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SHORT COMMUNICATION
Compensatory dendritic growth of CA1 pyramidal cells following growth impairment in the neonatal period

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Abstract

In the neonatal rat, inhibition of hippocampal neural activity *in vivo* by tetanus toxin results in a severe growth impairment of the basal dendrites of CA1 pyramidal cells. Here we tested whether this early growth impairment results in a permanent reduction of the basal dendritic tree or whether recovery processes are recruited later in development when synaptic activity has fully recovered. Quantitative analysis of dendritic parameters and spine density from reconstructed CA1 pyramidal cells showed that young adult CA1 pyramidal (postnatal day 31–34) cells that were exposed to activity deprivation in the neonatal period were almost indistinguishable from control cells of the same age. These results suggest that the early hippocampal activity controls the growth rate but is not necessary for the generation of an adult normal basal dendritic tree.

Introduction

It is well established that neural activity is necessary for appropriate structural and functional development of the nervous system (Shatz, 1990; Cline, 2001). From developmental studies in the visual and somatosensory systems, it seems also critical that proper neural activity occurs at critical periods to promote appropriate development. In the developing hippocampus the neural activity in the neonatal period is characterized by intrinsically generated network-driven discharges (Ben Ari *et al*., 1989; Leinekugel *et al*., 2002). This type of neural activity is largely restricted to the first postnatal week (Ben Ari *et al*., 1989) and it could be involved in promoting various forms of structural and functional maturation in the neonatal hippocampus. We have recently examined the importance of this neonatal hippocampal neural activity for the dendritic growth of CA1 pyramidal neurons. A local injection of tetanus toxin, which blocks release of synaptic vesicles by cleaving synaptobrevin (Schiavo *et al*., 2000; Rizo & Sudhof, 2002), was used to inhibit neural activity *in vivo* (Groc *et al*., 2002b). A single injection of tetanus toxin 1 day after birth [postnatal day (P)1] produced a strong reduction (80–50%) in glutamatergic and γ-aminobutyric acid (GABA)ergic synaptic activity throughout the first postnatal week (Groc *et al*., 2003). When examined at the end of the first postnatal week, this activity deprivation had resulted in a severe impairment of the basal dendritic growth of CA1 pyramidal cells (Groc *et al*., 2002b). Thus, the basal dendrites of these cells grow in an activity-dependent manner and the neural activity in the neonatal hippocampus has obviously an important function to promote this growth. However, whether this activity-dependent dendritic growth is tightly restricted to the first postnatal days, i.e. this period represents a critical period, remains an intriguing and open question. In the present study we have therefore investigated whether the growth impairment of the basal dendrites induced by blockade of neonatal neural activity leads to a smaller dendritic tree also at the young adult stage or whether the subsequent re-establishment of neural activity can initiate a compensatory growth, leading to a normally developed basal dendritic tree.

Materials and methods

Surgery and tetanus toxin efficiency

Surgical procedures associated with the injection of tetanus toxin were in accordance with the guidelines of the ethical committee for animal research in Göteborg. As described previously (Groc *et al*., 2002b), Wistar rats (Möllegaard, Denmark; $n = 7$ rats), 1 day after birth (P1) were anaesthetized by inhalation of isofluoran. The stereotaxic coordinates for injection were: anteroposterior, −1.4 mm;mediolateral, 2.0 mm; dorsoventral, 2.0 mm from the cortical surface (corresponding to the stratum oriens/pyramidale). Tetanus toxin (Alomone Laboratories, Israel) was dissolved in phosphate-buffered saline (PBS, 0.1 M, pH 7.4) and 5 ng of toxin was injected into the hippocampus using a fused silica needle (outer diameter 150 µm; Skandinavia Genetec AB). The incision was chemically sutured (Vet-Seal, B.Braun Medical).

