

Quantitative Morphology of Human Hippocampus Early Neuron Development

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ABSTRACT

Previous findings in adults revealed significant hemispheric asymmetry in the size of neuronal somata in hippocampal subfield CA2 (the resistant sector) with no age-related changes. A paucity of quantitative data on the developmental status of these protected neurons has led to the investigation of their morphology in comparison to neurons in adjacent subfield CA3, bilaterally.

Bilateral coronal sections from postmortem hippocampus, 24 to 76 weeks postmenstrual age (gestational age plus postnatal age), were studied. The neurons were digitized and measured on a computer.

Soma size correlated positively and significantly with age in CA2 and CA3, bilaterally. CA2 somata were significantly larger (left, 34%; right, 32%) than adjacent CA3 somata. Variability in soma form or size increased appreciably with age, in both subfields, bilaterally, while variability in soma orientation was weakly correlated with brain growth.

The results suggest that in early development there are similarities in hemispheric growth patterns in CA2 and CA3. Large CA2 soma size implies axonal connectivity to distantly located targets very early in development. The results have functional implications, including memory, to brain development. *Anat Rec* 254:87-91, 1999. © 1999 Wiley-Liss, Inc.

Key words: CA2; CA3; morphogenesis; hemispheric specialization; age

The classic investigations of Lorent de No (1934) distinguished hippocampal subfield CA2 from its neighboring subfields in Ammon's horn, CA3 and CA1, on the basis of layer arrangement, neuronal size and shape, and axonal growth patterns. Subsequent immunohistochemical techniques (Leranth and Ribak, 1991), Golgi tracing (Tamamaki et al., 1988), Timm staining (Cassell and Brown, 1984), a variety of neurotransmitter labeling (Rosene and Van Hoesen, 1987), and morphometry (Zaidel et al., 1997b) have all clearly distinguished between CA2 and its adjacent subfields in primates and humans. The growth of CA2 in the early course of human brain development, particularly in the left and right sides, has not been investigated systematically until now. Computer measurements of digitized tissue can provide quantitative data not easily detected with microscopy alone, and this was undertaken in the present study.

For unknown reasons, in human neonates and in very young infants, hippocampal subfield CA2 neurons are qualitatively the most form-differentiated (Friede, 1972; Gilles, 1983), and CA3 neurons are qualitatively close

behind CA2 in form differentiation; CA1 neurons appear in comparison immature, with poor definition between the cytoplasm and nucleus (Gilles, 1983), rendering them difficult to measure quantitatively.

Previously, morphology of the hippocampus in surgically resected tissue from temporal lobe epilepsy patients (Zaidel et al., 1993, 1994) revealed left-right asymmetry in neuronal regional differentiation, and the finding has motivated the investigation of the hippocampus in ontogenesis bilaterally (Zaidel et al., 1997a). A subsequent computer-assisted quantitative study of normal postmortem hippocampal tissue revealed asymmetry in the size of neuronal

