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Neurotransmitters as growth regulatory signals: role of receptors and second messengers

Jean M. Lauder

In the adult nervous system, neurotransmitters act as chemical mediators of intercellular communication by the activation of specific receptors and second messengers in postsynaptic cells. This specialized role may have evolved from more primitive functions in lower organisms where these substances were used as both intra- and intercellular signalling devices. This view derives from the finding that a number of 'classical' neurotransmitters are present in primitive organisms and early embryos in the absence of a nervous system, and pharmacological evidence that these substances regulate morphogenetic activities such as proliferation, differentiation, cell motility and metamorphosis. These phylogenetically old functions may be reiterated in the developing nervous system and in the humoral functions of neurotransmitters outside the nervous system. This review will provide evidence for this hypothesis based on the commonality of signal transduction mechanisms used in primitive organisms, early embryos and non-neuronal cells, and relate these relationships to the functions of neurotransmitters in the developing nervous system. This discussion has generally been limited to neurotransmitters where non-neuronal functions have been studied and information regarding the involvement of receptors and second messenger pathways is available.

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Neurotransmitters such as the monoamines, acetylcholine and GABA appear to be endogenous growth regulatory signals in primitive organisms (reviewed in Ref. 1). In *Tetrahymena*, intracellular concentrations of serotonin (5-HT) and dopamine vary inversely during logarithmic and stationary phases of growth[2,3]. These substances are released into the extracellular milieu, probably in response to elevated intracellular Ca^{2+}, where they can increase intracellular levels of cAMP, presumably via activation of cell surface receptors[2,4]. Evidence that GABA could play a similar role comes from the finding that treatment with diazepam, a GABA receptor ligand, elevates the growth rate of *Tetrahymena*[5]. Likewise, hydrozoan larvae produce catecholamines that can act extracellularly to regulate metamorphosis[6]. However, these functions are not limited to unicellular organisms. Serotonin, dopamine and norepinephrine are present in flatworms such as *Planaria*, where they appear to play roles in regeneration[7]. Serotonin and dopamine may play different, but complementary roles in this regrowth process, since 5-HT inhibits RNA synthesis, and promotes DNA synthesis, whereas dopamine restores RNA synthesis to normal levels following inhibition by 5-HT. Norepinephrine does not affect synthesis of RNA or DNA, but appears to promote regeneration by other mechanisms[7]. These functions may be mediated by specific receptors linked to particular G proteins, since 5-HT and dopamine stimulate Ca^{2+}-independent and Ca^{2+}-dependent adenylyl cyclases, respectively[7]. *Planaria* have been shown to express D_1 and D_2-like receptors, which, unlike their vertebrate counterparts, both mediate increases in cAMP (Ref. 8). A growth regulatory role for endogenous acetylcholine in invertebrates is suggested by the severe growth defects observed in choline acetyltransferase-deficient nematode and *Drosophila* mutants[9].

Neurotransmitters provided by symbiotic or parasitic hosts may act as intercellular regulatory signals. Motility is an important function in parasitic worms, allowing them to remain in place in spite of movements of tissues and body fluids in the host. Parasitic trematodes and cestodes, which do not express tryptophan hydroxylase, apparently depend on 5-HT released from the host to maintain motility. This action appears to be mediated by receptors positively coupled to adenylyl cyclase (reviewed in Ref. 10). Regulation of ciliary activity and cell motility may be a

general function of neurotransmitters in primitive organisms and early invertebrate embryos (see below and Ref. 1). Two particularly interesting examples of transmitter signalling between primitive organisms and symbiotic hosts are found in the metamorphosing larvae of abalone and the honeycomb worm. Planktonic abalone larvae settle on red algae and undergo metamorphosis due to a typical receptor–ligand interaction (reviewed in Ref. 11). Red algae produce a GABA-like peptide that becomes tightly bound to their surface. Abalone larvae that come into direct contact with red algae can bind to this peptide because they express GABA-like receptors. Metamorphosis is apparently triggered by increased adenylyl cyclase activity and the opening of Cl^- channels, since substances that elevate cAMP or open Cl^- channels mimic the effects of red algae. A similar scenario appears to apply to the metamorphosis of the honeycomb worm. Adult worms build tubes by cementing together grains of sand and other materials. The cement of the 'honeycomb' is a protein, stabilized by quinone cross-linking, that contains peptide-repeats rich in DOPA, the precursor for the neurotransmitter dopamine. Larvae bind to the DOPA-rich cement, possibly by a receptor–ligand interaction, and undergo metamorphosis. Cyclic AMP appears to be involved in this process since compounds that elevate cAMP can substitute for the honeycomb signal[11].

Monoamines and acetylcholine regulate morphogenesis of early embryos

The timely presence of neurotransmitters such as the monoamines and acetylcholine, together with the ability of pharmacological agents to interfere with developmental progression, has led to the suggestion that early embryos use these substances as intracellular signals to regulate cell proliferation and morphogenetic cell movements during cleavage and gastrulation (for reviews see Refs 12, 13).

