (1992) *Neuroreport* **5**, 409–412.
14. Dickie, B. G. M., Lewis, M. J. & Davies, J. A. (1992) *Neurosci. Lett.* **138**, 145–148.
15. Prast, H. & Philippu, A. (1992) *Eur. J. Pharmacol.* **216**, 139–140.
16. Malinski, T. & Taha, Z. (1992) *Nature (London)* **358**, 676–678.
17. Buck, L. B. (1992) *Curr. Op. Neurobiol.* **3**, 282–288.
18. Graziadei, P. P. (1990) in *Development of Sensory Systems in Mammals*, ed. Coleman, J. R. (Wiley-Interscience, New York), pp. 519–566.
19. Zhang, J. & Snyder, S. H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9382–9385.
20. Kots, A. Y., Skurat, A. V., Sergienko, E. A., Bulargina, T. V. & Severin, E. S. (1992) *FEBS Lett.* **300**, 9–12.
21. Dimmeler, S., Lottspeich, F. & Brune, B. (1992) *J. Biol. Chem.* **267**, 16771–16774.