Cholinesterases in Neural Development: New Findings and Toxicologic Implications

Stephen Brimijoin and Carol Koenigsberger

Department of Pharmacology, Mayo Clinic, Rochester, Minnesota

Developing animals are more sensitive than adults to acute cholinergic toxicity from anticholinesterases, including organophosphorus pesticides, when administered in a laboratory setting. It is also possible that these agents adversely affect the process of neural development itself, leading to permanent deficits in the architecture of the central and peripheral nervous systems. Recent observations indicate that organophosphorus exposure can affect DNA synthesis and cell survival in neonatal rat brain. New evidence that acetylcholinesterase may have a direct role in neuronal differentiation provides additional grounds for interest in the developmental toxicity of anticholinesterases. For example, correlative anatomic studies show that transient bursts of acetylcholinesterase expression often coincide with periods of axonal outgrowth in maturing avian, rodent, and primate brain. Some selective cholinesterase inhibitors effectively suppress neurite outgrowth in model systems like differentiating neuroblastoma cells and explanted sensory ganglia. When enzyme expression is altered by genetic engineering, acetylcholinesterase levels on the outer surface of transfected neurons correlate with ability to extend neurites. Certain of these "morphogenic" effects may depend on protein–protein interactions rather than catalytic acetylcholinesterase activity. Nonetheless, it remains possible that some pesticides interfere with important developmental functions of the cholinesterase enzyme family. — Environ Health Perspect 107(Suppl 1):59-64 (1999).

http://ehpnet1.niehs.nih.gov/docs/1999/Suppl-1/59-64/brimijoinabstract.html

Key words: acetylcholinesterase, butyrylcholinesterase, organophosphorus, anticholinesterase, echothiophate, chlorpyrifos, neurite outgrowth, brain development, neural cell adhesion molecules, cholinergic toxicity

Carbamate and organophosphorus pesticides probably owe both their agricultural effectiveness and their primary toxicity to inhibition of acetylcholinesterase (AChE, EC 3.1.1.7). The toxicology of anticholinesterase agents in adults is relatively well understood, but less is known about their effects on the young. When considering the developmental toxicology of anticholinesterase agents, three questions need to be asked: a) Are immature organisms likely to be more sensitive than adults to acute anticholinesterase toxicity, experiencing greater dysfunction at lower levels of exposure? b) Could anticholinesterases cause lasting neurochemical and neurobehavioral deficits in younger organisms? c) Might anticholinesterases even disturb the structure of emerging nervous systems by affecting development itself?

A short answer to all three questions is that we know too little to draw firm conclusions, and hard data are notably scarce. Nonetheless, there are theoretical grounds for hypothesizing that anticholinesterases might be more dangerous in the young than previously suspected. One reason for concern is new evidence that AChE and the related enzyme, butyrylcholinesterase (BChE, EC 3.1.1.8), also targeted by pesticides, may both have roles in neurologic development. That evidence will be addressed in the next section. First we should review current data on the sensitivity of young animals to toxicity from environmentally relevant anticholinesterase pesticides. Chlorpyrifos, a widely used organophosphate precursor, is an interesting case in point. Neuropathologic studies used to support chlorpyrifos registration with the U.S. Environmental Protection Agency uncovered no evidence of teratogenicity or gross damage to brain structure. However, sophisticated investigations directed at subter developmental effects have only begun in the past few years.

New results do not disprove the idea that chlorpyrifos is relatively benign, at least in adults, but they raise concerns about its effects on the young. It is now clear that newborn rodents are more sensitive than adults to the acute toxicity of chlorpyrifos in pharmacologic doses (1–3). Specifically, rats given chlorpyrifos at postnatal day 17 show behavioral changes. ChE inhibition, and downregulation of muscarinic receptors in the brain at single oral doses of 15 mg/kg, 5-fold lower than required for similar effects in adults (4). More disturbing are two reports of biochemical and morphologic effects in the newborn rat from chlorpyrifos in very low doses evoking no systemic toxicity. Although AChE activity was not determined, one study showed depressed DNA synthesis throughout the brain when day-old rats received 2 mg/kg chlorpyrifos subcutaneously (5). Another demonstrated net loss of neurons in the forebrain of 2-week-old rats treated with only 1 mg/kg (6).

