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Short communication

Effects of early ethanol exposure on dendrite growth of cortical pyramidal neurons: inferences from a computational model

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Abstract

A computational model has been used to infer rules governing dendritic growth of layer 2/3 associative pyramidal neurons in a rat model of foetal alcohol syndrome. Basal dendrites were studied in adult rats exposed to ethanol during the first postnatal week. Results suggest that ethanol exposure during early postnatal life affects mainly the branching of dendrites rather than their elongation.

Theme: Development and regeneration

Topic: Process outgrowth, growth cones, and sprouting

Keywords: Dendrites; Pyramidal neuron; Biotinylated dextran amine; Growth model; Foetal alcohol syndrome; Rat

The exposure to ethanol during brain development is currently recognized as one of the main causes of mental retardation in humans [1,4]. Experimental models of foetal alcohol syndrome (FAS) have established that early exposure to ethanol is able to induce important structural and functional alterations of the cerebral cortex, e.g. [11,12], as well as a strong rearrangement of its connections [7]. However, even if dendritic anomalies represent a consistent anatomical correlate of mental retardation [9], little is known about the dynamics of growth and reshaping of cortical dendrites during FAS. Here we report the results of an investigation concerning basal dendrites of layer 2/3 associative pyramidal neurons in a rat model of FAS. The quantitative parameters were analyzed using a computational model framework which provides inferences about the rules governing dendritic growth during development [20–22].

Newborn Wistar rats received ethanol by inhalation from the second postnatal day (P2; P0 is the birthdate) through P6. The method of ethanol administration was slightly modified from Ruwe et al. [15]. Briefly, an air pump (air flow=3 liters/min) was connected to a vaporization chamber into which ethanol (95% v/v) was injected at a rate of 2.5 ml/min. The ethanol atmosphere was then conveyed to a sealed plexiglass cage in which the pups were placed, after separation from the mothers, for 3 h a day. At the end of each session the blood alcohol concentration (BAC) was measured in one rat per litter using an enzymatic kit (332-A, Sigma, St. Louis, MO, USA). The BAC ranged from 185 to 290 mg/100 ml (mean±S.D.=249.90±32.51). Control animals of matched age were removed from the mothers for 3 h a day, omitting the ethanol inhalation procedure. After the experimental or control procedure the pups were regularly suckled by their own mothers. Animals were weighed at P7 and P14. The mean weight values of ethanol treated and control animals were not significantly different (P7: 13.97±1.01 g and 14.35±0.84 g for the ethanol and control group, respectively; ANOVA: $F_{1,16} = 0.73$. P14: 31.39±2.59 g and 32.80±2.48 g, respectively; $F_{1,16} = 1.36$). At P90, rats from the ethanol and the control group were deeply anaesthetized (ketamine 90 mg/kg, xylazine 10 mg/kg i.p.) and received cortical injections (0.1 μl) of a solution containing 10% biotinylated dextran amine (Molecular Probes, Eugene, OR, USA) and 10 mM *N*-methyl-D-aspartic acid (Sigma, St. Louis, MO, USA). Injections were

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aimed at the sensori-motor cortex of the right side (1.20–0.70 mm anterior to the bregma; 2–4 mm lateral to the midline; 1.5 mm below the pial surface). After a survival time of 72 h, animals were anaesthetized and transcardially perfused with phosphate buffered saline followed by 4% buffered paraformaldehyde. This tracing technique yields a Golgi-like filling of retrogradely labeled pyramidal neurons of layer 2/3 (Fig. 1A and B). Details of the histological procedure adopted to evidence labeled somata and dendrites are given in [6]. Some completely filled basal dendrites were entirely reconstructed across adjacent sections using Neurolucida (Microbrightfield, Colchester, VT, USA).

