

Neurotransmitters as inducers of differentiation

Stephen X. Arthur

Differentiation is the generation of cell diversity by a continual process of cell specialization, usually by stages of differential gene expression, including changes in cell morphology and biochemistry. An inducer is an extracellular chemical signal that triggers a stage of differentiation in a cell that is competent to respond. Differentiation is distinguished from controlled growth, in which a cell is told when to divide or die. Differentiation may also be distinguished from changes in morphology that do not depend on differential gene expression, but in neurobiology this distinction is not so clear, since the process of specifying the "singularity" of an individual neuron's connections may be considered to be differentiation even though it may not involve a direct intervention by the genome. In this essay I look at neurotransmitters as both neurotrophic factors—inducers of differential growth of processes, affecting final morphology of connections—and as "true" inducers of gene expression.

Neurotrophic effects

The idea of neurotrophism includes not just effects on growth and survival but also the regulation of connections. Overproduction and selective elimination of terminals occurs as neurons compete for the neurotrophic factor. The neurotrophic factor is released differentially by target neurons, affecting terminal arborization and dendritic growth of presynaptic neurons (Purves review, 1986). Nerve growth factor (NGF) is the well known model (Davies review, 1988), where *in-vivo* anti-NGF reduces survival and exogenous NGF promotes it, with the NGF receptor-ligand complex transported retrogradely to the nucleus. Similar effects are seen on different neuron types by brain-derived neurotrophic factor and ciliary neurotrophic factor (which is not homologous).

As reviewed by Parnavelas and Cavanagh (1988), in developing cortex there is transient expression of neurotransmitters in transient subplate neurons, and in transient projections, when an area is about to be innervated. Transient expression of neurotransmitter binding sites is also found. These observations suggest that neurotransmitters may act as neurotrophic factors in the orchestration of cortical connections. *In vitro*, direct effects of neurotransmitters are seen on neuronal survival—such as with vasoactive intestinal peptide (Brenneman and Eiden, 1986)—and on mitosis, for example via muscarinic receptors on cultured astrocytes and via cloned 5-HT receptors (reviewed by Hanley, 1989). Hanley suggests that mitogenic neurotransmitters may act on post-mitotic neurons for other forms of growth control, such as process extension, so that "the notion that trophic forms of growth responses lie on one end of a continuum which has cell division at the other implies an appealing genetic unity to all forms of neuronal growth". Last July, at the same time as the Hanley review, Lipton and Kater (1989) re-

viewed the most recent evidence on the trophic effects of neurotransmitters on neuronal cytoarchitecture, as follows.

In vitro studies on snail ganglia show that serotonin, dopamine, and glutamate can act on different cell types to promote withdrawal of filopodia and cessation of elongation. One neurotransmitter can inhibit the effects of another. The direction of effect depends on membrane potential: excitatory neurotransmitters inhibit outgrowth, while inhibitory neurotransmitters or hyperpolarization alone negate the inhibitory effect of the excitatory neurotransmitter. Note that this is not a retrograde effect from a target neuron, as with NGF, but is rather an anterograde effect on the axon from presynaptic dendritic or somatic receptors. Anterograde effects on dendritic growth have also been shown: (1) mammalian retina releases endogenous acetylcholine (ACh) in culture, and nicotinic antagonists promote sprouting of ganglion cell dendrites, implying that tonic ACh is stabilizing the dendritic arbors; and (2) glutamate is released from entorhinal cortical explants and affects co-cultured hippocampal pyramidal cells by inhibiting dendritic outgrowth and promoting synaptogenesis via kainate and quisqualate receptors.

On the other hand, local, brief application of glutamate to older hippocampal pyramidal cells or cerebellar granule cells stimulates dendritic protrusions or dendritic outgrowth, respectively, via NMDA receptors. Thus a neurotransmitter can have different effects in different cell types or stages, depending on the receptor and the mode of action. Lipton and Kater state that the evidence to date suggests a continuum of actions for excitatory neurotransmitters, where at low levels they stimulate sprouting, at higher levels they halt outgrowth, and at still higher levels they prune dendritic morphology.

In-vitro experiments during development show similar effects of neurotransmitter on morphology. In amphibian third-eye transplants, optic tectum inputs segregate, and these "neighbor relations" are shown to depend on the activity of NMDA receptors. In snail ganglia, treatment with 5,7-dihydroxytryptamine to reduce serotonin content resulted in serotonin-sensitive cells showing aberrant morphology and connections.