Heightened chlorpyrifos sensitivity in newborns does not reflect postnatal persistence of an intrinsically susceptible, "immature" AChE (7). Instead the explanation appears to be a relative deficiency of organophosphorus hydrolase enzymes in brain and liver (8). Issues insufficiently explored are fetal vulnerability and the ability of maternal detoxification and placental barriers to offer meaningful protection in utero. Meanwhile, it is becoming clear that anticholinesterase toxicity may directly or indirectly affect a variety of neural systems. Song et al. (9) found that chlorpyrifos in a subtoxic dose (1 mg/kg, subcutaneously) caused no mortality or weight loss in neonatal rats but inhibited brain AChE activity by 25%. Although the inhibition recovered within a few days, it was followed by sizeable reductions of adenyl cyclase expression in several brain regions. G-protein linked signaling cascades were also impaired. Chlorpyrifos may therefore be able to disturb multiple neurotransmitter pathways in the developing central nervous system.

Despite these indications of acute susceptibility to anticholinesterases, it is only fair to point out that AChE activity in younger animals typically recovers quickly and drops less than in adults when subtoxic doses are repeated (3,4). Such observations probably reflect a relatively robust turnover
of AChE in the immature organism, where rapid protein synthesis speedily replaces inactivated enzyme. Even so, the collective animal data justify continued attention to the risks of anticholinesterase toxicity in human infants. The toxicologic rationale is further strengthened by new data suggesting novel functions of cholinesterases in neural development.

Nonclassical Functions of Cholinesterases

In the classical view, AChE has evolved to regulate cholinergic neurotransmission by hydrolyzing synaptic acetylcholine, whereas BChE represents a less important enzyme with an uncertain role in metabolizing xenobiotic esters. Consistent with this view, AChE predominates in neurons and muscle cells wherever cholinergic synapses are found. BChE on the other hand occurs primarily in nonneural or nonneptic sites like liver, lung, plasma, and neuroglia. But the full picture is more complex, since AChE also occurs in nonneural and embryonic tissues like red blood cells, megakaryocytes, and migrating neural crest cells. Similarly, BChE appears in limited groups of neurons (10,11), although its physiologic function at such sites is unknown (12).

The wide cellular distributions of AChE and BChE have fueled speculation that cholinesterases function in ways unrelated to cholinergic neurotransmission—a topic of several recent reviews (13,14). Proposed noncholinergic roles for AChE range from neuromodulation by secreted forms (15,16) to promotion of cell proliferation in tumor growth and hematopoiesis (17,18). It has also been suggested that AChE facilitates axonal outgrowth and synapse formation, in other words, that it serves as a "morphogenic molecule" in neurons (19). If this suggestion is valid, then we must consider whether anticholinesterase pesticides might harm immature organisms by hindering the architectural development of their nervous systems. That is admittedly a speculative proposition, especially when it involves judging the risks of low level environmental exposures. The point is, however, that more data are needed on the involvement of AChE in neural development and the potential influence of anticholinesterases on that process.

Cholinesterases and Development of the Nervous System

One reason to suspect a developmental role for AChE is the growth-related shift in molecular forms generated by alternative 3'-mRNA splicing. The developing brain is rich in monomeric and dimeric AChE—forms that are mainly intracellular (19-21). Adult brain AChE, however, occurs mostly as tetramers anchored to the outer neuronal surface by a hydrophobic peptide. Transition from small intracellular to large extracellular AChE forms occurs during and after synapse formation (20). The meaning of this shift is unclear, but it might relate to a transition between two roles: first in generating and stabilizing synaptic structures, then in promoting synaptic function.