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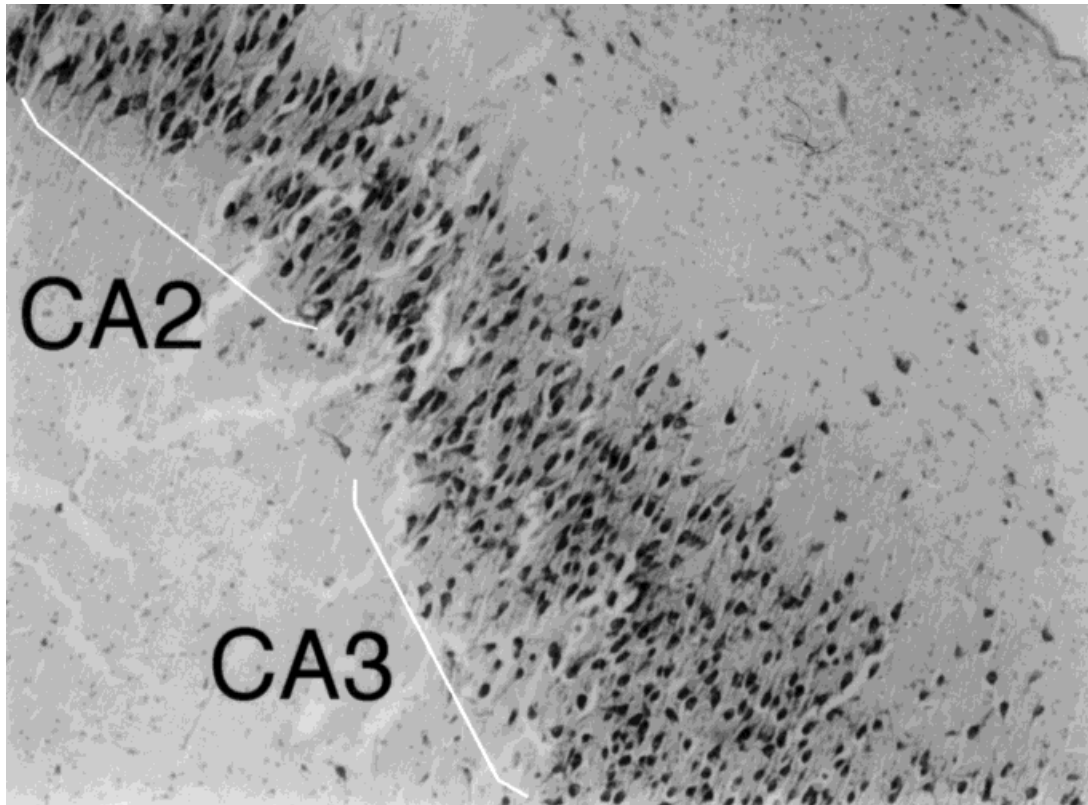


Fig. 1. Example of neuron size in CA2 and CA3 in a 1-month-old case. Neurons in CA2 are larger than neurons in CA3 in both sides.

somata, particularly in subfield CA2 (Zaidel et al., 1997b). The present study measured on a computer CA2 and CA3 neuron soma size, form, and orientation angle in brain development.

MATERIALS AND METHODS

Fifteen (seven males, eight females, all postpartum) postmortem brains underwent routine neuropathological examination in the neuropathology laboratory of the Radcliffe Infirmary, Oxford, England, following unexpected infant death. No pathological abnormalities were found in the brains which were removed within the first 24 h after death. Each brain was formalin-fixed (10% buffered solution), left and right hippocampal tissue was wax-embedded and coronally sectioned, and 20 μm specimens from the anterior part of the body of the hippocampus (see Duvernoy, 1988) were stained with cresyl violet. Their postmenstrual age (gestation age + postnatal age) ranged from 24–76 weeks. Their growth was normal, as can be seen in the strong correlation between age and brain weight ($r = .91$, $P < 0.0001$). Figure 2 illustrates the relationship between age and brain weight.

Neurons in CA2 and CA3 from hippocampal sections (on microscope slides) were digitized on a computer with a digital video camera (Sony DKC-5000, 3CCD) attached to an Olympus BH2 light microscope and connected to a Macintosh computer. A clear image of the tissue on the computer screen with good contrast between the neurons

and neuropil as judged by the human eye was calibrated and maintained throughout the study. A $\times 10$ microscope objective was used to visualize and digitize the neurons.

In each hippocampal subfield, a single zone from the center of the subfield was digitized. The neurons were then measured with NIH-Image (version 1.60) on a Macintosh. In subfield CA3, the captured zone was from the genu (where the subfield curves, rather than the hilar region of CA3). With each digitized sample, the background was first filtered with the 2D rolling ball. Then, while thresholding was enabled, each neuron was selected manually with the wand tool (for the measurement). The spatial scale was calibrated to micrometers. This procedure was used in previously published studies in adults (Zaidel et al., 1996, 1997b).

