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A Comparative Analysis of Perinatal Development of Barrel Cortex in Rat, Mouse and Guinea Pig Using Acetylcholinesterase Histochemistry

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Abstract: The role of acetylcholinesterase (AChE) in the developing neocortex was reexamined by comparing its expression in rats, mice and guinea pigs, following the protocol for acetylcholinesterase histochemistry (described in Sendemir et al., 1996) in order to determine the suitability of the breeding colony at Uludağ University for use as an animal model.

A total of 103 pups as well as two adult animals of each species were used.

In the rat pups, acetylcholinesterase-rich patches were distributed in a vibrissa-related array in the somatosensory cortex soon after birth, whereas regions of the cortex lying between individual patches and between rows of patches in the were depleted enzyme. Mice on postnatal day 3 and older mice revealed lightly stained, acetylcholinesterase positive spots in the center of barrel cores, while the barrel walls remained devoid of acetylcholinesterase; septae that divided the individual barrels were densely enzyme positive. The barrels in the guinea pigs formed before birth. Well-formed barrels were observed by postnatal day 0, and acetylcholinesterase activity gradually decreased by postnatal day 10 but did not fade away. The differential enzyme location in different rodents indicates that its role in the development of thalamocortical connectivity is distinctly different in rats as opposed to mice and guinea pigs.

Key Words: Thalamocortical development, barrel cortex, rodent, and acetylcholinesterase histochemistry.

Introduction

More than a decade ago, Kristt (1,2,3) showed that high levels of acetylcholinesterase (AchE) - an enzyme commonly known for its function in degrading the neurotransmitter acetylcholine which is also localized in the regions of the developing nervous system and that is not necessarily associated with cholinergic transmission (4, 5) - are present inthe ventrobasal thalamus and barrel cortex of perinatal rats. He showed that the enzyme is transiently distributed in whisker-related cortical patches during development, but is not detectable in the adult animal.

More sensitive histochemical methods for visualizing the presence of AchE have been introduced (6, 7), although the latter of these proved to be contradictory in previous studies (8, 9). The newer techniques have primarily made use of the rat as an animal model, and led to the supposition thta AchE has an important role in the formation of thalamocortical connectivity (10, 11, 12). However, for any such role to be generally true, one would expect the enzyme to have a similar distribution in all species that share a similar cortical organization.

This has been tested before by comparing the maturational expression of Ache in the somatosensory cortex (SI) of rat, mouse and hamster (9), with the conclusion that the roles suggested for AchE during the development of connectivity in the rat SI cannot be generalized to other rodent species without significant remediation.

In order to determine the suitabilty of our breeding colony (Uludağ University, Experimental Animals Research and Breeding Center) for use as an animal model for similar processes, the expression of AchE was retested in the rat, mouse and also guinea pig, as it is a special having a longer gestational period than the other two (mouse, 19; rat, 21; and guinea pig, aproximately 64 days).

Materials and Methods

The data reported in this study were collected from six litters of rats (Sprague-Dawley, n=48), four litters of mice (h=34) and eight litters of guniea pigs (n=21) as well as two adult animals of each species. The day of birth was designated as postnatal day (PND) 0 for all animals.

Pups aged PND 0, 1, 2, 3, 5, 8, 10, 15 and 20 and adult animals were overdosed with sodium pentobarbital and perfused transcardially with cold heparinized saline, followed by 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). The brains were removed and postfixed overnight at 4 ºC. Cortices from two or more animals at each time point were removed, flattened between glass slides, and cryoprotected. The tissue was cut parallel to the pial surface at a thickness of 60 μm. Forebrains from other animals (at all time points) were cut along the coronal plane. All sections were mounted on gelatin-subbed slides and air dried.

The protocol for AchE histochemistry described by Sendemir et al. (9) was used. The sections were immersed for 18-20 hours in the substrate incubation solution containing 0.002 M copper sulfate, 0.115% acetylthiocholine iodide, 0.078 M magnesium chloride, 0.002 M copper glycine and 24% sodium sulfate, in 0.05 M maleate buffer (at 37ºC; pH 6.0). The slides were transferred to 20% and 10% solutions of sodium sulfate for 5 minutes and 1 minute, respectively, washed in distilled water, and developed for 1-10 minutes in 4% ammonium sulfide in 0.1 M phosphate buffer and perchloric acid (pH 6.0). After washing, the tissues were fixed in 4% buffered (0.1 M, pH 7.4) parafolmaldehyde (20 minutes), rinsed again, toned with 0.2% gold chloride (5 minutes), immersed in 5% sodium thiosulfate (5 minutes), and, after a final rinse, the sections were coverslipped for light microscopy using a glycerol jelly as the mounting medium.