Monoamines may play roles in regulating cleavage divisions in blastulae. Serotonin and norepinephrine are synthesized and released by yolk granules following fertilization of sea urchins and chicks, and are present in blastulae of these animals as well as those of rodents[13–16]. Experimental evidence suggests that these neurotransmitters regulate cleavage divisions of the blastula. Exogenously applied norepinephrine can stimulate or inhibit specific cleavage divisions in the fertilized mouse ovum *in vitro*[17]. This finding is compatible with work in sea urchins implicating 5-HT-like compounds in the regulation of cleavage[12,13,18]. Levels of 5-HT and the 5-HT-like compound 5-methoxytryptamine (5-MT) have been found to vary inversely beginning with the first cleavage division, such that 5-HT progressively decreases while 5-MT increases. This could mean that 5-MT is more involved in regulating cleavage than 5-HT, or that the changing ratio of the two amines is the most important factor in controlling this process. Relatively non-selective 5-HT receptor antagonists, such as gramine and metergoline, block cleavage, but not other aspects of proliferation (e.g. DNA synthesis). These effects are accompanied by decreased intracellular levels of cAMP and increased Ca^{2+} efflux, which can be reversed by application of either 5-HT or 5-MT (Ref. 18). It has been proposed that intracellular receptors mediate these effects and may be associated with cytoskeletal elements located in the cleavage furrow[13,18]. This is supported by the finding that 5-HT binds to microfilaments and microtubules in the neural tube of the chick embryo[14].

Gastrulation in sea urchins may be coordinately regulated by 5-HT and acetylcholine. In the sea urchin, gastrulation is preceded by hatching of the blastula, which depends on cell motility. Ciliary activity in sea urchin blastulae is stimulated by 5-HT, dopamine and acetylcholine. The effects of these monoamines are accompanied by changes in intracellular Ca^{2+} and adenylyl cyclase activity[19]. After hatching of the blastula, primary mesenchyme cells (that will differentiate into the primitive skeleton) migrate into the blastocoel. This is followed by gastrulation, which involves invagination of the ectoderm through the blastopore (into the blastocoel) to form the archenteron or primitive gut. Serotonin may regulate the migration of primary mesenchyme, since treatment of gastrulating embryos with 5-HT antagonists causes skeletal defects. Acetylcholine antagonists, on the other hand, interfere with formation of the gut. It has been suggested that 5-HT, located in the region of the blastopore, initiates gastrulation, whereas acetylcholine released from ectoderm controls invagination of the archenteron[20].

Monoamines may also regulate morphogenetic cell movements in vertebrate embryos. Norepinephrine and 5-HT are synthesized by the notochord in the chick and frog[1,21–23]. During neurulation, these transmitters are transiently taken up into the floorplate of the neural tube and the neural folds where they are accumulated in non-overlapping regions, which cover most of the neuraxis[1,21]. Inhibitors of monoamine uptake or receptor antagonists produce a number of malformations in the chick, including neural tube defects (see Ref. 1). Together with the observation that 5-HT binds to cytoskeletal elements in chick neuroepithelial cells[14], these findings suggest that monoamines may regulate changes in cell shape and morphogenetic cell movements important for neural tube closure in amphibians and avians. This does not appear to be the case in the rodent embryo, where these neurotransmitters may subserve other morphogenetic functions (discussed below).

Recent evidence suggests that blood-borne 5-HT regulates craniofacial and cardiac morphogenesis in the mouse. Sites of 5-HT uptake are transiently expressed in craniofacial epithelia[24] and myocardium[25] during critical periods of morphogenesis. Degradation of 5-HT at uptake sites appears to regulate levels of 5-HT in underlying mesenchyme. Exposure of cultured mouse embryos to inhibitors of 5-HT uptake produces craniofacial malformations, reduces cell proliferation and increases cell death in both craniofacial and cardiac mesenchyme[25,26]. Craniofacial defects are similar to those reported after injection of serotonergic agents into pregnant rats[27] or mice[28]. Some of the dysmorphology that is caused by inhibition of 5-HT uptake may result from the inhibitory effects of high levels of 5-HT on cell migration, since this neurotransmitter has dose-dependent effects on migration of cardiac mesenchyme[25], craniofacial mesenchyme[29,30] and cranial neural crest (Moiseiwitsch, J. R. D. and Lauder, J., unpublished observations). Serotonin could regulate cell migration