The strongest circumstantial evidence for a morphogenic role of AChE lies in the timing of enzyme expression and axonal outgrowth. Neural AChE typically appears when axons are growing and before synaptic connections form (21). Thalamocortical projection neurons of the neonatal rat brain provide a particularly striking example. These sensory relay neurons are neither cholinergic nor cholinceptive, and they express virtually no AChE in either the embryonic or the mature state. Nonetheless, they begin exhibiting intense AChE activity shortly after birth, then cease again within another 2 to 3 weeks. This transient AChE expression coincides with the period when thalamic axons (from medial and lateral geniculate, ventral postero lateral and ventral postero medial nuclei) are growing into primary sensory cortex (22-24). Similar correlations of axonal growth and enzyme expression appear in other systems, including the developing human brain (25). Therefore, we suggest that transient AChE expression is no accident of development, but a means of promoting axonal outgrowth or synaptic connection.

Developmental patterns of cholinesterase expression are most thoroughly documented in avian nervous systems. Layer and co-workers (14,26) showed that a switch from BChE expression to AChE expression in embryonic chick cultures regularly accompanies the switch from cell proliferation to neural differentiation. In other words, neuroblasts express BChE as they cease dividing, but committed neurons express AChE as they generate axonal processes. Mutually exclusive spatiotemporal expression of AChE and BChE also occurs in vivo. For example, during limb formation in later stage chick embryos, long efferent nerve fibers originate from AChE-rich areas and grow through Schwann cell-lined pathways foreshadowed by BChE expression (27). Similarly, during ontogenesis of muscle tissue, motor neurons emerging from the neural tube express AChE as they extend toward the myotome through BChE-positive sclerotomal space (26,28). BChE thus precedes AChE in neurons and along the trajectory of their axons, which suggests a coordinated but reciprocal regulation of these two enzymes.

Data also support a developmentally regulated switch from BChE to AChE expression in spinal sensory or dorsal root ganglia (DRG). The DRG form a chain within the vertebral column beside the spinal cord. Each ganglion consists of pseudo-unipolar sensory neuronal cell bodies and satellite (glial) cells. Although the ganglia have no synapses of any kind, all neuronal subpopulations express the full range of cholinergic markers, including choline acetyltransferase (29-31). In embryonic chick DRG, total AChE activity per ganglion accumulates steadily with increasing age, but specific AChE activity (units/milligram) rises to a peak at gestational day 12 and then slowly declines to the adult level (32). In contrast, BChE activity begins high and then drops quickly as the DRG mature (14,19). There is a similar pattern of expression in the rat (33). At embryonic day 9 (E9), before DRG are formed, the neural tube stains heavily for BChE activity (Figure 1). In early DRG as well (E11-12), quantitative video microscopy shows prominent BChE activity while AChE activity is barely evident. From E13 on, however, AChE activity predominates and reaches high levels in the neuronal cell bodies. At these later stages BChE activity disappears from neurons but becomes highly expressed in glial cells of the surrounding neuropil and ganglionic roots. This distribution of cholinesterases resembles the adult pattern. Not surprisingly, AChE mRNA levels, as determined by polymerase chain reaction (PCR) assay from DRG collected at days E12-E19, vary in parallel with the intensity of enzyme stain. These temporal changes imply that AChE is a postmitotic differentiation marker that replaces BChE in sensory neurons when cell proliferation is complete.