Results

Developmental Changes in Patterns of AchE Staining in Rat, Mouse and Guinea Pig Cortex

Coronal Sections

On the first and second day following birth, Layer I and emergent infragranular layers were identified in the neocortices of the rats and mice: future Layer II-IV cells were still incorporated in the cortical plate. By PND0 and PND1 in the mice, a few fibers were beginning to enter the cortical plate, and similar sections from the rats revealed dense AchE-positive fibers in layer VI and part of Layer V.

Over the next few days, increasing numbers of enzyme-containing fibers entered the neocortical walls of the mice and rats. In the rats a densely stained band in the lower part of the cortical plate/Layer IV broke up into multiple patches, and became restricted to the differentiated Layer IV. The discrete patches of enzyme gradually disappeared by PND 20. In contrast to the enzyme distribution described for the rats, the expression of AchE in Layer IV low through the first few days after birth. In the guinea pigs, AchE-negative patches and positive septa were clearly visible from PND 0, and this continued even in the adult form. In the mice and guinea pigs a dense band of AchE staining located at the border of layers IV/V, became more pronounced but then thinner by PND8 (Figure 1).

Sections Parallel to the Pial Surface

Patterns relating to the periphery in SI cortex can be more clearly visualized in sections that are cut tangential to the cortical surface. In rats, AchE-containing patches overlie the barrels, whereas regions between individual barrels and between whisker-related rows, like the agranular cortex surrounding the barrels, do not express the enzyme. In mice, AchE staining in Layer IV gave an overall negative image of that seen in rats: for the most part, the barrels were low in enzyme, whereas the septa and agranular cortical areas became progressively more AchE positive over time, thus outlining individual barrels. This was the case from the first day in the guinea pigs.

The bands of staining along the septa surrounding each barrel in the mice and guinea pigs were much thinner than the enzyme-negative regions between barrels in the rats. Faint spots AchE staining were also present in the barrel cores. In the guinea pigs the barrels were more diagonal in shape, and clearly delineated by their AchE-positive septa. In contrast to the findings of previous literature (13), the barrels seemed to be larger that rat and mouse barrels, and they were not round (Figure 2).

AchE Staining in the Ventrobasal Complex of the Thalamus

The ventral posterior thalamus of all rodents is divided into the ventroposteromedial nucleus (VPM), which receives trigeminal input including sensory information from the vibrissa pad, and the ventroposterolateral nucleus (VPL), which receives input from dorsal column nuclei. In perinatal rats AchE staining was very dense in both of these cell groups. The whisker-representation modules of the diencephalon the - the barreloids - in the thalamus were identified with enzyme histochemistry after PND 3. Correlated with this staining