Altogether, 62 basal dendrites were completely reconstructed (31 belonging to 15 neurons from 3 ethanol animals; 31 belonging to 13 neurons from 3 controls). For each dendrite, several quantitative data were evaluated: total dendritic length, length of intermediate and terminal

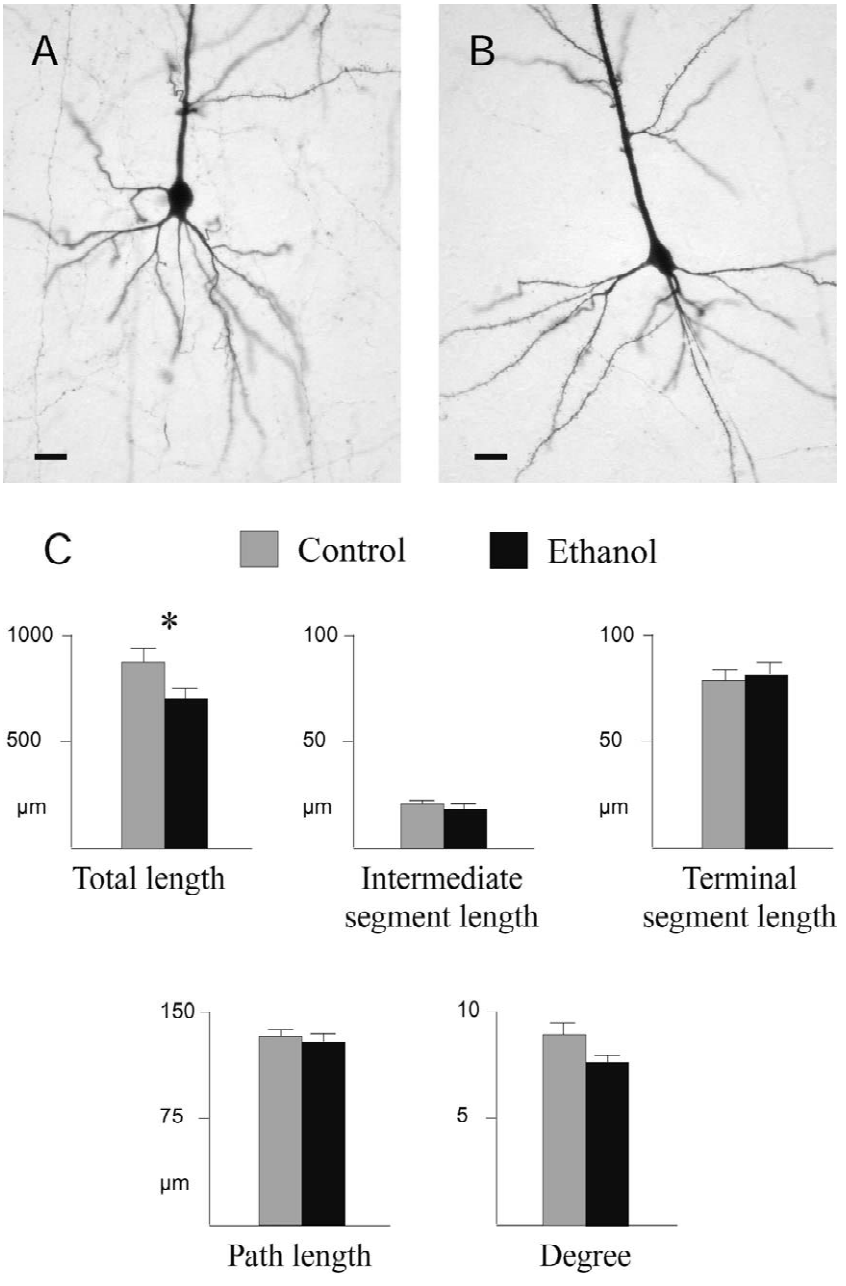

Fig. 1. A, B: Microphotographs showing retrogradely labeled associative neurons of layer 2/3 from a control case (A) and from an ethanol treated case (B). Note the complete filling of basal and apical dendrites. Scale bars = 15 µm. C: Bar graphs showing the mean and standard error of mean for the quantitative data concerning the basal dendrites of layer 2/3 neurons. *: $P < 0.05$ (Mann–Whitney test). Difference between ethanol and control cases for the degree are at the edge of significance ($P = 0.055$). $n = 62$ dendrites.

segments, path length (the distance from the soma to each end point), number of terminal segments (degree), centrifugal order of segments, tree asymmetry index [23].