Molecules Affecting Neurite Outgrowth

When AChE was cloned from Torpedo (34), mouse (35), and human sources (36), surprising sequence homologies came to light, with certain molecules implicated in morphogenic phenomena. Perhaps more than any other discovery, this observation has driven research into potential developmental functions of the cholinesterases.
Morphogenic proteins are classified according to mechanism of action and shared protein motifs. The most interesting molecules from our standpoint are those with homology to AChE, such as glutactin, neurotactin, gliotactin, and neuroligins. These nonenzymatic proteins belong to the broadly defined serine esterase domain family. They are all extracellular matrix components that influence cell–cell interactions by promoting cell adhesion. A dendrogram of serine esterase domain proteins with defined adhesive functions (Figure 2) illustrates their relationship to each other and to cholinesterases from representative species. Although the adhesive proteins are catalytically inactive, their sequence similarities with AChE range from 42 to 60% (Table 1). Conserved regions, including those that flank the “missing” catalytic serine, may be key to unraveling the morphogenic properties of this family.

Glutactin, neurotactin, and gliotactin are *Drosophila* proteins, at least two of which resemble AChE in showing transient expression during key stages in development. Glutactin is a secreted basement membrane glycoprotein of unknown function. Neurotactin is a transmembrane glycoprotein, transiently expressed on the surface of differentiating neuronal and epithelial cells (36). A three-dimensional model predicts that neurotactin’s N-terminal domain is structurally similar to *Torpedo* AChE, although the topology of membrane anchoring is different. In vitro studies with truncated neurotactin show that the extracellular region is critical for adhesive functions. When this region was replaced with the homologous domains from *Torpedo* AChE, *Drosophila* AChE (d-AChE) or glutactin, chimeric neurotactin still supported cell adhesion in transfected S2 cells (37). However, full-length glycospholipid-anchored dimers of d-AChE did not support adhesion on their own (38). Therefore, neurotactin’s ability to promote cell–cell interactions may require participation from cytoplasmic and transmembrane domains, which are of course lacking in AChE.

Another example of a cholinesterase-like molecule with structurally based morphogenic properties is gliotactin, a transiently expressed transmembrane protein in peripheral glia of *Drosophila*. Gliotactin may participate in cell–cell interactions that help glia to envelop nearby axons and form a tight blood–nerve barrier (39). Gliotactin-knockout insects have an intact peripheral nervous system, but the blood–nerve barrier does not form properly. This deficiency exposes nerves to high extracellular K+, leading to paralysis and other physiologic abnormalities.

Neuroligins also have significant homology to AChE (Table 1) and a potential, indirect role in cell–cell interaction. Rat neuroligin-1 (40) is a ligand for β-neurexins, putative neuronal surface receptors believed to contribute to synaptic organization. Studies of *Drosophila* mutants show that glial neurexin participates in forming the blood–brain barrier. Lack of neurexin expression causes defects similar to those in gliotactin knockouts (41). Interestingly, neurexin binds protein 4.1, an intracellular ligand that promotes interaction between transmembrane proteins.

![Figure 1](image_url)  
**Figure 1.** AChE and BChE enzyme activity in representative DRG. Enzymatically stained cryostat sections from rat embryos at embryonic days 9 (E9) to 17 (E17). Top panels show predominance of BChE in neural tube. After onset of neural differentiation (E13), BChE staining declines transiently and AChE staining increases markedly, starting in the ventral pole of the DRG. Later, AChE activity spreads throughout the DRG neurons, and BChE activity returns, but mostly in the roots and neuropil. From Koenigsberger et al. (33), with permission of Elsevier Science Publishers. Scale bar = 250 μm.

![Figure 2](image_url)  
**Figure 2.** Relationships among selected members of the serine esterase domain family. This dendrogram was generated on the basis of primary amino acid sequence identity using the PileUp program (55).
Table 1. Serine esteraseslike proteins compared by percentage identity and similarity. a

| r-AChE | m-BChE | d-ACHe | r-Neur | d-Glact | d-GLut | d-Nrta |
|--------|--------|--------|--------|--------|--------|--------|
| r-AChE | 52 (62) | 40 (47)| 30 (48)| 34 (42)| 36 (45)| 40 (42)|
| m-BChE | - | 38 (65)| 33 (60)| 31 (59)| 24 (53)| 26 (55)|
| d-ACHe | - | 30 (60)| 26 (58)| 26 (56)| 22 (54)| - |
| r-Neur  | - | 29 (58)| 25 (53)| 16 (43)| 10 (50)| - |
| d-Glact | - | - | 20 (50)| 24 (54)| - | - |
| d-Neuract | - | - | - | 19 (51)| - |