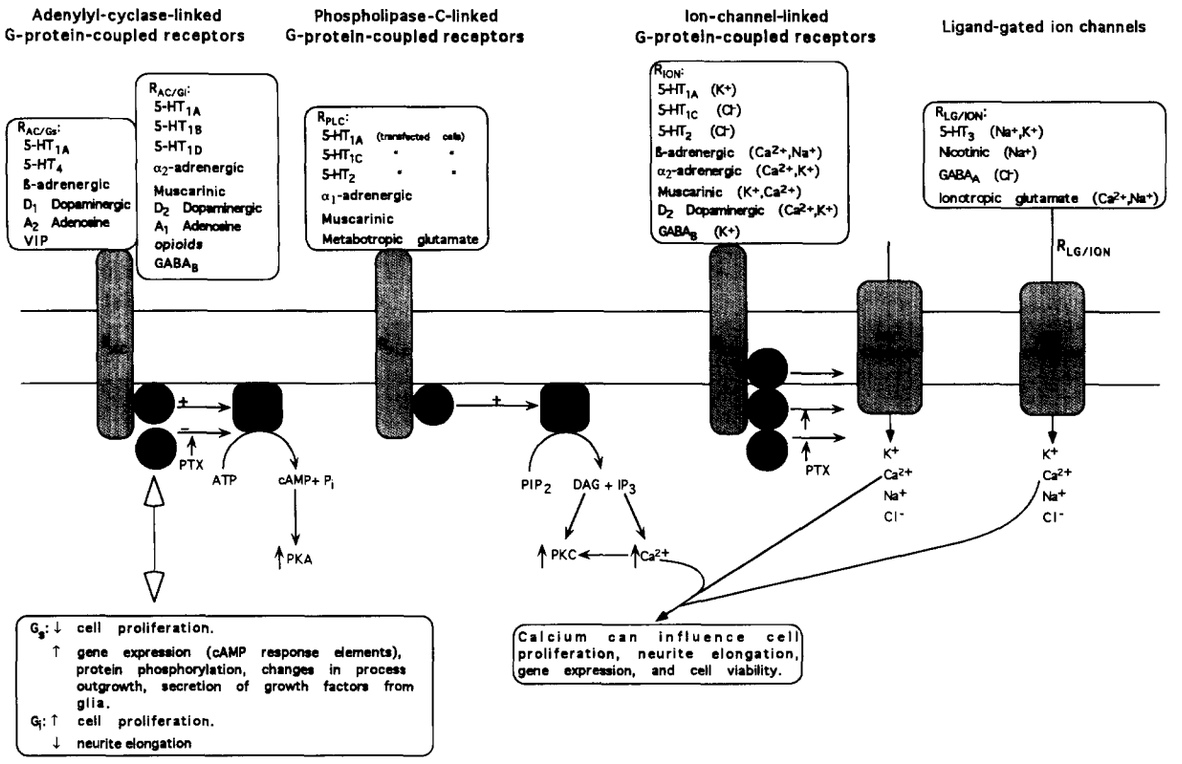

Fig. 1. *Neurotransmitter receptors linked to second messengers mediating growth responses in neuronal and non-neuronal cells. Abbreviations:* $R_{AC/Gs}$, *receptors coupled to G proteins that stimulate adenylyl cyclase (AC) activity, leading to cAMP formation and enhanced activity of protein kinase A (PKA);* $R_{AC/Gi}$, *receptors coupled to pertussis toxin (PTX)-sensitive G proteins that inhibit adenylyl cyclase activity;* R_{PLC}, *receptors promoting the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) – IP_3 increases intracellular Ca^{2+}, while DAG activates protein kinase C (PKC);* R_{ION}, *receptors indirectly promoting ion fluxes due to coupling to various G proteins;* $R_{LG/ION}$, *receptors that promote ion fluxes directly because they are structurally linked to ion channels (members of the superfamily of ligand-gated ion channel receptors).* (Figure based on Refs 31–46.)

by several possible mechanisms, including activation of receptors[31], uptake and binding to cytoskeletal elements[14], or binding to components of the extracellular matrix[32].

Neurotransmitters regulate cell proliferation and second messengers in non-neuronal cells

Neurotransmitters stimulate or inhibit proliferation of vertebrate non-neuronal cells by activating receptors coupled to different second messenger pathways (see Fig. 1). Stimulation of proliferation is most often associated with activation of G proteins negatively coupled to adenylyl cyclase (G_i), or positively coupled to phospholipase C [(PLC); which mediates phosphoinositol (PI) hydrolysis] (G_q) or to pertussis-toxin-sensitive pathways (G_o, G_i). In contrast, activation of neurotransmitter receptors positively coupled to cAMP usually inhibits cell proliferation and causes changes in cell shape indicative of differentiation. However, it should be kept in mind that multiple second messenger pathways are probably involved in the receptor-mediated regulation of cell proliferation, making actual mechanisms far more complex than these correlations would suggest[33,34]. Similar signal transduction mechanisms may underlie roles played by neurotransmitters in the developing or injured nervous system, as discussed below.

Neurotransmitters that promote cell proliferation may do so by inhibiting adenylyl cyclase or by activating PLC. Neurotransmitters that promote cell proliferation include 5-HT, adenosine (A_1 receptors), norepinephrine (α-adrenergic receptors) and acetylcholine (muscarinic receptors). Serotonin may function as a mitogen by several different second messenger pathways. In vascular smooth muscle cells, mitogenicity can involve cellular uptake of 5-HT (Ref. 31), interaction with 5-HT_4 receptors positively coupled to cAMP (Ref. 35), or with 5-HT_{1D} receptors coupled to a pertussis-toxin-sensitive pathway independent of cAMP (Ref. 36). Serotonin synergizes with mitogenic growth factors in fibroblasts via activation of 5-HT_{1B} receptors coupled to an inhibitory G protein for adenylyl cyclase (G_i; Fig. 1)[37,38]. However, when fibroblasts are transfected with genes coding for 5-HT_{1A}, 5-HT_{1C} or 5-HT_2 receptors, 5-HT appears to exert mitogenic effects either by inhibition of adenylyl cyclase or by other pathways involving PLC or pertussis-toxin-sensitive G proteins[38–42].

