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Developmental and experiential changes in dendritic symmetry in n. laminaris of the chick

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Early acoustic experience affects the structure of neurons in the brainstem of young chickens. In the binaurally innervated cells of the nucleurs laminaris the symmetry of the dorsal and ventral dendritic trees normally increases during the embryonic and early postnatal period. Unilateral ear plugs disrupt that development. This study shows that balanced stimulation plays an important role in the development of symmetrical neuronal structures in the central auditory pathway.

Accumulating evidence leaves little doubt that experience affects the structure of neurons in the central nervous system[1–7,11–20,24–26]. In particular, the shape of neurons is thought to be related to their stimulation[1–4,6,9].

The nucleus laminaris (NL) of birds is a group of neurons arranged in a plane. This sheet of cells, like the medial superior olive in mammals, receives binaural excitatory input from the cochlear nucleus[18]. The cells of NL send dendritic processes dorsally and ventrally, perpendicular to the plane of cells[20]; the dorsal dendrites receive input from the ipsilateral ear while the ventral dendrites receive input from the contralateral ear[14]. Thus, any manipulation of one ear can be expected to affect the dorsal dendrites on the same side of the head and the ventral dendrites of NL on the opposite side of the brain.

We examined developmental changes in the symmetry of the bipolar dendritic configuration and the roles of balanced stimulation of the two ears on the observed developmental changes. We assumed that normally occurring sound stimulation provides, over time, symmetrical signals to both ears which will result in an age-related increase in the symmetry of the dorsal and ventral dendrites. Conversely, unbalanced stimulation of the two ears, by plugging one external auditory meatus, would result in asymmetrical dendrites.

The initial analysis of dendritic symmetries as a function of age involved 230 cells from 10 chickens of 3 different ages. Animals were sacrificed at embryonic days 15 and 19, and on postnatal day 25[19]. After processing by a modification of the Golgi–Kopsch method[21] (see Fig. 2), fully impregnated cells were traced using a camera-lucida attachment on a conventional light microscope; at high magnification a line was drawn down the center of each dendritic arbor. Finally, the total lengths of the dorsal and of the ventral dendritic arborizations were determined using a digital planimeter[20]. All sections were coded to remove bias in tracing or measurement.

Dendritic symmetries were quantified by a ratio of the total lengths of the dorsal and of the ventral dendritic arbors. That is, there were two total lengths from each cell, one from the dorsal dendrites and one from the ventral dendrites; the smaller length was

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divided by the larger to form a ratio. Thus increasing ratios, up to the maximum of 1.0, indicate increasing dendritic symmetry.

The normal development of dendritic symmetry ratios is shown in Fig. 1 (solid points and lines). After 15 days of incubation, NL cells have bipolar dendrites but on the average the dorsal and ventral dendrites differed in length by about 27% (ratio = 0.73). After 31 more days of development (postnatal day 25), this ratio increased to 0.80. Thus, a small but significant increase in cellular symmetry is seen between embryonic day 15 and postnatal day 25 (Wilcoxon's S = 1218, $P < 0.025$).

At embryonic day 15 the auditory system has been functional for 4 days[8], but the embryo is still surrounded by fluid, and thresholds are high. The system has had little time for adjustments of structure to function, and dendritic symmetries are relatively low and highly variable. By day 19, just before the embryos enter the air space of the egg and first hear normal air-born sounds, the symmetries of the dorsal and ventral dendrites have increased and their variability has decreased. Chickens hatch on day 21. Following 25 days of posthatch acoustical experience the symmetries of the dorsal and ventral dendrites appear to have reached an asymptote. This normal age-related increase in dendritic symmetry suggests that as the organism is experiencing balanced sensory input to the two ears, the dendrites are also becoming progressively matched in terms of the amount of membrane devoted to input from each ear.

These results lead to the obvious question of whether the developmental changes in dendritic symmetry are related to symmetrical stimulation of the two ears, and thereby to matching afferent activity impinging on the dorsal and ventral dendrites of the NL cells. A test of this hypothesis is to unbalance the stimulation to the two ears and determine if a corresponding asymmetry of the dendrites occurs. This manipulation was performed by inserting a plastic ear plug in one ear on embryonic day 19 and measuring dendritic symmetries on postnatal day 25. This experiment was replicated under two rearing conditions described below.