Abbreviations: r, rat; d, Drosophila; m, mouse. a Calculations used the BestFit program (55). Comparisons with AChE were based on the extracellular domains of the homologous proteins. Cross-comparisons among gliotactin, glutactin, neurtactin, and neulin were based on entire amino acid sequences.

and the cytoskeleton in erythrocytes (42). Members of the 4.1 family are also expressed in vertebrate brain (41). Neurilogsins can therefore be linked to important pathways of cellular communication and adhesion in the nervous system.

Still other morphogenic molecules show a different type of homology with cholinesterases through common patterns of glycosylation. For example, the carbohydrate HKN-1 epitope is found on AChE in addition to myelin-associated glycoprotein, NCAM, L1, and members of the tenasin family (43-46). Although HKN-1 is not a unique marker for cell-adhesion proteins, this shared occurrence provides yet another reason to hypothesize that AChE itself engages in protein-protein interactions.

Experimental Test of Developmental Role

Having surveyed the circumstantial evidence that AChE is involved in neural development, we can now consider the results of experiments designed to test this hypothesis. Most of the early attempts along these lines were pharmacologic. That is, various anti-cholinesterases were examined in neuronal culture to determine whether enzyme inhibition led to abnormal differentiation and outgrowth of axons. Unfortunately, few pesticides or environmentally relevant toxicants were selected for study, but generic agents have helped to clarify morphogenetic roles and mechanisms.

A consistent finding by several research groups concerns morphogenic effects of BW284c51 [1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide], a selective, rapidly reversible, bis-quaternary AChE inhibitor. This agent strongly suppresses neurite outgrowth in several model systems, including cultured chick neurons (47), and cultured rat DRG (48). In our laboratory, that effect was replicated in differentiating murine neuroblastoma cells (49) as well as rat DRG (50). The morphologic effects of BW284c51 cannot be solely due to AChE inhibition, because equally potent AChE inhibitors like echothiophate do not affect neurite outgrowth in vitro (47,49,50) or in neonatal rats (51). Such results do not necessarily mean that the drug effects are nonspecific. As Bigbee argues (52), interaction of bulky ligands with AChE, especially at the peripheral site, may distort protein shape or surface charge. That could in turn disturb critical protein-protein interactions, including those of an adhesive nature. Therefore, the pharmacologic data lead naturally to the hypothesis that AChE's morphogenic functions need not involve catalytic activity at all. In other words, the critical property may be structure.

To explore a structural role for AChE in neural differentiation, one requires a system in which AChE protein levels can be modulated, preferably in both directions. Seeking a convenient, isolated system in which acetylcholine hydrolysis should not be a factor, we selected the differentiating N1E.115 cell line. This murine neuroblastoma cell line is classified as adrenergic (expressing tyrosine hydroxylase, but not choline acetyltransferase), but it exhibits moderate levels of AChE activity. Upon serum deprivation, most N1E.115 cells extend neurites whose rate of outgrowth can be easily measured.

First, we demonstrated that AChE expression in wild-type N1E.115 cells could be transiently downregulated with antisense oligonucleotides (49). Next, to enhance the underexpression and make it permanent, we engineered stable transfectants to express antisense AChE cDNA under the direction of a β-actin promoter. AChE overexpressers were also created by stable transfection of full length sense AChE cDNA. In all, we produced six antisense lines, seven sense lines, and one control line (empty vector transfection). The relationship between AChE expression in these cells and their ability to differentiate into a clearly neuronal phenotype was then characterized.