Activation of α_2-adrenergic receptors inhibits the stimulation of adenylyl cyclase by other agents[43], and promotes cell proliferation in fibroblasts and astrocytes[43,44]. In fibroblasts transfected with the α_2-adrenergic receptor gene, α_2-agonists decrease cAMP and promote proliferation in synergy with fibroblast growth factor (FGF)[43]. Serotonin has similar effects, which are not additive with those of α_2-agonists, suggesting that these ligands activate a common inhibitory G protein (G_i)[43]. Stimulation of muscarinic cholinergic receptors by carbachol

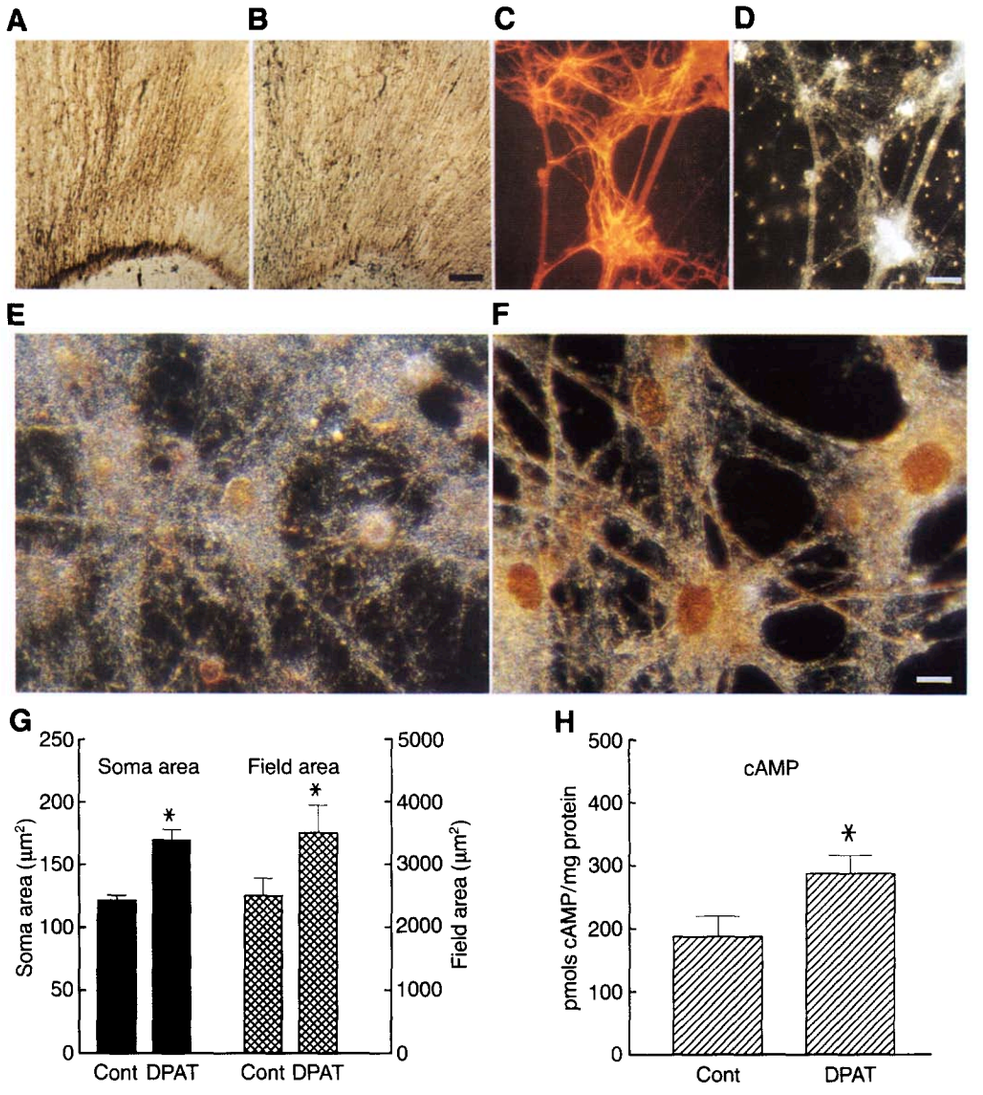

Fig. 2. *Serotonergic neuronal–glial interactions in embryonic rat brain. Expression of S100β and 5-HT$_{1A}$ receptors in radial-like glia and astrocytes from embryonic rat rostral brainstem.* **(A),(B)** *Adjacent sagittal sections through embryonic day 16 (E16) rostral brainstem showing radial-like glia stained with* (A) *anti-S100β (Sigma) and* (B) *anti-5-HT$_{1A}$ (gift from John Raymond; data from Lauder, J. M., Wilkie, M. B., Liu, J. P. and Raymond, J., pers. commun.).* **(C),(D)** *Radial glia/astrocytes cultured from E14 rostral brainstem, double-labelled with* (C) *antibodies against glial fibrillary acidic protein (GFAP) and* (D) *^{35}S-labelled oligonucleotide recognizing 5-HT$_{1A}$ mRNA (Ref. 56).* **(E),(F)** *Regulation of intracellular S100β in radial glia/astrocytes exposed for 24 hours to 10 nM 8-OH-DPAT.* (E), *Control;* (F), *treated with 8-OH-DPAT (Ref. 95).* **(G)** *Conditioned media from radial glia/astrocytes treated for 24 hours with 8-OH-DPAT promotes growth of E14 raphé 5-HT neurons[95]. Soma area represents area of cell bodies; field area represents area of dendritic arbors.* **(H)** *Exposure of radial glia/astrocytes for 10 minutes to 10 nM 8-OH-DPAT in the presence of isobutyl-methyl-xanthine stimulates cAMP formation[95]. * Represents significance at $p < 0.05$, Student's T-test. Scale bars in* (A),(B) *are 57 μm; in* (C),(D) *are 27 μm; in* (E),(F) *are 10 μm.*

promotes DNA synthesis in cultured astrocytes, and in fibroblasts transfected with genes coding for m1, m3 and m5 subunits of the human muscarinic receptor[45]. In both cases mitogenic responses are correlated with increased activity of PLC.

Neurotransmitters that elevate cAMP inhibit cell proliferation and promote differentiation. Muscarinic receptor subtypes M_1, M_3 and M_5 can also stimulate adenylyl cyclase and inhibit cell proliferation in a number of cells. These effects appear to occur by an indirect mechanism, independent of PI hydrolysis[46], and may mediate the inhibitory effects of carbachol on proliferation of fibroblasts transfected with these genes (see Ref. 45). Agonists of β-adrenergic and 5-HT$_{1A}$ receptors promote adenylyl cyclase activity by activation of stimulatory G proteins (G_s), inhibit proliferation, and promote