Slice preparation

For preparation of slices, P31–34 rats ($n = 6$) were decapitated, the brain was removed and placed in ice-cold solution containing (in mM): 124 NaCl, 3.0 KCl, 2 $CaCl_2$, 6 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and

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10 glucose. Transverse hippocampal slices (300 μm) were cut using a vibrating tissue slicer (Campden Instruments). Slices were individually transferred to a recording chamber where they were perfused at 30–32 °C. The extracellular solution contained (in mM): NaCl, 124; KCl, 3.0; $CaCl_2$, 4; $MgCl_2$, 4; NaH_2PO_4, 1.25; $NaHCO_3$, 26; and glucose, 10. CA1 pyramidal cells were identified visually using IR-DIC videomicroscopy (Hamamatsu, Nikon) and whole-cell patch-clamp recordings were performed with an EPC-9 patch-clamp amplifier (HEKA, Lambrecht, Germany). Whole-cell recordings were performed as described previously (Groc *et al*., 2002a, 2002b). The pipette solution contained (in mM): Cs-gluconate, 120; NaCl, 2; QX-314, 5; EGTA, 10; HEPES, 10; and 0.5% neurobiotin (solution was 280–300 mosm, pH 7.4). The cell capacitance was estimated using the capacitance compensation procedure of the Pulse program (HEKA). As described previously (Groc *et al*., 2002a, 2002b), analysis of spontaneous postsynaptic currents was performed using Mini Analysis Program (Synaptosoft 5.1.4., Justin Lee, USA).

Morphology

The morphological analysis was performed blinded as described previously (Groc *et al*., 2002b). Briefly, the slices were fixed overnight at 4 °C in a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After fixation, slices were rinsed in phosphate buffer, cryoprotected overnight in sucrose and frozen. To neutralize endogenous peroxidase, slices were pretreated in a 1% H_2O_2 solution. After rinses (0.1 M PBS), slices were incubated for 24 h at 4 °C in 1 : 100 avidin-biotinylated peroxidase complex diluted in PBS containing 0.3% Triton X-100. After rinsing, slices were processed with 0.06% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.006% H_2O_2 diluted in PBS, rinsed, mounted on gelatine-coated slides and coverslipped in an aqueous medium. Only neurons that were morphologically identified as CA1 pyramidal cells were analysed. The three-dimensional reconstruction of dendritic and axonal tree of pyramidal neurons (40 ×), including a full spine count, were performed using Neurolucida 2000 software (Microbrightfield Inc.) and an automatic dendrite measuring system as described previously (Groc *et al*., 2002b). The cell body was drawn in the two-dimensional plane. The apical dendrite was drawn in the following sequence: main shaft of apical dendrite originating from cell soma, with their terminal ramification in stratum lacunosum moleculare was reconstructed first; then the apical dendrite side (oblique) branches originating from main shaft located in stratum radiatum were reconstructed. The following parameters were analysed (Uylings *et al*., 1989): (i) number of basal dendrites; (ii) total number of segments per neuron separately for basal and apical dendrite, indicating the branching frequency; (iii) total length per neuron separately for basal and apical dendrites, including the length of individual incomplete segments; (iv) mean length of individual intermediate and terminal segments, separately for basal and oblique branches of apical dendrite; (v) somatic cell surface, i.e. the area of the cell soma projected onto the plane of sectioning to indicate its size; and (vi), total spine number and density (presented as spine per μm, calculated as total spine number divided by total dendritic length) separately for basal and apical dendrites. Intermediate segments are the segments between the dendritic origin and the first bifurcation point, or between two consecutive bifurcation points. Terminal segments are segments between the terminal tip of dendrites and the last bifurcation point before the terminal tip.

Statistical analysis

Data are expressed as mean ± SEM, or mean ± SD if specified. Morphological parameters were averaged per neuron and compared statistically between control and tetanus toxin groups using an analysis of variance (ANOVA) followed by *post hoc* Newman–Keuls tests. The statistical analysis of cell capacitance was performed using Student's *t*-test.