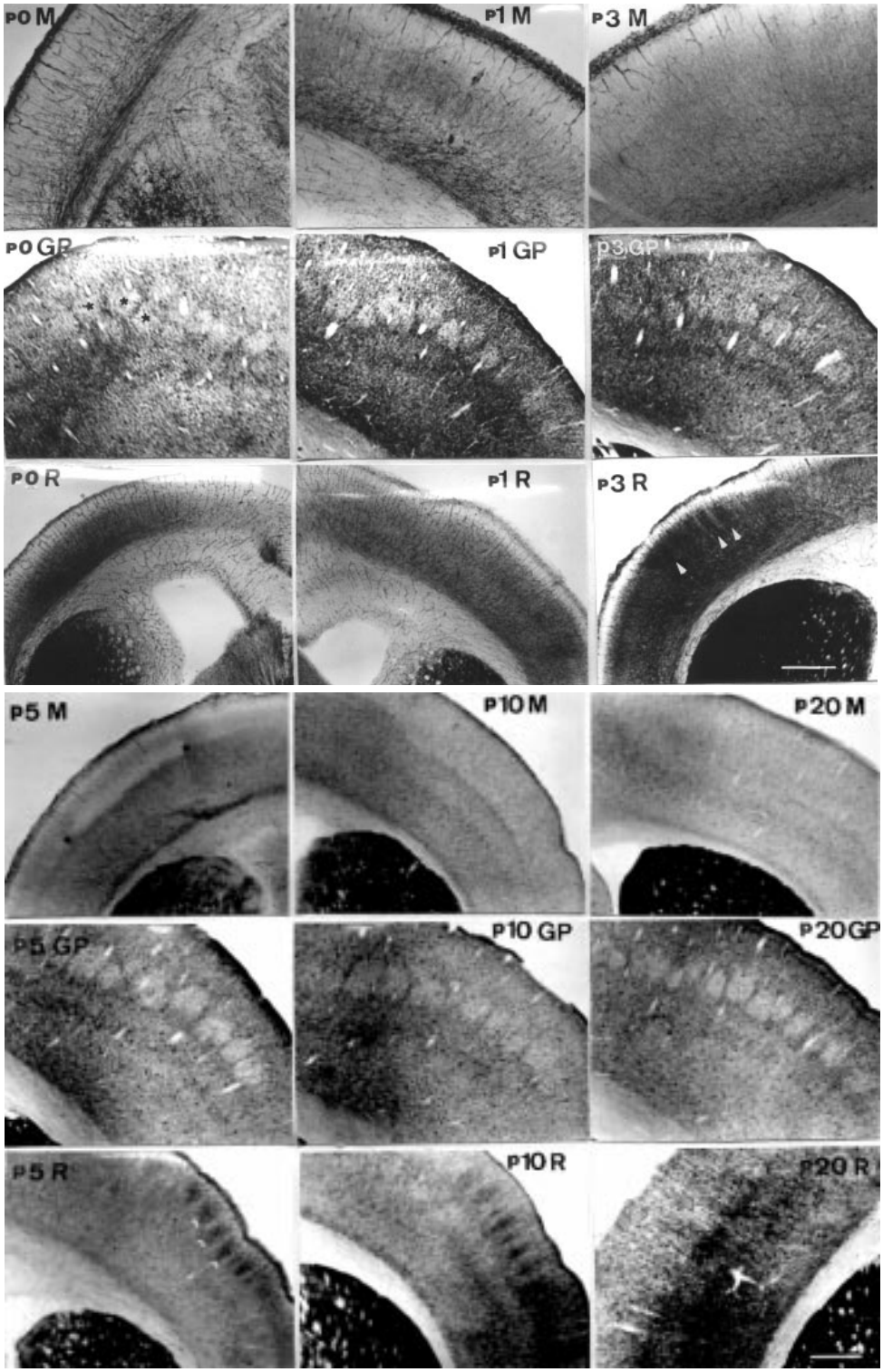

Figure 1. Acetylcholinesterase (AchE) staining patterns in the barrelfield cortex of the developing mouse (M), guinea pig (GP) and rat (R). By potnatal day (PND) 0, an enzyme-positive band is present in the lower part of the cortical plate in rats. By PND 3, this band becomes segregated into patches. The best patchy scene is in p5R (white arrowheads). The equivalent region in the mouse is depleted in AchE (between stars-p5M), and a negative image of the rat can be seen in the guinea pig-barrels are devoid of the enzyme, and AchE positive septa (p0 GP-stars). Guinea pig has adult appearance by birth and AchE activity does not show any changes. Scale bar, 500 µm.