Prior to testing for differences between control and ethanol groups, the individual segments variables have first been averaged per individual tree. As shown in Fig. 1C, there is a significantly decreased total dendritic length in animals treated with ethanol compared to controls. This seems to be the consequence of a reduced number of terminal segments per dendritic tree, with the difference between groups at the edge of significance ($P = 0.055$, Mann–Whitney test).

The experimental data were entered into the dendritic growth model proposed by Van Pelt et al. [20–22]. This computational model aims to describe quantitatively the geometry of dendritic trees as the outcome of a growth process of neurite elongation and branching. The model shows, by simulation, how given rules for elongation and branching of individual terminal segments result in specific dendritic geometries. Vice versa, experimentally reconstructed dendritic geometries can be analyzed in terms of the most probable rules for elongation and branching that produce similar geometries. The model uses a stochastic approach which is appropriate because of the large number of processes involved in neurite outgrowth. That means that the geometric variation in dendritic shapes becomes an integral part of the analysis. Briefly, the branching probability of a terminal segment $p(t)$ in a tree with n terminal segments is assumed to depend on the centrifugal order γ of the terminal segment (i.e., the number of branch points on the path from the soma to the segment), the number of terminal segments $n(t)$, and a baseline component $D(t)$ via $p(t) = D(t) \; . \; n(t)^{-E} \; . \; 2^{-S\gamma} \; / \; C(t)$, with parameter E representing the strength of the dependency on the terminal segment number (also called competition parameter), parameter S representing the strength of the order-dependency, and $C(t)$ a normalization factor [20–22]. For $E = 0$, the branching probability of terminal segments is independent of the (increasing) number of terminal segments during outgrowth. For $E = 1$, the branching probability becomes inversely proportional to this increasing number, making the summed branching probability of all terminal segments independent of this number. For $S = 0$, the branching probability is independent of the proximal/distal position of the segment with respect to the soma. A value of $S = +1/-1$ lets the branching probability decrease/increase with a factor of two across each branch point. Thus, a positive value of S means that proximal terminal segments have higher branching probability than distal ones. The time integral B of the baseline branching rate function over the period of growth T, $B = \int^T D(t)dt$, is taken as a third parameter for the branching process [22]. Parameter B can be interpreted as the total expected number of branching events during outgrowth under the condition $E = 1$ [22]. An exponential decreasing basal branching rate function was used [22], with dendritic growth starting at postnatal day 1 (P1) with neurite branching and elongation up to P14, followed by a phase of neurite elongation only up to P18 [18]. The elongation rates in these two phases, v_{be} and v_e, respectively, were allowed to differ in the model. Optimized growth parameters are given in Table 1. These results were obtained by additionally assuming a mean initial length of 5 μm for daughter segments after a branching event [21], and a decay constant of 10 days for the exponential baseline branching rate function [22]. Fig. 2 illustrates how well the distributions for the different dendritic shape parameters of model trees match those of the observed ones. Comparing the growth parameters between the control and ethanol group in Table 1, the largest difference is found in the branching parameter B, with the other parameters showing no or relatively less difference.

Our data suggest that the ethanol treatment in the early days of dendritic development especially decreases the baseline branching rate of the outgrowing neurites. The dependence of the branching rate on the segment position and on the total number of segments, as well as the elongation rates appear to be not or much less affected by the ethanol treatment. The observed simplification of dendritic growth is in substantial agreement with a previous report on basal dendrites of layer 3 neurons in developing rats exposed to ethanol [16]. However, other populations of cortical neurons could be differently affected by early ethanol exposure, as demonstrated by the increased dendritic complexity of layer 5 corticospinal neurons [13]. During neurite outgrowth, there is a reciprocal interplay between branching and elongation, which is mainly influenced by the relative concentrations of phosphorylated and dephosphorylated microtubule associated proteins (MAPs, see [19], for review). Notably, it has been demonstrated that ethanol is able to interfere with MAPs phosphorylation, possibly in a dose-dependent manner [2,17].

