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In vivo blockade of neural activity alters dendritic development of neonatal CA1 pyramidal cells

Laurent Groc,[1†] Zdravko Petanjek,[2,3†] Bengt Gustafsson,[1] Yehezkel Ben-Ari,[2] Eric Hanse[1] and Roustem Khazipov[2]
[1]Institute of Physiology and Pharmacology, Department of Physiology, Göteborg University, Göteborg, Sweden
[2]INMED/INSERM U29, 13273 Marseille Cédex 09, France
[3]Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia

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Abstract

During development, neural activity has been proposed to promote neuronal growth. During the first postnatal week, the hippocampus is characterized by an oscillating neural network activity and a rapid neuronal growth. In the present study we tested *in vivo,* by injecting tetanus toxin into the hippocampus of P1 rats, whether this neural activity indeed promotes growth of pyramidal cells. We have previously shown that tetanus toxin injection leads to a strong reduction in the frequency of spontaneous GABA and glutamatergic synaptic currents, and to a complete blockade of the early neural network activity during the first postnatal week. Morphology of neurobiotin-filled CA1 pyramidal cells was analyzed at the end of the first postnatal week (P6–10). In activity-reduced neurons, the total length of basal dendritic tree was three times less than control. The number, but not the length, of basal dendritic branches was affected. The growth impairment was restricted to the basal dendrites. The apical dendrite, the axons, or the soma grew normally during activity deprivation. Thus, the *in vivo* neural activity in the neonate hippocampus seems to promote neuronal growth by initiating novel branches.

Introduction

In the rat hippocampus, CA1 pyramidal cells are generated before birth, at the embryonic days E16–20 (Bayer, 1980a; Bayer, 1980b), and they progressively grow during the first postnatal month (Minkwitz, 1976; Pokorny & Yamamoto, 1981). The first postnatal week is the period of the most intensive growth of the pyramidal cells. During this period, the apical and basal dendrites of the pyramidal cells ramify and grow rapidly, expanding through the layers of stratum radiatum and stratum oriens, respectively (Pokorny & Yamamoto, 1981; Steward & Falk, 1991). Axons of CA1 pyramidal cells also display an intensive growth during this period, with a formation of a coarse local axonal arbor (Aniksztejn *et al*., 2001). Already at birth, CA1 pyramidal cells start to receive GABAergic and glutamatergic synaptic inputs (Tyzio *et al*., 1999). During the first postnatal week, the activity of CA1 pyramidal cells *in vivo* are synchronized in spontaneous network-driven discharges (Leinekugel *et al*., 2002). This neural activity is also observed in the hippocampal slice and intact hippocampus *in vitro* preparation as giant depolarizing potentials (Ben Ari *et al*., 1989; Khalilov *et al*., 1997). This early synaptically driven neural activity produces synchronous oscillations of intracellular calcium (Leinekugel *et al*., 1997; Garaschuk *et al*., 1998), providing conditions for a number of developmental processes including neuronal growth (Shatz, 1996; Ben-Ari, 2001).

One major difficulty in investigating the involvement of neural activity in neuronal growth has been the lack of an effective method to produce long-term block of *in vivo* neural activity in the neonate hippocampus. Thus, conventional pharmacological procedures would require a continuous infusion that is not feasible in a rapidly growing head. Genetic approaches to block synaptic transmission have resulted in premature death (Verhage *et al*., 2000; Varoqueaux *et al*., 2002), precluding analysis of postnatal *in vivo* development.

A possible approach for producing a prolonged reduction of neural activity is to block synaptic transmission using one of the clostridial toxins, such as tetanus toxin (Schiavo *et al*., 2000). This toxin, which specifically cleaves the vesicle-associated membrane protein (VAMP)/synaptobrevin 2 that is necessary for presynaptic vesicle exocytosis (Schiavo *et al*., 1992), blocks both spontaneous (TTX-sensitive) and miniature (TTX-insensitive) excitatory postsynaptic currents in hippocampal cell culture (Capogna *et al*., 1997; Renger *et al*., 2001). We have found, using whole cell recordings of acutely prepared slices, that a single injection of tetanus toxin into the hippocampus at postnatal day 1 (P1) *in vivo* strongly reduces (down to 20% of control) the frequency of glutamatergic and GABAergic synaptic events in CA1 pyramidal cells for several days (Groc, Gustafsson, Hanse, unpublished, Groc *et al*., 2001). This strong reduction of synaptic activity together with the fact that the endogenous network activity is totally disrupted by fairly modest interventions (Ben Ari *et al*., 1989; Garaschuk *et al*., 1998) suggest that such tetanus toxin injection will effectively block the early postnatal neural network activity *in vivo*. Slices from tetanus-injected hippocampi do also lack any sign of such network activity (Groc, Gustafsson, Hanse, unpublished, Groc *et al*., 2001). In the present study, we have used this experimental model to test the involvement of neural activity in the morphological development of CA1 pyramidal cells.