The method of ear plugging has been previously described as has its sound attenuating characteristics[10]. A small hole was made in the shell and the

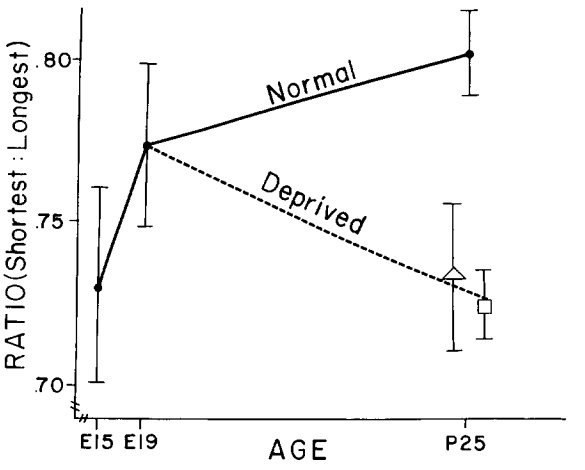

Fig. 1. The development of dendriti symmetry in n. laminaris in embryonic and hatchling chickens. Means and standard errors of the smaller ratio of total dendritic lengths on the dorsal and ventral sides of each cell are plotted. Increasing ratios indicate increasing symmetries. The solid lines and points show normal development. Sample sizes were 39 cells at embryonic day 15 (E15), 61 at embryonic day 19 (E19) and 130 at postnatal day 25 (P25). The open triangle and square indicate the first and second replications of the unilateral plug treatment. Sample sizes were 46 and 241 cells respectively.

embryo's head was pulled through the membranes far enough to dry one ear canal. The external auditory meatus was then filled down to the tympanum with silicone plastic. These embryos hatch a few hours earlier than expected but otherwise appear and behave normally. The ear plugs produce a 40 dB conductive hearing loss which is nearly flat across the frequency range of the chicken and appears fully reversible at least out to 10 days posthatching[10]. Earplugs were checked daily and replaced when loose or at least every 5 days. The impression of the tympanic membrane could be seen on the end of the removed plugs. Therefore in each animal one ear was subjected to 40 dB of conductive deficit from prior to hatching until 25 days after hatching, at which time the animal was sacrificed and the brain was processed as described above.

In the first experiment, unilaterally ear-plugged animals were raised in a normal laboratory environment. Thus, after ear plugging on embryonic day 19 these animals were returned to a standard laboratory incubator. Twenty-four hours after hatching they were moved to a commercial brooder where they lived with other chicks until being sacrificed. Calibrations of the noise in the brooder revealed a

broad sprectrum of sounds dominated by low frequencies. Two chickens with plugs in their right ears and two with plugs in their left ears were used. Forty-six cells were drawn and measured. These cells were sampled equally from the middle region of NL on both sides of the brains.

As shown in Fig. 1 by the open triangle, the unilateral ear plugs significantly altered dendritic structure by decreasing the symmetries of cells in the nucleus laminaris ($S = 1308$, $P < 0.02$). The ratio of 0.74 shows that dendritic length of one side of these cells averaged 26% less than the length on the other side. These results are consistent with a similar effect in mammals[6].

To more thoroughly examine this effect, the unilateral deprivation was replicated under more controlled conditions. Two chickens had their right ears plugged and two had the left ears plugged at 19 days of incubation. These birds were incubated, hatched, and reared in pairs for 27 days in 305 × 336 × 254 mm double-walled chambers which attenuate outside sounds 45 dB at 100 Hz, 65 dB at 300 Hz and > 75 dB at higher frequencies. Speakers inside the boxes broadcast pulsing pure tones from 200 to 4600 Hz at 50 dB SPL for 36 min out of each hour for 12 h per day. The animals were sacrificed 25 days after hatching and the brains were processed as described above. Measurements of the dorsal and ventral dendrites were made on 241 cells sampled from throughout the nucleus, about half from each side of the brain.

As shown in Fig. 1 by the open square, the symmetry of the treated cells is clearly less than that of normal cells of the same age ($S = 8062$, $P < 0.00005$). Again the ratio of longest to shortest dendrite averaged 0.73 indicating an average 27% difference in size between the two sides of the cell. The two replications of the ear-plugging manipulation therefore yielded similar results; a manipulation that unbalanced the stimulation to the two ears led to unbalanced dendritic structures. Fig. 2 shows examples of asymmetrical cells from this experiment and a normal cell for comparison.

As another way of looking at these same data, the correlation of the dorsal and ventral dendrites was computed across all the cells in each group. The correlation coefficient measures the strength of the relationship between dendritic length on the two