a more differentiated morphology in astrocytes and other non-neuronal cells[31,47,48]. Agonists of β-adrenergic receptors also promote phosphorylation of glial fibrillary acidic protein (GFAP), which may underlie changes in cell shape in astrocytes (reviewed in Ref. 47). Other neurotransmitters having growth-related effects on non-neuronal cells include adenosine (A_2 receptors), dopamine (D_1 receptors), vasoactive intestinal peptide (VIP), somatostatin, histamine, substance P and opiates (see Refs 49–51).

Neurotransmitters may regulate neurogenesis by similar signal transduction mechanisms

Most studies *in vivo* indicating that neurotransmitters regulate neurogenesis are based on correlative ontogenetic relationships, or altered development following lesions or pharmacological treatments. Studies *in vitro* provide more direct evidence for such functions, and suggest that effects of neurotransmitters on neural development are mediated by specific receptors linked to particular second-messenger pathways. The following examples were chosen because they provide evidence both *in vivo* and *in vitro* for neurotransmitters as developmental signals. (For a recent comprehensive review of neurotransmitters as neurotrophic factors see Ref. 51.)

Serotonin may regulate development of raphé neurons and their embryonic target cells. Appropriate levels of 5-HT appear to be important for normal development of both serotonergic raphé neurons and their embryonic target cells. Studies *in vitro* suggest that 5-HT regulates growth of raphé neurons in a dose-dependent manner (see Refs 48, 52 for reviews). Serotonin also appears to initiate and auto-amplify its own synthesis in cultured hypothalamic neurons[53]. Evidence for an autoregulatory function of 5-HT is further supported by the observation that *Drosophila* mutants incapable of 5-HT synthesis contain serotonergic axons with aberrant growth patterns[54]. Similarly, abnormal growth of 5-HT axons occurs in rats treated prenatally with the 5-HT receptor agonist 5-MT (Ref. 48). Taken together, these *in vivo* and *in vitro* studies indicate that altered levels of 5-HT may be deleterious to developing serotonergic neurons.

This also appears to be true for targets of developing 5-HT axons. Depletion of 5-HT in embryonic raphé neurons by maternal administration of *p*-chlorophenylalanine (*p*CPA; an inhibitor of 5-HT synthesis) leads to delayed neurogenesis in regions receiving an early innervation by 5-HT axons (see Ref. 52 for a review). This effect could be receptor mediated, since several 5-HT receptor subtypes are expressed in these target regions[55,56]. Although it is not known whether these embryonic receptors are functional, they appear to be regulated by 5-HT because maternal administration of *p*CPA or the agonist 5-MT alters the numbers of 5-HT binding sites expressed postnatally[57,58].