Although the main purpose of this study was the quantitative measurement of the neurons on a computer, it was considered that determination of neuronal density in these regions would complement the data and may shed additional light. Thus, neuronal density was determined with a light microscope (Olympus BH2). The identical methods for counting neurons in neuropathology material as described earlier were used here as well (Zaidel et al., 1993). Said succinctly, the method consisted of counting neurons through the entire thickness of the 20 μm tissue as a 10 \times 10 grid in the eyepiece was positioned over the center of each subfield.

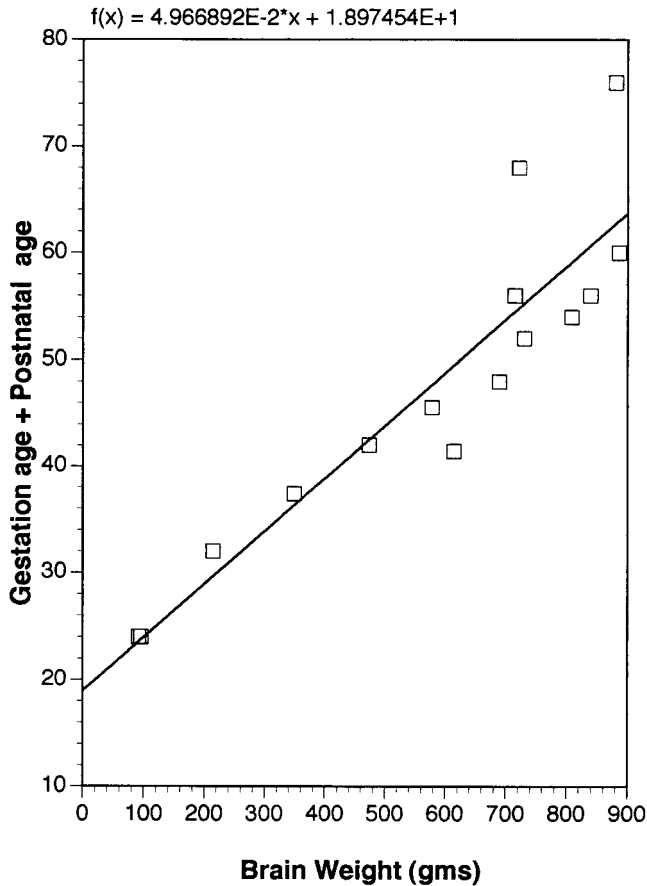


Fig. 2. Relationship between brain weight and age (gestational age plus postnatal age) in the sample of cases reported in the present study. $r = .91$; $P < 0.0001$.

Experimental Design and Logic of Data Analysis

From each digitized zone, both soma size and variability in size (standard deviation) were compared to brain weight. Brain weight was considered a finer measure of developmental marker than postvoluntary age. For the statistical analysis of soma orientation, the standard deviation of the angles (the angle between the major axis of the best fitting ellipse and a line parallel to the x-axis of the computer screen) was calculated. This determined the status of regularity in soma orientation. For shape, the length of the minor axis was divided by the length of the major axis of the best fitting ellipse, and the standard deviation of the quotients was entered into statistical calculations—that is, the standard deviation of (length minor \div length major). This formula for determining shape accounts for any differences due to soma size.

RESULTS

Figure 1 shows an example of CA2 and CA3 neurons. CA2 somata were 34% and 32% larger than CA3 somata in the left and right sides, respectively. Soma size increased linearly with brain growth, bilaterally (Fig. 3). Similarly, the question of variability (standard deviation) in soma size was investigated as well; size variability increased as

the brain grew, (left CA2, $r = .87$, $P < .0001$; left CA3, $r = .79$, $P < .0002$; right CA2, $r = .75$, $P < .0006$; right CA3, $r = .80$, $P < .0001$). Likewise, soma shape variability increased systematically (left CA2, $r = .63$, $P < .0009$; left CA3, $r = .77$, $P < .0004$; right CA2, $r = .53$, $P < .03$; right CA3, $r = .63$, $P < .009$). On the other hand, variability in soma orientation was somewhat weakly correlated with brain weight, bilaterally, and consistently in the negative direction (left CA2, $r = -.36$, $P < .1$; left CA3, $r = -.27$, $P < .3$; right CA2, $r = -.46$, $P < .08$; right CA3, $r = -.32$, $P < .2$).