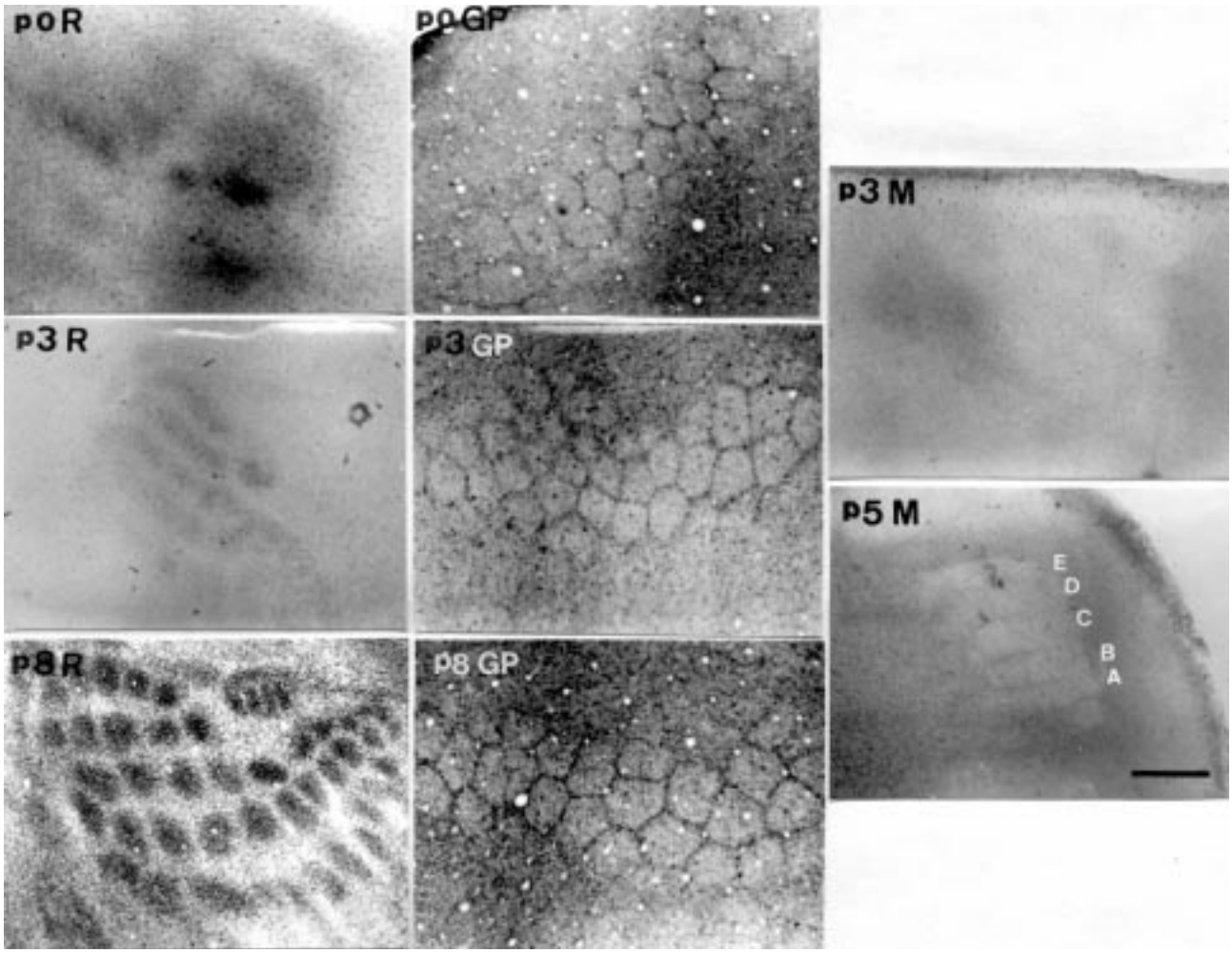

Figure 2. Patterning of periphery-related modules in flattened somatosensory cortex of rat, mouse and guinea pig as revealed by acetylcholinesterase (AchE) histochemistry. AchE-positive patches become clear as rat and mouse develop from postnatal day 0, but in guinea pig, barrels keep their initial shape that can be seen formed even by postnatal day 0. AchE positive patches occupy barrel centres in rat (left column). In mouse (right column) and guinea pig (middle column), barrels are for the most part devoid of enzyme while dense staining is present along septa separating indivudual barrels. (Rows of barrels from A to E are shown in p5M figure). Scale bar, 500 µm.

pattern, the vibrissa-specific barrels, like the non-whisker barrels, were rich in AchE. In contrast, in both the mice and guinea pigs VPM was depleted AchE at all postnatal ages, and the VPL contained high levels of the enzyme. No photographs of the guinea pig thalamus were taken, because they were too big to photograph with a microscope (Figure 3).

Discussion

We have documented that a complex expression of AchE prevails in the developing somatosensory cortex of rodents and that specific differences exist in the laminar and tangential localization of the enzyme in neonatal mice and guinea pigs as opposed to rats.