Dopamine and acetylcholine regulate neurogenesis of their synaptic targets. Several aspects of postnatal striatal development are altered by neonatal depletion of dopamine by reserpine or destruction of dopaminergic afferents using 6-hydroxydopamine. These effects, which include decreased expression of peptidergic genes[59], altered neuronotrophic activity of striatal cells[60], hyperinnervation by serotonergic afferents[61] and retarded growth of striatal neurons[62], suggest that dopaminergic neurons exert multiple influences on striatal development. Cholinergic neurons and their receptors appear early in the embryonic rat brain where they develop in approximate synchrony[63,64], suggesting that acetylcholine could also play a role in neurogenesis. Moreover, in the chick optic tectum, a subunit of the nicotinic receptor is transiently expressed during innervation by optic nerve fibers, raising the possibility that acetylcholine could be involved in development of the retinotectal projection[65]. Convincing evidence for a developmental role of acetylcholine *in vivo* is the finding that neonatal lesions of rat forebrain cholinergic neurons produce abnormal cortical lamination and positioning of pyramidal cells[66]. Studies *in vitro*, discussed below, further support this view.

Monoamines and acetylcholine regulate growth of cultured target neurons mediated by cAMP and Ca^{2+}. Serotonin, dopamine and acetylcholine influence growth of their normal synaptic target cells *in vitro* (reviewed in Refs 52, 67). This growth regulation may be inhibitory or stimulatory depending on the method of administration, developmental state of neurons, second messenger systems involved or the presence of glial cells. Serotonin and dopamine inhibit neurite elongation and elevate intracellular Ca^{2+} when applied directly to *Helisoma* neurons, which are their natural targets[67]. Consistent with this finding, depletion of 5-HT in *Helisoma* embryos by 5,7-dihydroxytryptamine causes increased dendritic growth in 5-HT target cells, suggesting that serotonergic afferents normally inhibit growth. However, 5-HT can promote neurite outgrowth in a subpopulation of embryonic *Helisoma* neurons (see Ref. 68). It has been suggested that inhibitory and stimulatory effects of 5-HT are due to different growth states and Ca^{2+} homeostasis of receptive cells[67,68]. Acetylcholine also inhibits neurite elongation when applied directly to appropriate *Helisoma* neurons, but can prevent the inhibitory effects of 5-HT on serotonergic target cells by adjusting intracellular Ca^{2+} and the membrane potential[67]. Acetylcholine is spontaneously released into the medium of cultured retinal neurons (presumably from amacrine cells), and appears to be an inhibitory growth signal for retinal ganglion cells, since nicotinic antagonists enhance neurite outgrowth by these neurons[69]. Inhibitory growth regulation by neurotransmitters, which appears to result from elevated intracellular Ca^{2+}, may constitute a means of stabilizing dendritic growth during synaptogenesis *in vivo*[67,69].

Although changes in intracellular Ca^{2+} appear to mediate many effects of neurotransmitters on neurite elongation, other evidence suggests that regulation of adenylyl cyclase activity may also be important (see Refs 49, 67). In addition, the presence of glia may be an important factor. For example, neurite outgrowth is inhibited by micromolar amounts of 5-HT added to dissociated cultures of cortical[70] or raphé neurons. However, in organotypic cortical or hippocampal cultures, neuronal differentiation and synaptogenesis are stimulated by the same concentration of 5-HT and this is accompanied by enhanced glial proliferation (see Ref. 52 for a review). The presence of glial cells may be largely responsible for the difference in neuronal development since glia can release neurotrophic factors in response to neurotransmitters. Like 5-HT, dopamine inhibits neurite outgrowth by cultured retinal and cortical neurons[71–73]. Inhibitory effects of 5-HT and dopamine have been linked to activation of 5-HT_{1A} (Ref. 70) or D_1 receptors[71–73]. No information is available regarding second messengers involved in 5-HT_{1A} activation in cortical cultures. However, this could result in stimulation of adenylyl cyclase, since cAMP is elevated in cultures of 5-HT neurons exposed to 5-HT_{1A} agonists (Liu, J. P. and Lauder, J., unpublished observations). Evidence suggests that dopamine may exert growth-suppressive effects via activation of D_1 receptors positively coupled to adenylyl cyclase[71–73], although stimulation of the PLC pathway has also been implicated[72]. Alternatively, dopamine may be converted to toxic free radicals that suppress growth or kill cultured neurons[74]. In contrast, activation of D_2 receptors negatively coupled to adenylyl cyclase appears to promote growth of cortical neurons[73].

GABA is a neurotransmitter appearing early in development that acts as a trophic factor. GABAergic neurons appear early in the development of embryonic brain and spinal cord[75,76]. GABAergic fibers, apparently ascending from the spinal cord, project through regions of brainstem, midbrain and forebrain where serotonergic, dopaminergic and peptidergic neurons are being generated, which suggests that GABA could influence their differentiation[75]. This possibility is enhanced by the finding that $GABA_A$/benzodiazepine receptors are expressed in approximate spatio-temporal coincidence with these early GABAergic pathways[77,78]. In addition, GABAergic neurons appear in the marginal zone and subplate during early phases of cortical development, where they are ideally positioned to provide trophic support for incoming cortical afferents[79]. These GABAergic neurons may also protect cortical neurons from excitotoxic effects of glutamate, as discussed below. In developing retina, kainate lesions of GABAergic horizontal cells alter development of the outer plexiform layer[80]. Development of the sexually dimorphic nucleus may also be influenced by GABA, since its size is decreased in males following perinatal treatment with the $GABA_A$ agonist muscimol[81]. Appearance of GABAergic afferents in cerebellum precedes the expression of $GABA_A$ receptors by granule cells,

which suggests that GABA might be able to induce the expression of its own receptors[82]. This possibility is supported by studies *in vitro* demonstrating trophic effects of GABA on cerebellar granule cells that include promotion of neurite outgrowth, synaptogenesis and the formation of low-affinity GABA receptors[50].