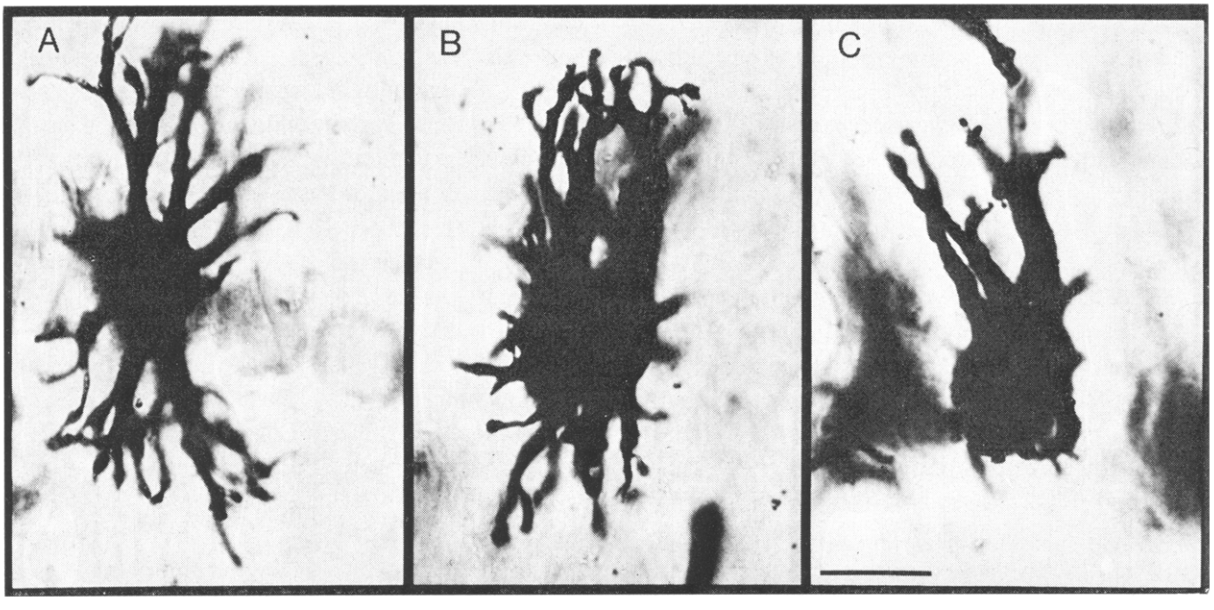

Fig. 2. Normal and asymmetrical cells in nucleus laminaris. A: a normal cell from the middle of the nucleus at 25 days posthatch. Two other cell bodies in the lamina are counterstained with thionin. B: a cell representative of the average asymmetry in the treated groups. Its ratio is 0.70; the dorsal dendrites (on top) total 456 μm, the ventral dendrites total 319 μm. C: an extremely asymmetrical cell from the second replication of the unilateral ear-plugging treatment. Its ratio is 0.45, 97/216 μm. Both abnormal cells, B and C are contralateral to the ear plug. Cells were treated by the Stensaas modification of the Golgi–Kopsch method which fully impregnates 10% of the cells throughout the nucleus. The calibration bar is 20 μm. Dorsal is up.

sides of a cell. Correlations normally increase from 0.57 to 0.71 between embryonic day 15 and posthatch day 25[19]. The correlation of the unilaterally ear-plugged animals at 25 days posthatch was 0.57. Thus, as in the analysis of symmetries, the unbalanced input makes the cells of 25-day-old chickens resemble much younger cells, in that there is a weak relationship between the lengths of dendrites on the two sides of the cells.

The analysis of dendritic symmetry simplifies what appears to be a much more complicated picture of the effects of manipulations of the acoustic environment on the absolute sizes of dendrites. Such analyses are complicated by the fact that at this time it is not possible to directly relate changes in conductive hearing to activity in nerve fibers across the frequency range of an organism. For example, our ear plugging procedure probably mass-loads the tympanic membrane[23] and may therefore *increase* the transmission of low-frequency bone conducted and internal noises while *decreasing* air conducted sounds of the same frequencies[22]. Preliminary analyses of these data provide some support for this hypothesis, and differential effects of ear plugging on cells in various frequency regions of the n. magnocellularis of the chick have been reported[5]. Further analyses are in progress to examine such interactions. The advantage of the analysis of symmetry is that regardless of how the ear plugs affected the electrical or chemical activity impinging on the dendrites of NL, the monaural occlusion was likely to have unbalanced that activity to the two sides of the cells.

In conclusion, dendritic symmetries increased with age, suggesting a correlation between balanced stimulation and the ontogeny of dendritic structure. Supporting this hypothesis, unbalanced stimulation arrested the development of dendritic symmetry in nucleus laminaris.