In data pooled from all cell lines, AChE enzyme activity correlated strongly with mRNA level as determined by Northern blot analysis (r = 0.98). On average, cells transfected with sense AChE cDNA increased their AChE expression (enzyme activity and mRNA level) 2.5-fold over wild type. Increased amounts of immunoreactive Tau protein, a neuronal differentiation marker, rose as well, and the sense-transfectants displayed significantly greater neurite outgrowth (Figure 3). In contrast, antisense cell lines exhibited halved the wild-type level of AChE activity (and mRNA), lower levels of Tau, and decreased neurite outgrowth. Overall, the rate of neurite outgrowth correlated strongly with the AChE level (r = 0.94). This correlation is good evidence that AChE expression directly or indirectly facilitates the genesis of axons in differentiating nerve cells. If that view is correct, and if the effect depends on protein-protein interaction or adhesive functions, one would make two predictions: a) some AChE should localize to the external surfaces of neural growth cones, and b) anti-AChE antibodies that bind external enzyme should impair neurite outgrowth. Confirming these predictions, a polyclonal antibody against AChE bound selectively to the surface of AChE-overexpressing cells and caused a 43% decrease in neurite outgrowth (49). Bigbee (52) recently reported even stronger antibody-mediated suppression of neurite extension in cultured rat DRG.

Other investigators have also obtained evidence that AChE promotes neural growth and differentiation by non-enzymatic means. For example, Small (53) reported that substrata containing native and irreversibly inactivated AChE were equally good in stimulating neurite elongation, although heparan sulfate proteoglycans had to be present as well. Similarly, Soreq's group found that increased process extension in glioma cells overexpressing various AChE constructs bore no relation to hydrolytic activity (54). Taking the information together, it seems that AChE's role in neural differentiation is more likely to involve structural than catalytic mechanisms.

Conclusion

Evidence is rapidly mounting that cholinesterases have a role in neural development: a) AChE is surprisingly homologous to proven morphogenic molecules. b) In
both mammals and birds, a developmentally regulated switch from BChE to AChE expression coincides with the onset of neural differentiation. c) Transient AChE expression in many neural pathways corresponds closely to the period of axonal outgrowth. d) Large, inhibitory effects on neurite outgrowth can be caused by in vitro exposure to certain anticholinesterases, particularly those such as BW284-c51, capable of interacting with AChE’s peripheral site. e) Neurite outgrowth and other measures of differentiation are enhanced in neuronal lines that overexpress AChE and depressed in lines that underexpress AChE. f) Specific AChE antibodies interfere with extension of neural processes after binding to external cell surfaces in culture.

This growing body of knowledge is exciting to neurobiologists. There are also important toxicologic implications. Although AChE may affect morphogenesis by noncatalytic mechanisms such as structural recognition, these mechanisms could certainly be vulnerable to pesticides. Any anticholinesterase that might alter AChE’s steric or electrostatic properties should be suspected as a growth inhibitor until proved innocent. Agents acting at the peripheral site of the enzyme deserve special scrutiny. Most pesticides fall outside this category. However, the toxicologic data reviewed earlier indicate that those agents may have additional actions that would be deleterious to a growing nervous system. It seems wise to re-evaluate the developmental risks of anticholinesterases as data become available from ongoing studies of environmentally relevant molecules in neuronal culture and sensitive embryologic models of neural development.

REFERENCES AND NOTES

1. Pope CN, Chakraborti TK, Chapman ML, Farrar JD, Arthun D. Comparison of in vivo cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. Toxicology 68:51–61 (1991).

2. Pope CN, Chakraborti TK. Dose-related inhibition of brain and plasma cholinesterase in neonatal and adult rats following sublethal organophosphate exposures. Toxicology 73: 35–43 (1992).

3. Chakraborti TK, Farrar JD, Pope CN. Comparative neurochemical and neurobehavioral effects of repeated chlorpyrifos exposures in young and adult rats. Pharmacol Biochem Behav 46:219–224 (1993).