Excitatory amino acids can act as trophic factors or excitotoxins. Glutamate is the most abundant excitatory amino acid (EAA) in the CNS and can act as a trophic neurotransmitter or neurotoxin, depending on its concentration[67]. Glutamate interacts with both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors. NMDA receptors are linked to Ca^{2+} channels and are preferentially activated by NMDA, whereas non-NMDA receptors are stimulated by quisqualate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) or kainate. Quisqualate/AMPA receptors are divided into ionotropic receptors (selectively activated by AMPA, linked to Na^+ channels and mediating fast synaptic responses) and metabotropic receptors (selectively activated by quisqualate and linked to second messenger pathways such as PLC). Both NMDA and non-NMDA receptors may play roles in CNS development[83]. Different types of EAA receptors have unique distributions and developmental expression patterns in the CNS. Periods of maximal expression occur during critical periods of synaptogenesis and plasticity, when these receptors may mediate growth regulatory effects of activity (see Refs 51, 83, 84 for reviews). However, since EAAs can also act as excitotoxins, maximal expression of EAA receptors during certain developmental periods may make particular brain regions especially vulnerable to excitotoxicity[83]. Toxicity of EAA may be ameliorated by trophic neurotransmitters (e.g. GABA) or glial cells, as discussed below.

Trophic neurotransmitters and glia may prevent toxicity of EAA during CNS development. GABAergic inhibition is already operational in kitten visual cortex at the time of eye opening, and appears to play a role in the development of ocular dominance columns by positively modulating the effects of neuronal activity and EAAs (reviewed in Ref. 84). In effect, this may mean that GABAergic afferents provide trophic support for cells that would otherwise be vulnerable to excitotoxicity. This possibility is strengthened by the finding that GABA prevents the growth-suppressive effects of glutamate on cultured hippocampal neurons by reducing Ca^{2+} influx (see Ref. 49). Glia also appear to protect neurons from growth suppressive/excitotoxic effects of EAA. This may occur by uptake and removal of EAA (Ref. 85) or by the release of neurotrophic factors such as basic FGF, nerve growth factor (NGF) or insulin-like growth factors (IGFs), which prevent excitotoxic effects of glutamate on cultured hippocampal neurons[49,86]. Another possibility is that glia may release trophic neurotransmitters such as GABA that have previously been taken up from adjacent neurons. Release may be regulated by stimulation of glial EAA receptors. This possibility is supported by the finding that type 2 astrocytes express non-NMDA receptors, and release GABA in response to agonists[87,88]. Neuroactive peptides such as VIP also have a number of trophic effects on developing neurons (see Ref. 89 for a review). One of their important functions may be to modulate the effects of EAA, either by enhancing the release of glial-derived growth factors[89] or by elevating intracellular cAMP in neurons[90].

Glial-derived growth factors may mediate some trophic functions of neurotransmitters

Astrocytes express a wide variety of neurotransmitter receptors (reviewed in Ref. 47) and provide region-specific growth signals for afferent neurons[91,92]. Synthesis and release of growth factors may be regulated by neuronal activity and neurotransmitters. Such neuronal–glial interactions could mediate certain trophic functions of neurotransmitters during development and in response to injury.

Glial receptors may mediate release of growth factors. One example of trophic signalling between neurons and glia is the serotonergic regulation of the calcium-binding protein S100β, which functions as a serotonergic growth factor (see Ref. 57 for a review). During development of the serotonergic system, interactions of raphé neurons with glia expressing 5-HT_{1A} receptors may elicit the release of S100β, which appears to be co-expressed with these receptors in radial glia located adjacent to raphé neurons and along the trajectory of their axons (see Refs 93, 94 and Figs 2A–D). This is a reasonable scenario since 5-HT_{1A} agonists promote the release of S100β from astrocytes, and S100β promotes the growth of 5-HT neurons[57,96]. Moreover, the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) promotes the growth of 5-HT neurons co-cultured with embryonic brainstem glia[95]. This effect could involve the release of S100β, since this protein is increased in such glial cells following exposure to 8-OH-DPAT (Ref. 95; see Figs 2E,F). It has been suggested that increased cAMP following activation of glial 5-HT_{1A} receptors could promote transcription of the S100β gene, which contains a cAMP response element[57]. This is consistent with the finding that cAMP is increased in embryonic brainstem glia exposed to 8-OH-DPAT (Ref. 95; Figs 2G,H).