One of the branching parameters possibly affected by ethanol administration is the E parameter, accounting for competitive effects which occurs during neurite maturation (for instance the competition for trophic factors). It has been suggested that the dendritic growth of cortical pyramidal neurons is characterized by strong competitive conditions [19,20]. The higher value of the E parameter observed in ethanol treated animals is in good agreement

Table 1
Optimized values for branching and elongation parameters to match the model predicted distributions of the different shape data with the observed ones, as shown in Fig. 2

	Branching parameters			Elongation parameters	
	B	E	S	v_{be} (μm/h)	v_e (μm/h)
Control	5.9	0.79	0.60	0.21	0.52
Ethanol	5.1	0.83	0.60	0.19	0.53

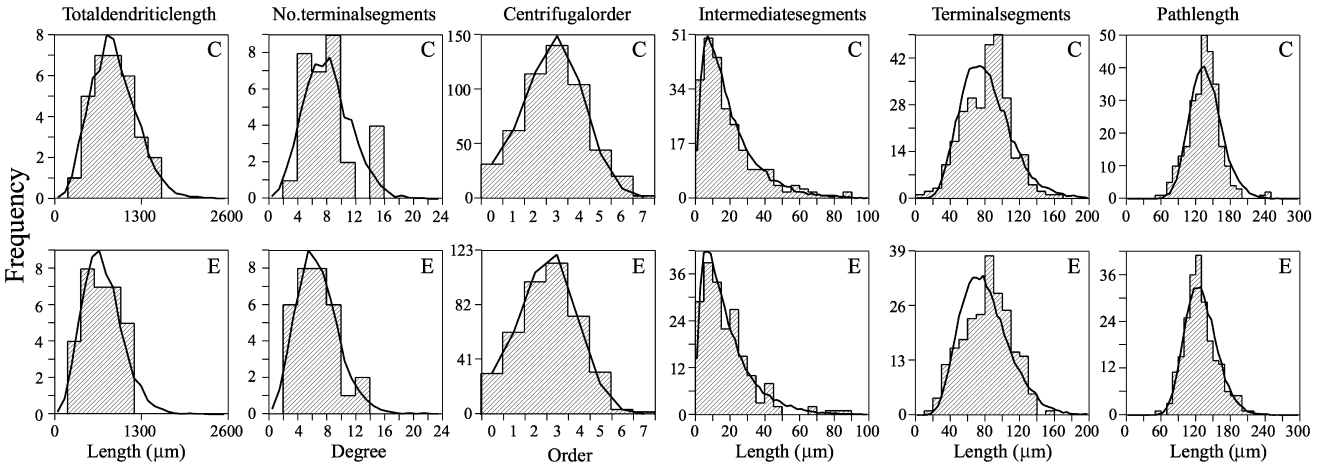

Fig. 2. Distributions of shape parameters of observed (shaded histograms) and modeled (continuous curves) dendritic trees. Dendritic trees have been analysed for their total dendritic length (1st column), number of terminal segments (2nd column), centrifugal order of the segments (3rd column), length of intermediate (4th column) and terminal segments (5th column), and for path length up to terminal tips (6th column). Results are obtained for dendritic trees under (C) control condition (1st row), and (E) ethanol treatment (2nd row). Note the correspondence between modeled and observed outcomes.

with the known effects of early alcohol exposure to reduce the production of neurotrophins [3,5]. An additional factor influencing the dendritic growth and whose availability might be reduced during FAS is represented by the afferent activity [10]. In fact, one of the consequences of experimental FAS is a marked reduction of the thalamic input onto layer four of the sensori-motor cortex [7].

It is not clear whether the reduced dendritic branching represents a constant feature of mental retardation (see [8,9,14], for review). The present findings indicate that the functional alterations observed in human FAS might be related to the dendritic simplification occurring in layer 2/3 associative neurons. Inferences obtained from the computational model could prompt further in vivo and in vitro studies as well as more detailed model assumptions to better clarify the dynamics of dendritic growth after exposure to ethanol.

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