Results

Dendritic morphology of adult CA1 pyramidal cells

We have shown previously that the injection of tetanus toxin at P1 produced a sustained inhibition of spontaneous synaptic activity during the first postnatal week (Groc *et al*., 2003), and that this injection strongly reduced the number of basal dendritic branches when examined at P6–10 in CA1 pyramidal cells (Groc *et al*., 2002b). To investigate whether the growth of the basal dendrites recover after this neonatal dendritic growth impairment, we compared the morphological features of P31–34 CA1 pyramidal cells from noninjected (control) and tetanus toxin-injected hippocampi. As previously shown (Groc *et al*., 2002b), noninjected hippocampi can reliably serve as control material as the surgical procedure *per se* does not affect the morphological development of CA1 pyramidal cells. At P31–34, both control and tetanus toxin exposed CA1 pyramidal cells exhibited well-developed apical and basal dendritic trees (Fig. 1A–D). As we previously found that tetanus toxin-exposed cells had many less primary basal dendrites when examined 1 week after injection of the toxin (P6–10), we specifically examined the number of basal dendrites originating directly from the soma. We found no significant difference in this parameter between the two groups (control, 3.8 ± 0.3 branches, $n = 6$; tetanus, 3.7 ± 0.2 branches, $n = 9$; $P > 0.05$), indicating compensatory growth of primary basal dendrites in tetanus toxin-exposed cells between P6–10 and P31–34. The total length of the basal dendritic tree, as well as the total number of basal dendritic branches, was also not significantly different between control and tetanus toxin-exposed cells (Table 1). It can be noted that the length of the terminal basal branches of neural activity deprived cells was found to be reduced significantly ($P = 0.04$; Table 1). Although this effect could be because of random fluctuation of the material, another possibility is that the increase in the number of basal newly generated branches in synaptic activity-deprived hippocampi happened, to some extent, at the expense of the extension of the branches (Wong & Ghosh, 2002). None of the other basal dendritic tree parameters were significantly different between control and tetanus toxin-exposed cells (Fig. 2, Table 1). Thus, these results indicate a complete recovery of the basal dendritic tree of cells that were exposed neonatally to tetanus toxin (Fig. 2). We found no difference in apical dendritic tree and its branch parameters (Table 1), consistent with the findings in P6–10 rats (Groc *et al*., 2002b). The spine number or spine density in all part of the dendritic tree (basal and apical) did not differ significantly between the two groups (Fig. 1, Table 1). It is noteworthy that the spine density observed in our staining condition (∼0.35 spine/μm, biocytin staining in light microscopy) is similar to some results obtained in similar staining conditions (Jiang *et al*., 1998), but lower when compared with those obtained using different staining protocols, such as electronic microscopy analysis (> 1 spine/μm; see Megias *et al*., 2001).

Consistent with the morphological analysis, the cell capacitance values from the reconstructed and nonreconstructed cells indicated no change between control and tetanus toxin-exposed cells (control, 37 ± 2 pF, $n = 8$; tetanus, 39 ± 3 pF, $n = 12$; $P > 0.05$). This can be compared to the 25% smaller cell capacitance of toxin exposed cells at P6–10 (Groc *et al*., 2002b). Finally, the frequency and the amplitude of the spontaneous excitatory postsynaptic currents from control (frequency, 7.2 ± 0.9 Hz; amplitude, 28.3 ± 1.9 pA, $n = 5$) and tetanus toxin-exposed (frequency, 6.3 ± 0.7 Hz; amplitude, 31.8 ± 1.6 pA,