In both mice and rats there is clear evidence that glutamate/aspartate, and not acetylcholine, is the neurotransmitter which subserves the thalamocortical connections (14, 15). Choline acetyltransferase is not detectable in the sensory thalamic nuclei of immature or adult rats (16, 17), lending support to the claim that thalamocortical projection neurons are not cholinergic. Yet during development, these thalamic nuclei stain intensely for Ache (2, 18). The expression of AchE in immature thalamic neurons, its presence in the cortical

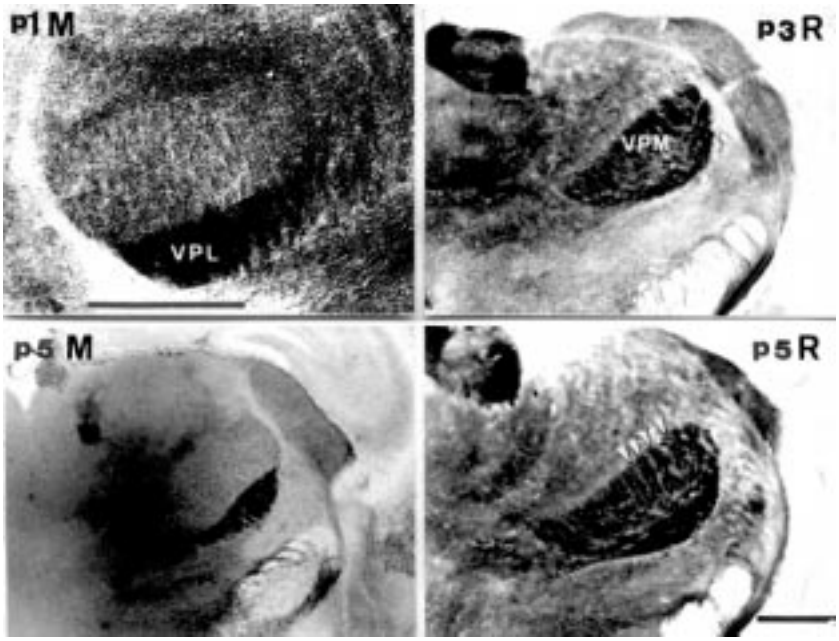

Figure 3. Acetylcholinesterase (AchE) staining in ventrobasal complex of postnatal day 5 mouse (M), and rat (R). In rat, ventroposteromedial (VPM) and ventroposterolateral (VPL) nuclei of ventrobasal complex are AchE positive; row of barreloids can be identified (arrowheads p5R). In mouse and guinea pig AchE-positive barreloids are visible in VPL (although they are not clear in these pictures), but VPM remains devoid of enzyme. Scale bar, 500 µm.

projection zones of these nuclei, and its virtual disappearance from thalamo-recipient zones of the neocortex after diencephalic lesions all indicate that much of the AchE localized in developing layers III/IV of the sensory neocortex derives primarily from neurons inthe thalamus. These observations have led to the suggestion that AchE of thalamic origin may serve an important function in the establishment of synaptic connectivity between the thalamus and cortex.

Robertson and Yu (12) advanced hypotheses with regard to how AchE might contribute to the establishment of thalamocortical connectivity. Comparative studies on the structure of cytoarchitectonic aggregates that comprise cortical barrels do show species-specific differences: barrels in the mouse have cell-sparse cores (hollows), and cell-dense walls; in the rat, the barrel cores (centers) are cell dense (13, 19). Thus, the presence of AchE-positive patches in the rat, and the enzyme containing septae and barrel cores in the mouse and guinea pig may somehow relate to the way in which granule cells become clustered in the immature SI cortex of these species. Such a relationship necessitates that AchE mediate cell-cell adhesions (18, 20, 21).

Unlike the findings in rats barrels form in utero in guinea pigs. The expression of the AchE-negative barrels plus Ache-positive septa do not show any changes following birth.

The role of Ache expressed in the septal regions of the guinea pig and mouse, but not of the rat, remains unexplained. Examining the developmental pattern of cytotactin, cytotactin binding proteoglycan, or other ECM molecules in the mouse and guinea pig barrelfield cortex could shed light on this.

In light of our comparative study, one would have to suppose that the mechanisms of thalamocortical connectivity and formation of barrels in rats are different to those in mice and guinea pigs, or that an alternate enzyme in mice and guinea pigs serves the function of AchE in rats.