As for a mechanism of these trophic effects, Lipton and Kater state that the evidence implicates changes in intracellular calcium ion concentration. Many different systems would produce this, via voltage-operated calcium channels, NMDA or nicotinic receptor channels, or inositol-tris-phosphate. It appears that an optimal amount of calcium is needed for sprouting, where less than 80 nM produces no growth, 100-300 nM produces growth, and over 500 nM inhibits growth and produces regression of processes. Protein kinase A and protein kinase C have also been implicated. With respect to "true" induction by gene expression, these mechanisms indicate that changes in morphology induced by neurotransmitters may be due instead to alterations in cytoskeletal components by way of direct phosphorylation events. Demonstration of "true" induction may require the presence of a biochemical phenotype switch.

Biochemical differentiation

A protein differentiation factor is known that can induce previously noradrenergic cultured neurons to synthesize acetylcholine and form cholinergic synapses (Fukada, 1985). Recently it has been shown that NGF can act as an inducer as well, regulating gene expression for substance P and calcitonin gene-related peptide (CGRP) (Lindsay and Harmar, 1989). Can neurotransmitters act this way? While the Lipton and Kater review of neurotransmitter effects on cytoarchitecture was in press (1989), two papers appeared that answer this question in the affirmative.

The first paper is by Denis-Donini (1989), showing the induction of a dopaminergic phenotype in olfactory glomerular interneurons by CGRP released by synapsing sensory epithelium neurons in co-culture. *In vivo*, tyrosine hydroxylase (TH) and dopamine uptake are first expressed by the interneurons at the time the sensory neurons synapse on them. Transection stops expression. Little TH is found when cultured separately. Olfactory nerve extracts and synthetic CGRP both induce the dopamine phenotype, and anti-CGRP antibodies blocks induction. From these experiments it is not clear whether this induction occurs *de novo* or is simply an increase (a five-fold increase was found), or whether an alternative neurotransmitter was expressed before, as in the case of the cholinergic factor (Fukada, 1985) (although GABA is co-expressed in some interneurons). In any case, this is an effect on gene expression, even if it occurs post-transcriptionally.

The second paper, by Moran and Patel (1989a), shows induction of the dopaminergic phenotype of cultured cerebellar granule cells by stimulation of their NMDA receptors, which *in vivo* receive input from glutaminergic mossy fibres. NMDA applied chronically to partially depolarized (with potassium) granule cells produces a three-fold increase in the specific activity of glutaminase. This effect is blocked by APV, MK801, and high Mg, as well as by inhibitors of protein and RNA synthesis. Again, it is not clear if this is *de-novo* gene expression or if a transmitter switch is involved, but it does require transcription.

Moran and Patel suggest a mechanism for their induction. As with trophic effects, they implicate increased internal calcium (in this case via NMDA channels). They had previously shown (1989b) that depolarization by high external potassium also induces glutaminase, via voltage-gated calcium channels. The effects of NMDA and high potassium are not additive, suggesting a common mechanism through transmembrane calcium entry. Thus the primary second messenger in both morphological induction and "true" gene induction by neurotransmitters may be the same.

Implications

Neurotransmitters appear to have an unexpectedly large range of roles to play in the development and plasticity of the nervous system, all intimately connected to their classical role in information transfer and processing. This range includes mitogen, morphogen, and inducer of biochemical phenotype. They may even be used for chemotaxis, as is shown for NGF (reviewed by Lockerbie, 1987).

Transient expression of neurotransmitters may play a major role in embryology, just as tonic release may play a major role in the plasticity and maintenance of connections. Mudge (1989) even suggests that the main role of the neuropeptides may turn out to be as inducers (particularly since they diffuse and are not broken down). There may be a large role for neurotransmitters in anterograde induction, as opposed to the retrograde effects of growth factors. This is supported by the fact that growth cones can secrete neurotransmitter prior to making synaptic contact (reviewed by Lockerbie, 1987), and that receptors can appear prior to synaptogenesis (Shaw et al., 1989).

The activity of information processing can drive the development of structure and function, including plasticity. Membrane potential is a mediator in both processes. Competence to respond to a neurotransmitter inducer would depend on the receptors available, and the kind of response induced could depend on the subtype of receptor, just as neurotransmission effects are determined.

Outside the nervous system there has been great difficulty in isolating inducer molecules. Since neurotransmitters are so well characterized, the nervous system may prove to be the model system for induction mechanisms. On the other hand, many aspects of the mechanisms employed by the nervous system for induction — the use of a simultaneously functional molecule, morphogenesis without gene-expression changes—may prove to be unique.

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