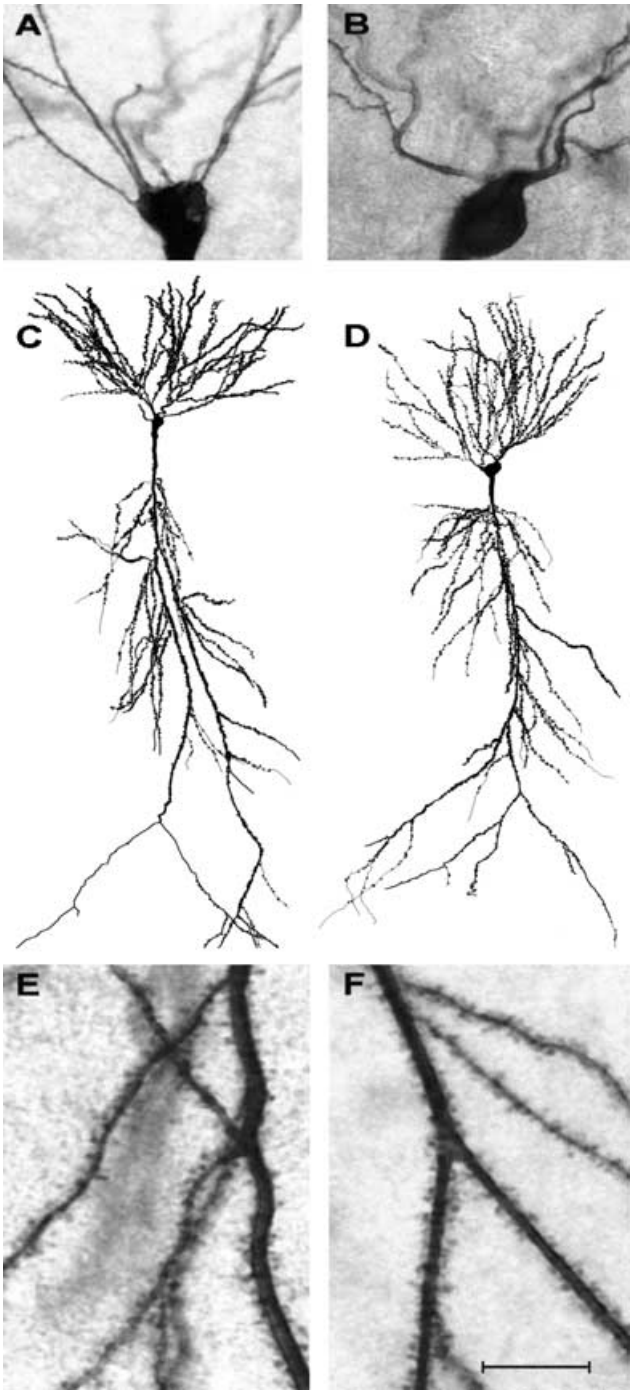

FIG. 1. Photomicrograph of young adult control (left panel) and tetanus toxin-exposed (right panel) CA1 pyramidal cells. (A and B) Enlarged primary basal dendrites that originated directly from the soma. Note that the branch number is similar between the control (A) and tetanus toxin-exposed (B) cells. (C and D) Camera lucida reconstruction of these cells show the overall basal (upper) and apical (lower) dendritic tree. The spines are indicated by small dots along dendrites. (E and F) Enlarged apical dendrite exhibiting numerous spines. Scale bars, 25 μm (A and B); 100 μm (C and D); 50 μm (E and F).

$n = 6$) cells were not significantly different, indicating that the synaptic activity fully recovered at the adult stage (see below).

Estimate of the synaptic and dendritic recovery

As shown previously (Groc *et al.*, 2002b, 2003), the injection of tetanus toxin at P1 produced a maximal inhibition of spontaneous synaptic activity of about 80% at P2–3 and at P6–10 this inhibition had decreased to about 50% of control. To obtain an indication of the relative rate of recovery of synaptic transmission and of basal dendritic morphology, we also examined control ($n = 2$) and tetanus toxin-exposed ($n = 2$) cells at P15. The frequency of GABAergic spontaneous postsynaptic currents were about the same in control (mean ± SD; 2.6 ± 0.5 Hz, $n = 2$) and tetanus toxin-exposed (2.1 ± 0.6 Hz, $n = 2$) cells indicating that the tetanus toxin-induced synaptic activity blockade had subsided at this age. However, the total length of the basal dendritic tree of the tetanus toxin-exposed cells was still only 26% of that of the controls (mean ± SD; control, 2663 ± 117 μm; tetanus, 682 ± 330 μm), because of a reduction in the number of basal dendritic branches (mean ± SD; control, 63 ± 3; tetanus, 14 ± 6). Thus, the relative growth impairment observed at P15 is of the same order as the one at P6–10 cells (33%; Table 1 from Groc *et al.*, 2002b), indicating that the morphological recovery is initiated well after the full recovery of synaptic transmission.

TABLE 1. Morphometric values from neurobiotin-stained CA1 pyramidal cells (P31–34)

	Control	Tetanus
Reconstructed cells (n)	6	9
Soma size (μm^2)	161 ± 12	177 ± 13
Basal dendrite		
Total length (μm)	3577 ± 210	3248 ± 242
Total branch number	61 ± 4	63 ± 3
Spine number	1220 ± 127	1103 ± 203
Spine density (spine/μm)	0.34 ± 0.03	0.33 ± 0.05
Terminal segment length (μm)	89 ± 4	77 ± 3*
Intermediate segment length (μm)	26 ± 2	23 ± 2
Apical dendrite		
Total length (μm)	5494 ± 201	4766 ± 337
Branch number	70 ± 7	61 ± 6
Spine number	1766 ± 201	1705 ± 234
Spine density (spine/μm)	0.32 ± 0.04	0.35 ± 0.04
Terminal segment length (μm)§	93 ± 5	91 ± 5
Intermediate segment length (μm)§	22 ± 3	28 ± 3

Mean values ± SEM. *$P = 0.04$ when compared with control values, ANOVA followed by Newman-Keuls test. §From oblique side branches.