A similar scenario may apply to trophic interactions between embryonic dopamine neurons and mesencephalic glial cells. It is known that mesencephalic astrocytes promote the growth of dopamine neurons (see Ref. 92 for a review). One glial-derived growth factor that might mediate these effects is insulin-like growth factor II (IGF-II), which is expressed by embryonic mesencephalic glia, and promotes growth of embryonic dopamine neurons *in vitro*[96]. It is not known whether this growth factor is released by mesencephalic glia in response to neurotransmitters. However, co-culture of dopaminergic neurons with such glial cells enhances the ability of 5-HT to promote their growth[92,95]. This raises the possibility that 5-HT could promote the release of a dopaminergic growth factor from embryonic mesencephalic glia.

Accumulated evidence indicates that neurons of different neurotransmitter phenotypes respond in characteristic ways to growth factors (reviewed in Ref. 92). If neurotransmitters released from developing axons stimulate glia to release neurotrophic factors *in vivo*, the timely expression of appropriate glial receptors, together with the selective responses of neurons, could impart a high degree of specificity to neurotransmitter regulation of neural circuitry construction or repair.

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New views of the thalamic reticular nucleus in the adult and the developing brain

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The thalamic reticular nucleus plays a crucial role in modifying the patterns of activity that can reach the cerebral cortex from the thalamus. Although the nucleus is generally viewed as a cell group with widespread and nonspecific thalamic and cortical connections, recent evidence has begun to stress the extent to which at least some of the reticular pathways transmit well-defined maps with a clear local sign from the cortex and the thalamus. Further, evidence from the adult structure of the nucleus and ongoing developmental studies suggest that the reticular nucleus plays an important part in organizing the earliest connections between cortex and thalamus and that the developmental sequence may explain the complex connections formed in the adult.

Most of the inputs that reach the cortex from the periphery (e.g. retina, skin) or from other parts of the brain do so by passing through one or more of the nuclei of the thalamus*. These thalamic relays themselves receive their heaviest innervation not from the periphery, but instead from axons that return from the cortex to the thalamus (Fig. 1)[1]. A thalamic nucleus receives afferents from the cortical areas to which it sends its axons, and also from other, functionally related cortical areas. These two-way connections provide a major, but often ignored pathway between cortical areas having different functions (e.g. see Ref. 2).

All of the axons that pass either way between the cortex and the thalamus must go through the thalamic reticular nucleus, and many of them, possibly all, give off collateral, excitatory branches that innervate the reticular nucleus (Fig. 1)[3–6]. The reticular cells in turn provide an inhibitory (GABAergic)[3,6–8] innervation back to the thalamic nucleus that provides their input (Fig. 1). In addition to these thalamic and cortical afferents, reticular cells receive afferents from various brainstem centres[9,10] and from the basal forebrain[9,11], and these are thought to relate reticular activity to levels of arousal[12–14]. Knowledge of the connections of the reticular nucleus should provide insights into how the afferents to the cortex are organized and help in understanding the functions of the thalamus and reticular nucleus, which at present are still rather mysterious.

The first part of this review considers two views of reticular connections. One argues that the nucleus lacks accurately mapped connections with the cortex and thalamus, and relates reticular activity in a global manner to the control of thalamic activity during different stages of arousal. The other recognizes that remarkably well-defined maps are established in the reticular nucleus and raises some new and currently unanswered questions about how cortical activity might be controlled or modified through its reticular connections. These two views are not mutually exclusive.

The second part of the article shows that an appreciation of how fibre systems relate to the reticular nucleus can lead one to question the developmental events that produce the adult structures. We will suggest that the reticular nucleus plays a key role in guiding fibres to and from the cortex and in converting cortical maps to thalamic maps; further, that this very process of fibre guidance and sorting may provide a framework upon which the complex adult patterns of connectivity can be built.

The reticular nucleus in relation to the thalamocortical pathways

The pathways connecting cortex and thalamus to the reticular nucleus establish distinct reticular sectors that relate to functionally distinct parts of the thalamocortical pathway. For example, the schematic diagram in Fig. 1 shows, in coloured shading, how three different dorsal thalamic nuclei relate to different sectors of the reticular nucleus[1,15].

The reticular cells are relatively large, with long dendrites running parallel to the reticular sheet, apparently spanning the sectors of the nucleus, and potentially extending across sectors[5,12,16–18] (Fig. 2). Reticular cells have large receptive fields and are mostly modality specific. Cells in the auditory sector, for instance, respond to auditory stimuli, whilst those in the somatosensory sector, respond to somatosensory stimuli. Some cells, however, have mixed receptive fields, for example, those that lie on the border of adjacent sectors[8].

Classically, the reticular nucleus was thought to form the rostral end of the brainstem reticular formation and make up part of a diffuse projection system to the cortex. This projection system, together with the midline and intralaminar nuclei of the thalamus, furnished a 'train of rhythmic waves, known as bursts, in widespread areas of the cortex'[19]. This 'global' function for the reticular nucleus was further supported by Jones and colleagues[15,17], who described the connections of the reticular nucleus with

* 'Thalamus' will be used throughout for dorsal thalamus.