4. Moser VC, Padilla S. Age- and gender-related differences in the time course of behavioral and biochemical effects produced by oral chlorpyrifos in rats. Toxicol Appl Pharmacol 149: 107–109 (1998).

5. Whitney KD, Seidler FJ, Slotkin TA. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. Toxicol Appl Pharmacol 134:53–62 (1994).

6. Campbell CG, Seidler FJ, Slotkin TA. Chlorpyrifos interferes with cell development in rat brain regions. Brain Res Bull 43:179–189 (1997).

7. Mortensen SR, Brimijoin S, Hooper MJ, Padilla S. Comparison of the in vitro sensitivity of rat acetylcholinesterase to chlorpyrifos-oxon: What do tissue IC₅₀ values represent? Toxicol Appl Pharmacol 149:46–49 (1997).
nucleus of the thalamus and its connections in the developing human and monkey brain. J Comp Neurol 335:431–447 (1995).

26. Layer PG. Cholinesterases preceding major tracts in vertebrate neurogenesis. BioEssays 12:415–420 (1990).

27. Alber R, Sporns O, Weikert T, Willbold E, Layer PG. Cholinesterases and peptide-glutamin binding related to cell proliferation and axonal growth in the chick forebrain. Anat Embryol 190:429–438 (1994).

28. Layer PG, Alber R, Rathjen FG. Sequential activation of butyrylcholinesterase in rostral half somites and acetylcholinesterase in motor neurons and myotomes preceding growth of motor axons. Development 120:287–296 (1996).

29. Biagini S, Odorico T, Poiana G, Scarsella G, Augusti-Tocco G. Acetylcholinesterase in the development of chick dorsal root ganglia. Int J Dev Neurosci 7:267–273 (1989).

30. Castrigniano F, De Stefano ME, Leone F, Mulato B, Tata AM, Fasolo A, Augusti-Tocco G. Ontogeny of acetylcholinesterase, substance P and calcitonin gene related peptide-like immuno-reactivity in chick dorsal root ganglia. Neuroscience 34:499–510 (1990).

31. Tata AM, Plateroti M, Cibati M, Biagini S, Augusti-Tocco G. Cholinergic markers are expressed in developing sensory neurons. J Neurosci 37:247–255 (1994).

32. Giacobini G, Marchisio PC, Giacobini E, Klosow SH. Developmental changes of cholinesterases and monoamine oxidase in chick embryo spinal and sympathetic ganglia. J Neurochem 17:1177–1185 (1970).

33. Koenigsberger C, Hammond PI, Brimiouin J. Developmental expression of acetyl- butyrylcholinesterase in the rat: enzyme and mRNA levels in embryonic dorsal root ganglia. Brain Res 787:249–258 (1998).

34. Schumacher M, Camp S, Maultet Y, Newton M, McPhee-Quigley K, Taylor SS, Friedmann T, Taylor P. Primary structure of Torpedo californica acetylcholinesterase deduced from cDNA sequence. Nature 319:409–409 (1986).

35. Rachinsky TJ, Camp S, Li Y, Ekstrom TJ, Newton M, Taylor P. Molecular cloning of mouse acetylcholinesterase: tissue distribution of alternatively spliced mRNA species. Neuro 5:317–327 (1990).

36. Sorèq H, Ben Aziz R, Prody CA, Seidman S, Gnaat A, Neville L, Lienman-Hurwitz J, Lev-Enichm G, Ginsberg D, Lapidot-Lisfon Y. Molecular cloning and construction of the coding region for human acetylcholinesterase reveals a G > C-rich attenuating structure. Proc Natl Acad Sci USA 87:9688–9692 (1990).

37. De la Escalera S, Bockamp ED, Moya F, Fiorent M, Jimenez F. Characterization and gene cloning of neuronal AChE in Drosophila transmembrane protein related to cholinesterases. EMBO J 9:3593–3601 (1990).