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1 Bergstrom, R. M., Entropy and information in fetal behavior. In G. Gottlieb (Ed.). *Studies on the Development of Behavior and the Nervous System, Vol. 2. Aspects of Neurogenesis*, Academic, New York, 1974 pp. 205–217.
2 Borges, S. and Berry, M., Preferential orientation of stellate cell dendrites in the visual cortex of the dark reared rat, *Brain Research*, 112 (1976) 141–147.
3 Coleman, J. R. and O'Connor, P. O., Effects of monaural and binaural sound deprivation on cell development in the anteroventral cochlear nucleus of rats, *Exp. Neurol.*, 64 (1979) 533–566.
4 Coleman, P. D. and Riesen, A. H., Environmental effects on cortical dendritic fields. I. Rearing in the dark, *J. Anat.*, 102 (1968) 363–374.
5 Conlee, J. W. and Parks, T. N., Age- and position-dependent effects of monaural acoustic deprivation in nucleus magnocellularis of the chicken, *J. comp. Neurol.*, 202 (1981) 373–384.
6 Feng, A. S. and Rogowski, B. A., Effects of monaural and binaural occlusion on the morphology of neurons in the medial superior olivary nucleus of the rat. *Brain Research*, 189 (1980) 530–534.
7 Gottlieb, G., The roles of experience in the development of behavior and the nervous system. In G. Gottlieb (Ed.), *Studies on the Development of Behavior and the Nervous System, Vol. 3. Neural and Behavioral Specificity*, Academic, New York, 1976, pp. 25–54.
8 Jackson, H., Hackett, J. T. and Rubel, E. W, In vitro electrophysiological analysis of n. magnocellularis and n. laminaris of the chicken, *Neurosci. Abstr.*, 4 (1978) 7.
9 Jacobson, M., *Developmental Neurobiology*, Plenum, New York, 1978, 562 pp.
10 Kerr, L. M., Ostapoff, E. M. and Rubel, E. W, Influence of acoustic experience on the ontogeny of frequency generalization gradients in the chicken, *J. exp. Psychol.*, 5 (1979) 97–115.
11 Killackey, H. P. and Ryugo, D. K., Effects of neonatal peripheral auditory system damage on the structure of the inferior colliculus of the rat, *Anat. Rec.*, 187 (1977) 624.
12 Mistretta, C. M. and Bradley, R. M., Effects of early sensory experience on brain and behavioral development. In G. Gottlieb (Ed.), *Studies on the Development of Behavior and the Nervous System, Vol. 4. Early Influences*, Academic New York, 1978, pp. 215–247.
13 Parks, T. N., Changes in the length and organization of nucleus laminaris dendrites after unilateral otocyst ablation in chick embryos, *J. comp. Neurol.*, 202 (1981) 47–57.
14 Parks, T. N. and Rubel, E. W, Organization and development of brain stem auditory nuclei of the chicken: organization of projections from n. magnocellularis to n. laminaris, *J. comp. Neurol.*, 164 (1975) 435–448.
15 Rogowski, B. A. and Feng, A. S., Normal postnatal development of medial superior olivary neurons in the albino rat: a Golgi and Nissl study, *J. comp. Neurol.*, 196 (1981) 85–97.
16 Rosenzweig, M. R. and Bennett, E. L., Experiential influences on brain anatomy and brain chemistry in rodents. In G. Gottlieb (Ed.), *Studies on the Development of Behavior and the Nervous System, Vol. 4, Early Influences*, Academic, New York, 1978, pp. 289–327.
17 Rubel, E. W, Ontogeny of structure and function in the vertebrate auditory system. In M. Jacobson (Ed.), *Handbook of Sensory Physiology, Vol. IX, Development of Sensory Systems*, Springer, New York, 1978, pp. 135–237.

18 Rubel, E. W and Parks, T. N., Organization and development of brain stem auditory nuclei of the chicken: tonotopic organization of n. magnocellularis and n. laminaris, *J. comp. Neurol.*, 164 (1975) 411–434.
19 Smith, Z. D., Organization and development of brain stem auditory nuclei of the chicken: dendritic development in n. laminaris, *J. comp. Neurol.*, 203 (1981) 309–333.
20 Smith, D. J. and Rubel, E. W, Organization and development of brain stem auditory nuclei of the chicken: dendritic gradients in n. laminaris, *J. comp. Neurol.*, 186 (1979) 213–240.
21 Stensaas, L. J., The development of hippocampal and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. I. Fifteen millimeter stage, spongioblast morphology, *J. comp. Neurol.*, 129 (1967) 59–70.
22 Tonndorf, J., Bone conduction. In J. V. Tobias (Ed.), *Foundations of Modern Auditory Theory, Vol. 2*, Academic, New York, 1972, pp. 197–237.
23 Tonndorf, J. and Duvall, A. J. III, Loading of the tympanic membrane: its effect upon bone conduction in experimental animals, *Acta Oto-Laryng.*, Suppl 213 (1966) 39–54.
24 Valverde, F., Structural changes in the area striata of the mouse after enucleation, *Exp. Brain Res.*, 5 (1968) 274–292.
25 Volkmar, F. R. and Greenough, W. T., Rearing complexity affects branching of dendrites in the visual cortex of the rat, *Science*, 176 (1972) 1445–1447.
26 Webster, D. B. and Webster, M., Effects of neonatal conductive hearing loss on brain stem auditory nuclei, *Ann. Otol.*, 88 (1979) 684–688.