The correlation between neuronal density and brain weight revealed a consistent pattern in both subfields (left CA2, $r = -.20$; left CA3, $r = -.49$; right CA2, $r = -.22$) with a strong, significant correlation only in the right CA3 ($r = -.92$, $P < 0.0001$). Similarly, the relationship between soma size and density was strong only in the right CA3 ($r = -.85$, $P < .0003$); r values in the left CA2 and CA3 were $-.17$ and $-.49$, respectively, and $-.12$ in the right CA2.

DISCUSSION

Similar growth patterns were observed in the two sides with respect to soma size and the variability of size, shape, or orientation. A strong focal and lateralized effect was found only in the right CA3. As the neurons in this hippocampal subfield grew larger, density declined, an outcome that suggests the maturation of neurons. It is widely accepted in early development that cell proliferation and programmed cell death precede cell maturation. A low correlation value would imply a slow neuronal maturation rate or the combination of cell proliferation, death, and maturation. Such a value was obtained in the left and right CA2 and left CA3.

The hemispheric directionality in neocortical growth rates is right side earlier than left side. Specifically, right superior frontal and superior temporal gyri were visualized 1–2 weeks earlier than in the left side, and their gyral complexity was apparent earlier in the right than in the left (Dooling et al., 1983; Turkewitz, 1988). Broca's area homologue in the right side developed dendrites before the left (Scheibel, 1984). There is also recent evidence for early functional development in the right as compared to the left hemisphere (de Schonon and Mathivet, 1989, 1990; Chiron et al., 1997).

In the adult hippocampus (age range 22–87 years), left CA2 neurons were only 11% larger than CA3 (genu) neurons, and right CA2 neurons were 3% smaller than CA3 neurons (Zaidel et al., 1996, 1997b), whereas in the present age range we found that CA2 neurons were 34% (left) and 32% (right) larger than CA3 neurons. CA2's large-sized somata in early development could reflect long-range axonal connectivity. Indeed, previous findings from Golgi and immunohistochemical studies in animals traced CA2 axons to the medial septal nucleus and to the contralateral hippocampus, both of which are a substantial distance away (Tamamaki et al., 1988; Leranthe and Ribak, 1991). In addition, from studies in monkeys we know that regions outside the hippocampus provide input to CA2 and CA3, namely from the supramammillary nucleus in the hypothalamus and the accessory basal nucleus of the amygdala (reviewed by Rosene and Van Hoesen, 1987). The mossy fiber system originating with the granule cells of the dentate gyrus makes synapses with