Correspondence: Dr Laurent Groc, as above.
E-mail: laurent.groc@physiol.gu.se

†L.G. and Z.P. contributed equally to this work

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Materials and methods

Surgery procedure

Surgical procedures were in accordance with the guidelines of the ethical committee for animal research in Göteborg. Wistar rat pups (Möllegaard, Denmark) (n = 8 rats), one day after birth (P1) were anaesthetized by inhalation of isofluoran. Pup heads were placed in a surgical mask to maintain the skull stable. A constant flux of a mixture of isofluoran/air was constantly applied inside the surgical mask during the surgical procedure. Body temperature was maintained constant using a heat generator placed beneath the animal. A midline incision was made on the head and a hole was drilled in the skull (outer diameter, 0.4 mm). The stereotaxic coordinates for injection were: anteroposterior, –1.4 mm; mediolateral, +2.0 mm; dorsoventral, –2.0 mm from the cortical surface (correspond to the stratum oriens/pyramidale). In some experiments (n = 2 rats), the toxin was injected –2.4 mm from the cortical surface (correspond to the stratum radiatum) (Fig. 1). Tetanus toxin (Alomone Laboratories, Israel) was dissolved in phosphate buffered saline (PBS, 0.1 M, pH 7.4) to a final concentration of 10 µg/mL 5 ng of tetanus toxin was injected into the hippocampus using a fused silica needle (outer diameter, 150 µm, Skandinavia Genetec AB). After injection, the needle was left *in situ* for at least 3 min to reduce reflux up the needle. The incision was chemically sutured (Vet-Seal, B.Braun Medical) and pups were allowed to fully recover before being returned to their littermates (< 10 min).

Slice preparation

P6–10 rats (n = 8) were decapitated, the brain was removed and placed in ice-cold solution composed of (in mM): 124 NaCl, 3.0 KCl, 2 $CaCl_2$, 6 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose. Transverse hippocampal slices (300 µm) were cut using a vibrating tissue slicer (Campden Instruments) and stored at 28 °C, for at least 30 min. For recording, slices were individually transferred to a recording chamber where they were perfused at 30–32 °C. Slices from tetanus toxin-injected hippocampi were located ± 900 µm from the injection site. Slices from control hippocampi were selected following the same procedure. The extracellular solution contained (in mM): 124 NaCl, 3.0 KCl, 4 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose.

Patch-clamp recordings

CA1 pyramidal cells were visually identified 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 previously described (Groc *et al.*, 2002). The pipette solution contained (in mM): 120 Cs-gluconate, 20 TEA-hydroxide, 2 NaCl, 5 QX-314, 4 Mg-ATP, 0.4 Na-GTP, 10 EGTA, 10 Hepes, and 0.5% neurobiotin (solution was 280–300 mosm with a pH of 7.4). The analysis of spontaneous PSCs (sPSCs) was performed using Mini Analysis Program (Synaptosoft 5.1.4., Justin Lee, USA). The cell capacitance was estimated using the capacitance compensation procedure of the Pulse program (HEKA, Lambrecht, Germany).

Morphology

The morphological analysis was performed blindly such that the analyzer was not aware of which slices had been taken from control or tetanus-exposed hippocampi. Only one cell (n = 24) was reconstructed per hippocampal slice. The slices were fixed overnight at 4 °C in a solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After fixation, slices were rinsed in PB, cryoprotected in sucrose for 16 h, and quickly frozen on dry ice. The detection of neurobiotin-filled neurons was performed on unsectioned slices. To neutralize an endogenous peroxidase, slices were pretreated for 30 min in 1% H_2O_2. After several rinses in PBS (0.1 M, pH 7.4), 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 30 min rinses in PBS, slices were processed with 0.06% 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St-Louis, MO) and 0.006% H_2O_2 diluted in PBS, rinsed, mounted on gelatine-coated slides and coverslipped in an aqueous medium (Crystal/Mount, Biomeda, Foster City, CA). We analyzed only P6–10 neurons that were morphologically identified as CA1 pyramidal cells. They were reconstructed for morphometric analysis using the Neurolucida 2000 software (Microbrightfield Inc., Colchester, VT) and automatic dendrite measuring system. This system consists of a three-dimensional motorized stage (MAC 2000, Ludl Electronic Products Ltd) placed on a Nikon Optiphot-2 microscope with an adjusted video camera (DEI-470, Optronics Engineering). In Neurolucida, neurons were drawn over the live