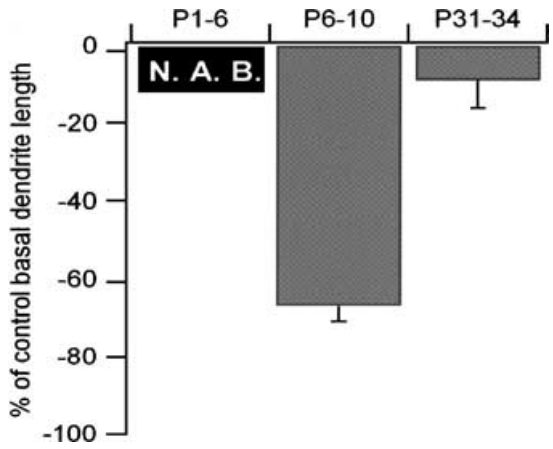

FIG. 2. Developmental profile of the tetanus toxin-induced basal dendritic growth impairment. After neural activity blockade during the first postnatal days using a local injection of tetanus toxin in the CA1 area, the basal dendritic growth of CA1 pyramidal cells is massively blocked at P6–10 (−67% from Groc *et al.*, 2002b). At P31–34, the basal dendritic tree is not significantly different (−9%).

Discussion

During the first postnatal week, the hippocampus is characterized by an oscillating network activity (Leinekugel *et al*., 2002) and the initiation of the basal dendrite growth in CA1 pyramidal cells (Minkwitz, 1976; Pokorny & Yamamoto, 1981). We have previously shown that an inhibition of this network activity during the first postnatal week (Groc *et al*., 2003) resulted in a severely impaired growth of the basal dendrites (Groc *et al*., 2002b). In the present study we show that this neonatal activity deprivation did not lead to a truncated basal dendritic tree in the young adult (P31–34) rat. Thus, the basal dendrites of adult CA1 pyramidal cells from previously activity-deprived hippocampi did not differ in any basal dendritic tree parameter (such as number of primary branches, total branch number and total dendritic length) from age-matched controls. This result indicates that the first postnatal week, with its characteristic oscillating network activity, does not represent a critical period for the growth of the basal dendrites in the sense that growth impairments during this period can be repaired by a compensatory growth later in development.

The present study is the first attempt to tackle the question of whether and how the dendritic growth of hippocampal pyramidal cells is controlled by synaptic activities during a developmental critical period, i.e. the first postnatal week. However, many studies, mainly in the visual system, have described that growth and/or remodelling of dendrites mostly takes place during critical periods of functional network refinements (Trune, 1982; Kalb, 1994; Schoop *et al*., 1997; Antonini *et al*., 1998; Deplano *et al*., 1999; Stern *et al*., 2001; Mataga *et al*., 2002). Although such correlations have suggested a direct relationship between dendritic growth/remodelling and functional network maturation other studies have shown clear exceptions. For example, despite a massive disruption of the functional maturation of visual system networks (e.g. axonal segregation) following activity-deprivation during critical periods (Wong *et al*., 1991; Dalva *et al*., 1994), dendritic growth proceeded normally under these conditions. This example also indicates that the present results have to be interpreted with caution, i.e. that the recovery of quantitative dendritic parameters not necessarily implies a full functional recovery.

In experiments in which tetanus toxin was injected into the hippocampus at P10 (Jiang *et al*., 1998), no change in the overall morphology of the adult dendritic tree (except a slight effect on the basal dendrites) was reported. However, the number and shape of spines on these young adult pyramidal cells were altered (Jiang *et al*., 1998). This result was not observed following our P1 injections of tetanus toxin, the density of spines being the same in adult cells from control and tetanus toxin-exposed hippocampi (whether the spine morphology was affected remains, however, unknown as our staining method was inappropriate to precisely and quantitatively track morphological changes in spines). An explanation for the difference in spine density could then be that spine development on CA1 pyramidal cells is specifically sensitive to activity-deprivation in the second postnatal week. Alternatively, the spine changes observed after the late synaptic activity blockade (P10) could be secondary to the recurrent epileptic activity that was observed during the recovery from the activity blockade under those conditions.

In conclusion, the growth impairment of the basal dendritic tree of CA1 pyramidal cells given by activity deprivation in the neonatal period can be compensated for by postponing the period of rapid growth to a later time (3rd−4th postnatal weeks). From this study and a previous one (Groc *et al*., 2002b), we propose that the neural activity observed in the neonatal hippocampus is important for the basal dendritic growth of CA1 pyramidal cells, but is not indispensable for the generation of an adult normal basal dendritic tree.

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Abbreviations

P, postnatal day; PBS, phosphate-buffered saline.

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