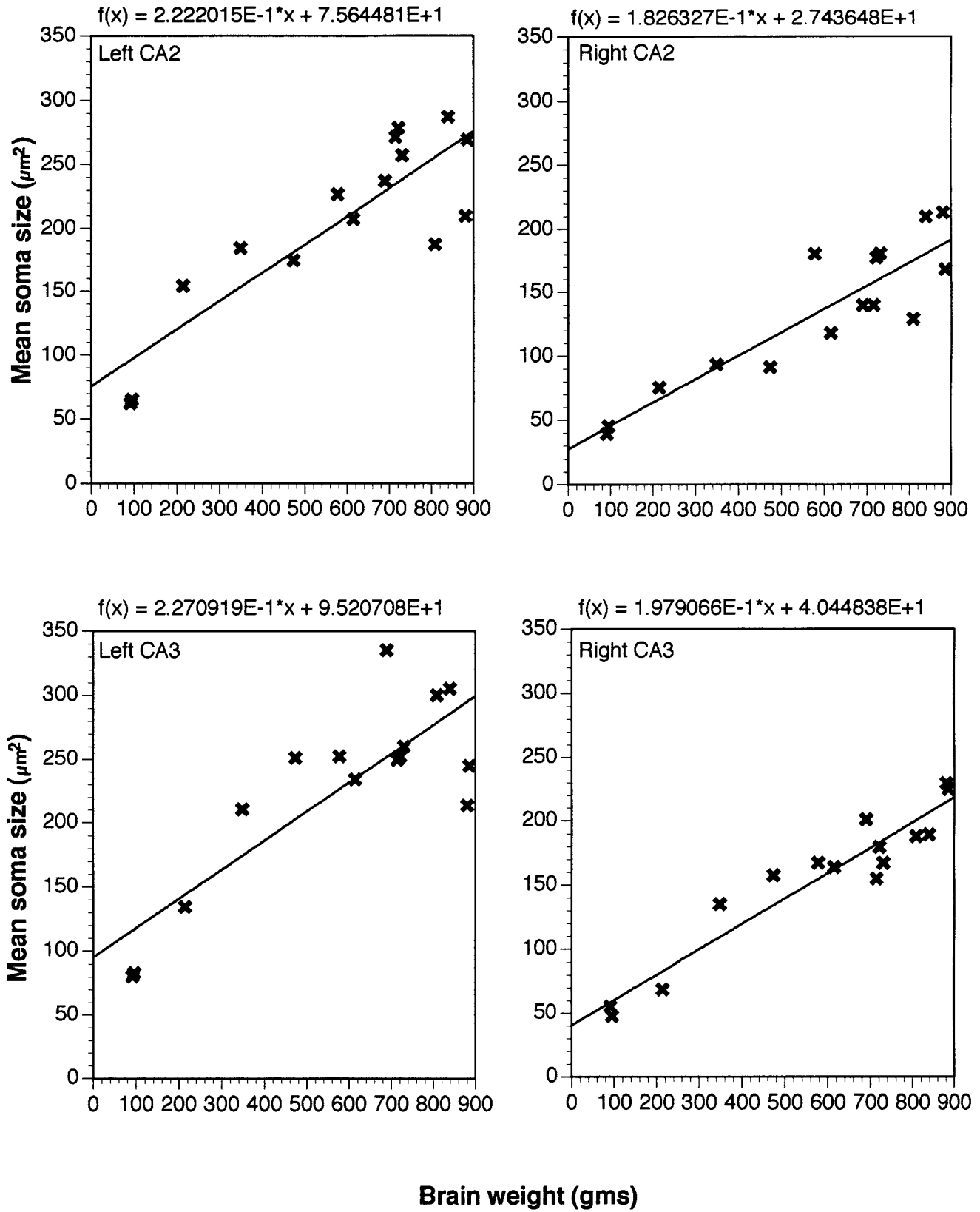


Fig. 3. Neuron size in development. Scatterplots of neuron size (µm²) in hippocampal subfields CA2 and CA3 as function of brain weight (g) in the left and right hemispheres of the brain. Left CA2, $r = .86$, $P < 0.0001$; left CA3, $r = .89$, $P < 0.0001$; right CA2, $r = .83$, $P < 0.0001$; right CA3, $r = .95$, $P < 0.0001$.

CA4 and CA3 but stops short of CA2 (Lorente de No, 1934; Cassell and Brown, 1984; Rosene and Van Hoesen, 1987). The principal intrinsic projections to CA2 originate in CA3. Considering the fact that abundant protective calcium-binding proteins are found in CA2, speculations on the functional and structural role this subfield plays in hippocampus development are warranted.

In sum, the findings revealed that in early development certain cytoarchitectural principles apply to both hemispheres equally. Neuron size and shape variance increased with brain weight (age) bilaterally, the regularity in neuronal orientation was the same bilaterally, and the CA2 > CA3 neuron size effect was left-right symmetrical. All of this is likely to have implications for the development of functional asymmetry in the brain observed in adulthood and to periods of vulnerability to disease or plasticity in normal growth. Future studies which would provide quantitative data on neurons in additional hippocampal subfields at an appropriate developmental stage could shed further light on the relationship between neuron morphology and hippocampal integrity.

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