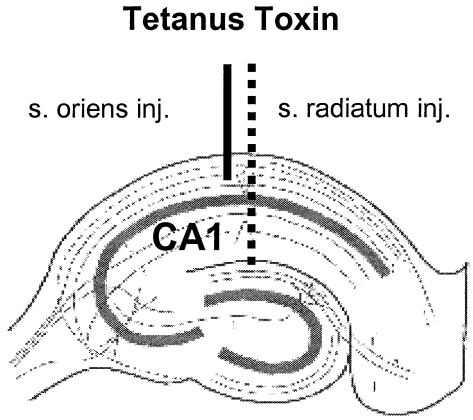

FIG. 1. Schematic diagram of the injection of tetanus toxin within the hippocampus. Tetanus toxin was injected at P1 in either the stratum oriens (solid line) or in the stratum radiatum (dashed line) part of the CA1 area.

TABLE 1. Morphometric values from neurobiotin-stained CA1 pyramidal cells (P6–10)

	Control	Tetanus
Reconstructed cells (n)	12	12
Cell capacitance (pF)	26.5 ± 2.5	16.8 ± 1.1[a]
Soma size (µm^2)	200 ± 22	169 ± 16
Axon length		
Total length (µm)	2308 ± 313	2030 ± 185
Segment number	28 ± 4	25 ± 5
Segment length (µm)	89 ± 9	86 ± 9
Varicosity number	213 ± 35	225 ± 25
Inter-varicosity distance (µm)	11 ± 1	9 ± 0.5
Basal dendrite		
Total length (µm)	798 ± 81	267 ± 34[a]
Segment number	30 ± 2	10 ± 2[a]
Segment length (µm)	26 ± 2	27 ± 2
Apical dendrite		
Total length (µm)	2075 ± 209	1849 ± 243
Segment number	52 ± 5	52 ± 7
Segment length (µm)	41 ± 3	37 ± 3

Mean values ± SEM.
[a]$P < 0.001$, compared with control values, t-test.