38. Darboux I, Barthalay Y, Piomont M, Hipeau-Jacquotte R. The structure-function relationships in Drosophila neurotoxin show that cholinesterases domains may have adhesive properties. EMBO J 15:1183–1184 (1996).

39. Auld VJ, Fetter RD, Brodie K, Goodman CS. Giotocin, a novel transmembrane protein on peripheral glia, is required to form the blood-nerve barrier in Drosophila. Cell 81:757–767 (1995).

40. Ichtchenko K, Hata Y, Nguyen T, Ulrich B, Missler M, Maxow M, Sudhof TC. Neuregulin-1: A splice-site-specific ligand for β-neureceptors. Cell 81:435–441 (1995).

41. Littleton JT, Bhat MA, Bellen HJ. Deciphering the function of neurexins at cellular junctions. J Cell Biol 137:793–796 (1997).

42. Japan JH. Blood: Textbook of Hematology. 2nd ed. Boston:Little Brown, 1998.

43. Schachter M, Martini R. Glycans and the modulation of neural recognition molecule function. Trends Neurosci 18:183–191 (1995).

44. Bon S, Melfa K, Musset F, Grassi J, Massoulle J. An immunoglobulin M monoclonal antibody, recognizing a subset of acetylcholinesterase molecules from electric organs of Electrophorus and Torpedo, belongs to the HNK-1 anti-carbohydrate family. J Neurochem 49:1720–1731 (1987).

45. Layer PG, Kaulich S. Cranial nerve growth in birds is preceded by cholinesterase expression during neural cell crest migration and the formation of an HNK-1 scaffold. Cell Tissue Res 265:393–407 (1991).

46. Weikert T, Layer P. The carbohydrate epitope HNK-1 is present on all inactive, but not on all active forms of chicken butyrylcholinesterase. Neurosci Lett 176:9–12 (1994).

47. Layer P, Weikert T, Alber R. Cholinesterases regulate neurite growth of chick nerve cells in vitro by means of a non-enzymatic mechanism. Cell Tissue Res 273:219–226 (1993).

48. Dupree J, Bigbee J. Retardation of neurite outgrowth and cytoskeletal changes accompanies acetylcholinesterase inhibitor treatment in cultured rat dorsal root ganglion neurons. J Neurosci Res, 37:267–273 (1994).

49. Koenigsberger C, Chiappa S, Brimiouin J. Neurite differentiation is modulated in neuroblastoma cells engineered for altered AChE expression. J Neurochem 69:1398–1407 (1997).

50. Chiappa S, Brimiouin J, Pharmacological tests of a role for acetylcholinesterase in promoting neurite outgrowth by dorsal root ganglia. In: Cholinesterases 96 (Doctor BP, Quinn D, Taylor P, eds). New York:Plenum, 1996.

51. Ling JJ, Yu J, Robertson RT. Sustained inhibition of acetylcholinesterase activity does not disrupt early geniculocortical ingrowth to developing rat visual cortex. Brain Res. 86:354–358 (1995).

52. Bigbee JW, Sharma KV, Gupta JJ, Dupree JL. Morphogenic role for acetylcholinesterase in axonal outgrowth during neural development. Environ Health Perspect 107(Suppl 1):81–87 (1999).

53. Small D, Reed G, Whitefield B, Norcombe V. Cholinergic regulation of neurite outgrowth from isolated chick sympathetic neurons in culture. J Neurosci 15:144–151 (1995).

54. KarpeL R, Sterrinfield M, Ginsberg D, Guhl E, Graessmann A, Sorèq H. Overexpression of alternative human acetylcholinesterase forms modulates process extensions in cultured glioma cells. J Neurobiol 26:114–123 (1996).

55. SCC. Wisconsin Sequence Analysis Package. Madison, WI:Genetics Computer Group, Inc., 1994.