References

1. Kristt DA. Development of neocortical circuitry: histochemical localization of acetylcholinesterase in relation to the cell layers of rat somatosensory cortex. J Comp Neurol, 186:1-16, 1979.

2. Kristt DA. Acetylcholinesterase in the ventrobasal thalamus: transience and patterning during ontogenesis. Neuroscience, 10:923-939, 1983.

3. Kristt DA. Acetylcholinesterase in immature thalamic neurons: relation to afferentiation, development, regulation and cellular distribution. Neuroscience, 29:27-43, 1989.

4. Kostovic I, Goldman-Rakic PS. Transient cholinesterase staining in the mediodorsal nucleus of the thalamus and its connections in the developing human and monkey brain. J Comp Neurol, 219:431-447, 1983.

5. Kostovic I, Rakic P. Development of peristriate visual projections in the monkey and human fetal cerebrum revealted by transient cholinesterase staining. J Neurosci, 4:25-42, 1984.

6. Bear MF, Carnes KM, and Ebner FF. Postnatal changes in the distribution of acetylcholinesterase in kitten striate cortex. J Comp Neurol, 237:519-532, 1985.

7. Schlaggar BL, and O'Leary DDM. Patterning of the barrelfield in somatosensory cortex with implications for the specification of neocortical areas. Perspectives on Dev Neurobiol, 1:81-91, 1993.

8. Erzurumlu RS, Sendemir E, and Jhaveri S. Species variations in patterns of AchE staining in SI cortex of developing rats. SN Abstr, 20:754, 1994.

9. Sendemir E, Erzurumlu RS, and Jhaveri S. Differential expression of acetylcholinesterase in the developing barrel cortex of three rodent species. Celebral Cortex, 6:377-387, 1996.

10. Robertson RT. A morphogenic role for transiently expressed acetylcholinesterase in thalamocortical development? Neurosci Lett, 75:259-264, 1987.

11. Robertson RT. Transiently expressed acetylcholinesterase activity in developing thalamocortical projection neurons. In: Cholinesterases: structure, function, mechanism, genetics, and cell biology (Eds.: Massoulia J, Bacou F, Barnard E, Chatonnet A, Doctor BP, Quinn DM), American Chemical Society Washington, DC, 1991, pp:358-365.

12. Robertson RT, and Yu J. Acetylcholinesterase and neural development: new tricks for an old dog? News Physiol Sci, 8:266-272, 1993.

13. Rice FL, Gomez C, Barstow C, Burnet A, and Sands P. A comparative analysis of the development of the primary somatosensory cortex: Interspecies similarities during barrel and laminar development. J Comp Neurol, 236:477-495, 1985.

14. Ottersen OP, Fischer B, Storm-Mathisen J. Retrograde transport of D-[^{3}H] aspartate in thallamocortical neurones. Neurosci Lett, 42:19-24, 1983.

15. Kharazia VN, and Weinberg RJ. Glutamate in thalamic fibers terminating in Layer IV of primary sensory cortex. J. Neurosci, 14:6021-6032, 1994.

16. Armstrong DM, Saper CB, Levey AI, Hainer BH, Terry RD. Distribution of cholinergic neurons in the rat brain demonstrated by the immunocytochemical localization of choline acetyltransferase. J Comp Neurol, 216:53-68, 1983.

17. Hauser CR, Crawford GD, Barber RP, Salvaterra PM, Vaughn JE. Organization and morphological characteristics of cholinergic neurons: an immunocytochemical study with monoclonal antibody to choline acetyltransferase. Brain Res, 266:97-119, 1983.

18. Schlaggar BL, De Carlos JA, O'Leary DDM. Acetylcholinesteraes as an early marker of the differentiation of dorsal thalamus in embryonic rats. Dev Brain Res, 75:19-30, 1993.

19. Welker C, and Woolsey TA. Structure of Layer IV in the somatosensory cortex of the rat: description and comparison with the mouse. J Comp Neurol, 158:437-454, 1974.

20. Drews U. Cholinesterase in embryonic development, Prog Histochem Cytochem, 7:1-52, 1975.

21. Sharma KV, and Bigbee JW. Acetylcholinesterase antibody treatment results in neurite detachment and reduced outgrowth from cultured neurons: further evidence for a cell adhesive role for neuronal acetylcholinesterase. J Neurosci Res, 53:454-464, 1998.