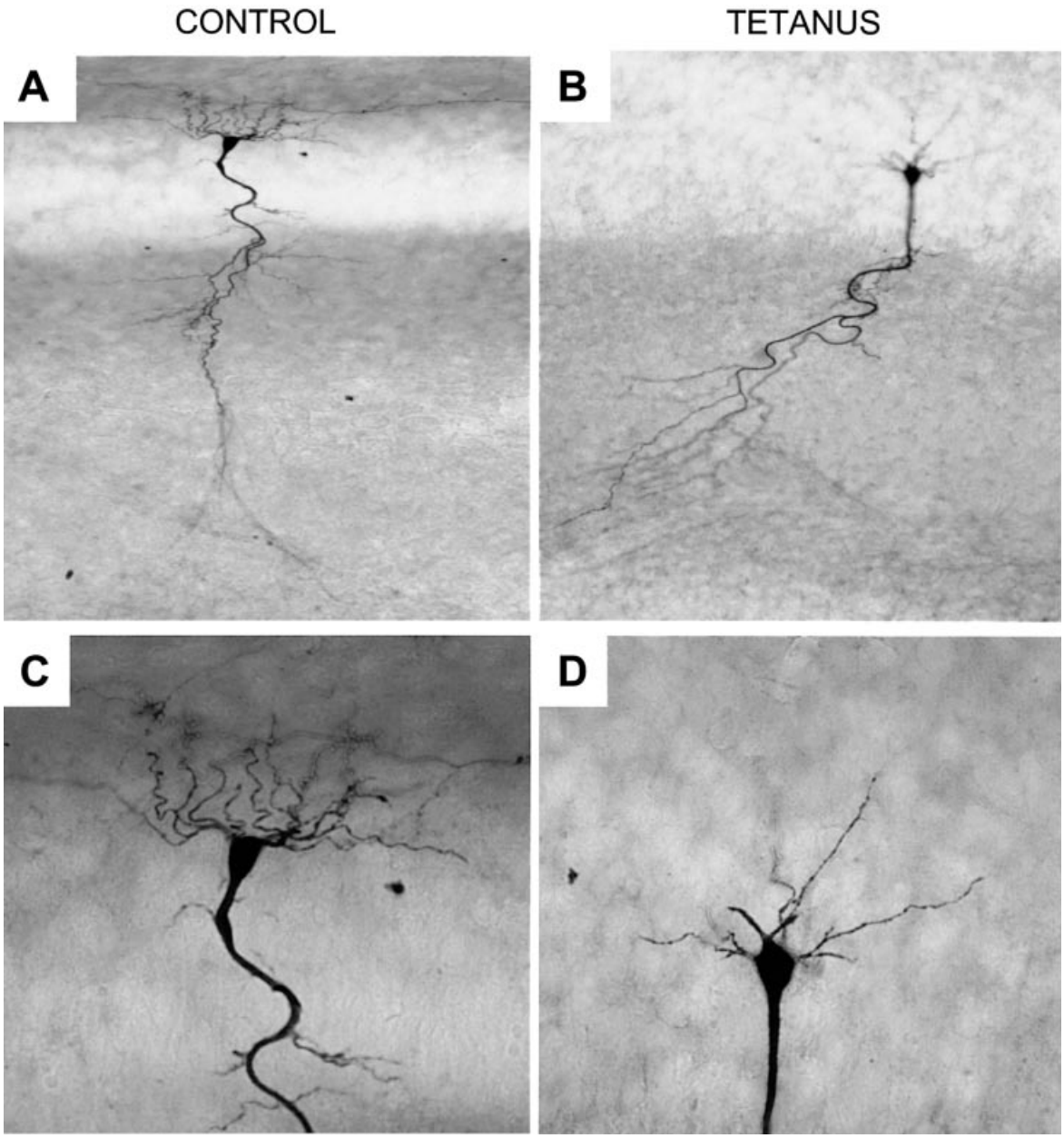

FIG. 2. Morphological features of control and tetanus-exposed CA1 pyramidal neurons. (A and B) Overview of a control (A) and a tetanus-exposed (B) neuron (P8). The apical dendrite in both conditions reached stratum lacunosum moleculare. (C and D) The basal dendritic tree of the control cell (C) is well developed compared to that of the tetanus-exposed (D) cell.

picture, bringing the signed point in the sharp focus when drawing. Changes in depth (z-dimension) were identified for each drawn point. Each point was also given x, y coordinates in relation to a reference point. The interface between the motorized stage and the PC is a stage controller (MAC 2000, Ludl Electronic Products Ltd), which is responsible for the synchronous automatic movement of the drawing. We then obtained three-dimensional data of the dendritic tree.

For each neuron, the dendritic tree was quantified as the total number of dendritic segments and the length of each such segment. To establish possible differences in the dendritic branching pattern, Sholl analysis was carried out for the apical dendritic tree using Neurolucida software (Sholl, 1953). The number of intersections of all dendritic branches within concentric circles (spacing of 50 μm around the cell body) was counted. The soma surface size was calculated by projecting its area on a two-dimensional plan (x–y). The axonal tree observed within the slice was used to calculate axonal features including total length and segment number. As the hippocampal slice does not contain the whole CA1 pyramidal cell axonal tree, it should be noted that the total axon length represented only a partial value of the whole axonal tree. However, this value was used to compare axonal trees between control and tetanus toxin-exposed hippocampi. Axonal varicosities were indicated, and the total number of varicosities as well as the intervaricosity distance was calculated.

Data are expressed as mean ± SEM. The statistical analysis was performed using Student t-test or ANOVA test, as appropriate.

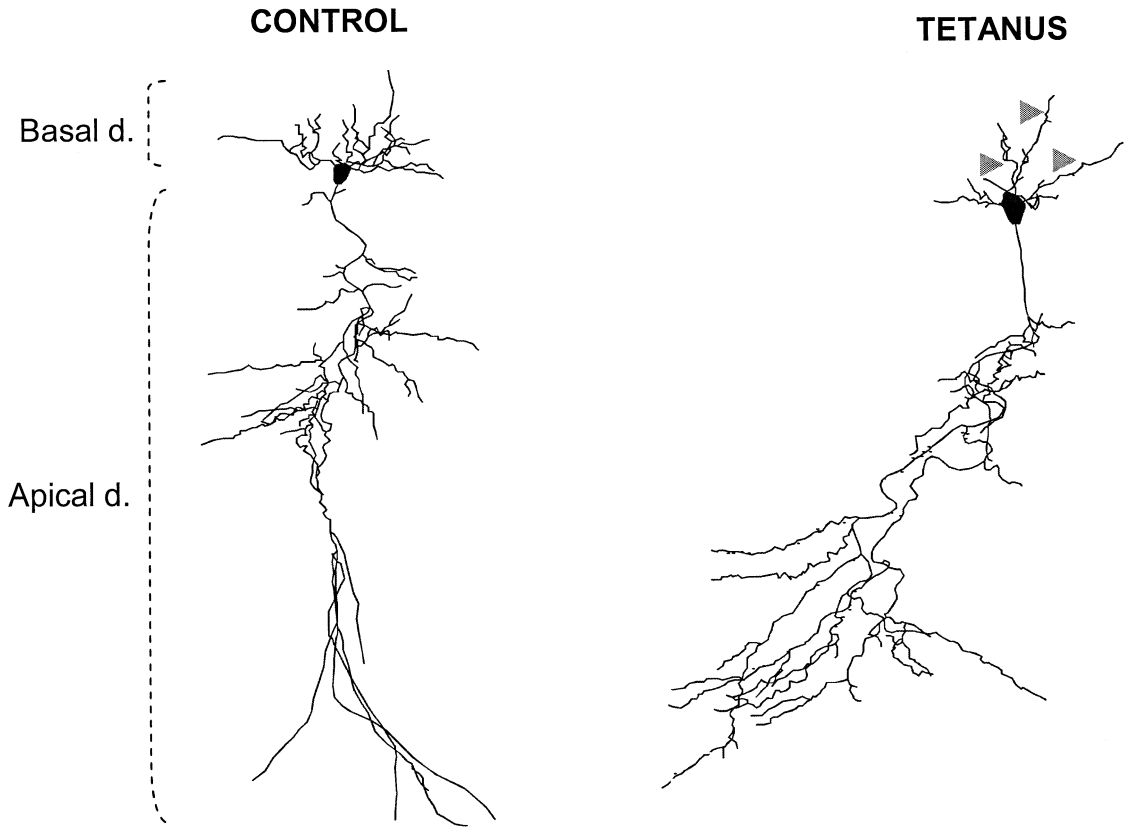

FIG. 3. Camera lucida reconstruction of the control (left) and tetanus-exposed (right) CA1 pyramidal cells shown in Fig. 2. The basal dendritic tree of the tetanus-exposed cell is less developed due to a reduction in the number of segments. Basal dendritic segments (arrow heads) were dramatically reduced in their relative number whereas their length was not affected.

Results

The surgical procedure does not affect the morphological development of CA1 pyramidal cells

In the first series of experiments, we studied whether the surgical procedure *per se* affects the development of CA1 pyramidal cells. Thus, morphological parameters of CA1 pyramidal cells from contralateral noninjected (n = 6 rats, P6–10, n = 7 cells) and saline-injected hippocampi (n = 2 rats, P7–8, n = 5 cells) were compared. There was no statistical difference in any of the analyzed parameters of CA1 pyramidal cells (cf. Table 1). For example, the basal dendritic total length (834 ± 169 μm (saline) vs. 769 ± 82 μm (noninjected), $P > 0.05$) or the apical dendritic total length (2160 ± 246 μm (saline) vs. 1990 ± 343 μm (noninjected), $P > 0.05$) was not different in saline or noninjected hippocampi. These results suggest that the surgical procedure *per se* does not affect the morphological development of CA1 pyramidal neurons. CA1 pyramidal cells from saline-injected and noninjected hippocampi were therefore pooled together as control material (n = 12 cells, Table 1).

Morphology of control CA1 pyramidal cells (P6–10)

At P6–10, control CA1 pyramidal cells exhibited relatively well-developed apical and basal dendritic trees (Figs 2A and C, and 3). The apical dendritic tree consisted of a ramified trunk that reached the stratum lacunosum moleculare (Figs 2A and 3). The basal dendritic tree was also ramified but to a less extent when compared to the apical one (Figs 2C and 3, Table 1). Thus, our observations are consistent with previous reports suggesting that the apical dendritic tree of CA1 pyramidal cell develops earlier than the basal dendritic tree (Pokorny & Yamamoto, 1981; Gaiarsa *et al*., 1992; Tyzio *et al*., 1999; Khazipov *et al*., 2001; Lopez-Gallardo & Prada, 2001). The axons arborized mainly within stratum oriens, with some neurons exhibiting a few axonal branches within the stratum pyramidale (Fig. 5). It should be noted that, at this age, no recurrent collateral axons within stratum radiatum was observed (Anikstejn *et al*., 2001). The axonal intervaricosity distance (approximately 10 μm, see Table 1) is consistent with previous reports (Gomez-Di Cesare *et al*., 1997; Jiang *et al*., 1998).

Blockade of hippocampal neural activity alters the dendritic development of CA1 pyramidal cells

Injection of tetanus toxin in the CA1 area at P1 causes a large reduction in the frequency of spontaneous synaptic transmission that is most prominent on days 2–3 (to less than 20% of control) and subsiding on days 6–8 (to approximately 50% of control), as well as a blockade of the endogenous synchronous neural activity (Groc, Gustafsson, Hanse, unpublished, Groc *et al*., 2001). In the present study, spontaneous synaptic transmission was only examined at P6–10, i.e. at the time of neurobiotin staining, demonstrating the same extent of synaptic activity blockade as was previously found at this age (42% and 65% of control for AMPA and $GABA_A$ PSC frequency, respectively; control values from 14 cells, tetanus toxin values from 20 cells).

Morphological analysis revealed a growth impairment following neural activity blockade that was restricted to the basal dendritic tree. Thus, the total length of the basal dendritic tree was reduced to

approximately one-third of that of control neurons (267 ± 34 μm vs. 769 ± 82 μm, $P < 0.001$) (Table 1; Figs 2 and 3). This reduction of the basal dendritic tree was due to a reduction of the segment number (10 ± 2 vs. 32 ± 2 $P < 0.001$), whereas the mean segment length was unaffected (Table 1). On the other hand, neither the apical dendritic tree nor the soma size was affected (Table 1). This differential effect was not dependent on the tetanus toxin injection site (stratum oriens vs. stratum radiatum) (see Fig. 1). The decrease of the total length of tetanus-exposed basal dendrite was similar after stratum oriens (269 ± 48 μm, $n = 8$) and stratum radiatum (242 ± 42 μm, $n = 4$) injections ($P > 0.05$). Similarly, the total length of the apical dendrite did not differ significantly when the injection was made in the stratum oriens (1670 ± 308 μm, $n = 8$) or in the stratum radiatum (2100 ± 293 μm, $n = 4$) ($P > 0.05$).

Some of the tetanus toxin-exposed cells displayed changes in their dendritic tree orientation (Fig. 5) (see also, Paul & Scheibel, 1986). In order to quantify the apical dendritic tree orientation, we performed a Sholl analysis on a subset of randomly selected control and tetanus toxin-exposed cells. We found no difference between control ($n = 6$) and tetanus-exposed ($n = 10$) apical dendritic arborizations ($P > 0.05$, ANOVA) (Fig. 4). Although total axonal length (see material and methods) tended to be shorter in tetanus toxin-injected hippocampi, there was no statistically significant difference in any of the parameters of axonal morphology (total length, number of varicosities and intervaricosity distance) (Table 1).

As can be calculated from Table 1, the total dendritic length of tetanus toxin-exposed cells was on average 74% of control. This reduction corresponds well to the previously observed decrease in cell capacitance (Groc, Gustafsson, Hanse, unpublished, Groc *et al*., 2001). In the present study, the cell capacitance values from reconstructed cells indicated a reduction to 65% of the control value (Table 1).

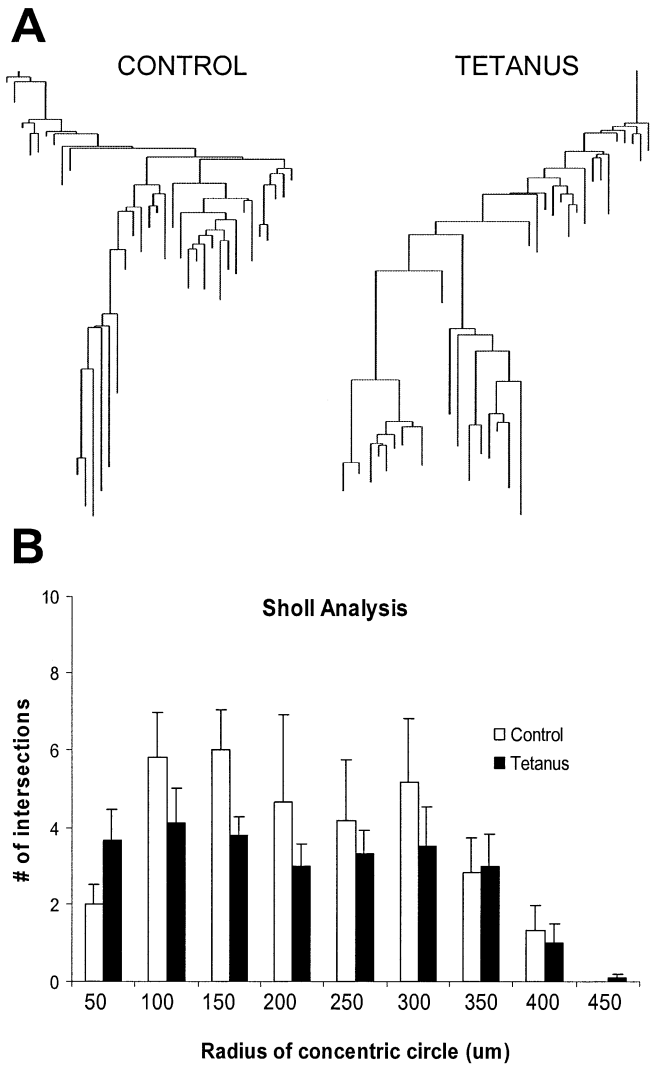

FIG. 4. Analysis of the apical dendritic morphology from control (left) and from tetanus toxin-exposed (right) CA1 pyramidal neurons. (A) Dendrogram of control and tetanus-exposed apical dendrites (same cells as shown in Figs 2 and 3). Note that there is no difference in the dendritic length between control and tetanus toxin-exposed cells. (B) Sholl analysis of apical dendrites from control and tetanus toxin-exposed CA1 pyramidal cells (P6–10). There was no significant difference between control ($n = 6$) and tetanus toxin-exposed ($n = 10$) apical dendritic arborizations ($P > 0.05$, ANOVA).

Discussion

Neural activity in the early postnatal period has been proposed to be an important factor in the control of neuronal growth (Cline, 2001). During the first postnatal week, the hippocampus is characterized by an oscillating network activity (Ben Ari *et al*., 1989; Garaschuk *et al*., 1998) and a rapid neuronal growth (Minkwitz, 1976; Pokorny & Yamamoto, 1981). In the present study, we tested *in vivo,* by injecting tetanus toxin into the hippocampus of P1 rats, whether this neural activity indeed promotes the neuronal growth of pyramidal cells. We found this tetanus toxin-induced neural activity deprivation to induce a strong impairment in the dendritic growth of CA1 pyramidal cells. This impairment was related to a reduction in the number, but not the mean length, of the dendritic branches. Surprisingly, this effect was restricted to the basal dendritic tree, which was reduced to one-third of the control value, whereas the apical dendritic tree, soma, and axons were unaffected. This result suggest that *in vivo* neural activity promotes dendritic branching (see also Li *et al*., 2002). However, our result also suggests that this role of neural activity can be either temporally or spatially delimited.

It should be noted that the above conclusions are based on the presumption that tetanus toxin produces no other effect than inhibiting neural activity by blocking synaptic transmitter release. This transmitter release may not exclusively of synaptic origin, but may also come from glial cells (Araque *et al*., 2000). Both neurite outgrowth and synaptogenesis depend on vesicle exocytosis and may thus be conceived to be additional targets for tetanus toxin. However, vesicle exocytosis involved in these processes relies on a tetanus toxin-insensitive form of synaptobrevin, VAMP-7 (Coco *et al*., 1999; Verderio *et al*., 1999; Martinez-Arca *et al*., 2001).

Although several previous studies have investigated the relationship between neural activity and dendritic growth of hippocampal neurons, they have used preparations of either cultured neurons or of organotypic slices, and neural activity has been blocked using either receptor antagonists or TTX (reviewed in McAllister, 2000). When blocking glutamatergic transmission in cultured hippocampal neurons using *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists, there was a growth impairment related to dendritic segment length reduction while branching was unaffected (Nuijtinck *et al*., 1997). On the other hand, NMDA receptor blockade in organotypic slice cultures led to a more complex dendritic arborization of CA1 pyramidal cells (Luthi *et al*., 2001). Other studies did not reveal any significant change in the dendritic growth following TTX or glutamate receptor antagonist treatments (Kossel

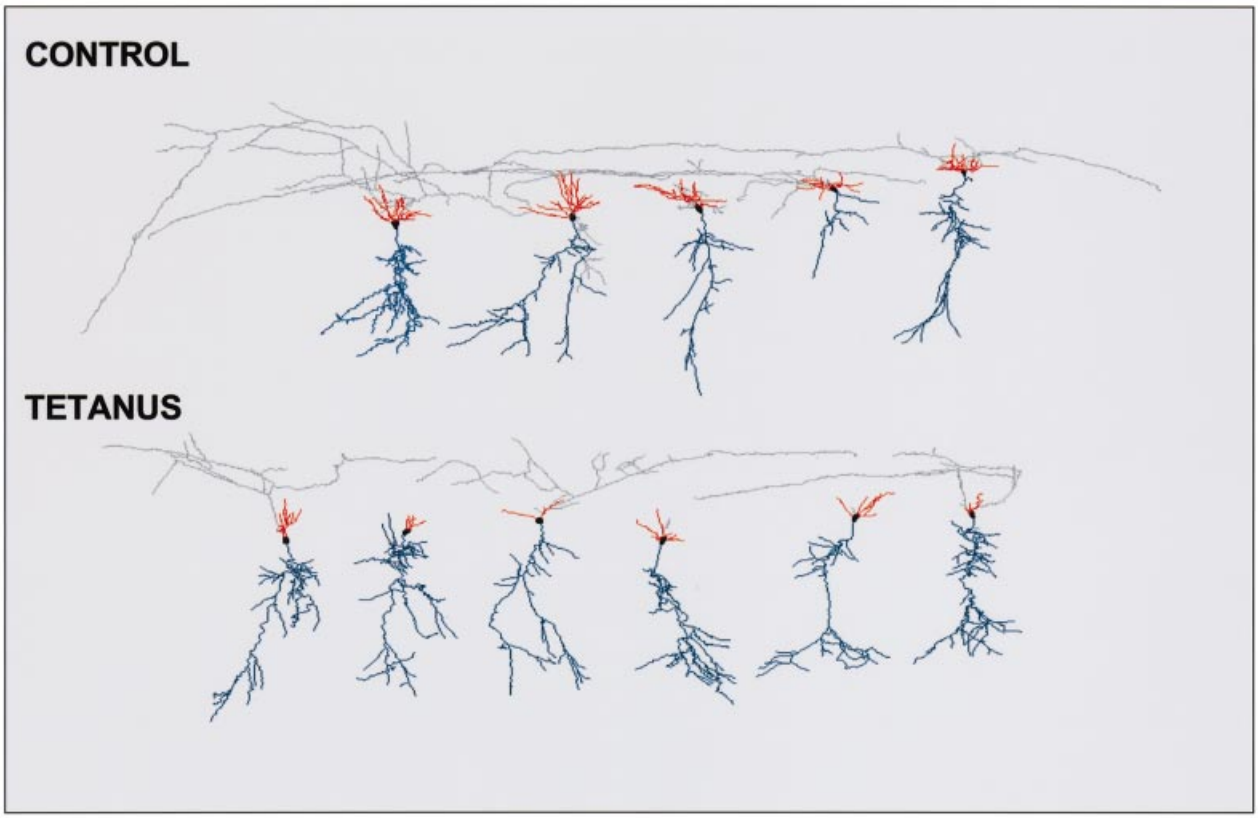

FIG. 5. Camera lucida reconstruction of control (five cells, top) and tetanus-exposed (six cells, bottom) CA1 pyramidal cells (P6–10). The basal dendritic tree (red) of tetanus toxin-exposed cells was dramatically reduced, whereas the apical dendritic tree (blue) was not affected. Axonal morphology (grey) was also not affected by tetanus toxin injection.

et al., 1997; Drakew *et al*., 1999; Frotscher *et al*., 2000). A block of $GABA_A$ receptors, likely reducing neural activity (Ben-Ari *et al*., 1997), produced a reduction in the number of primary neurites and branching points of cultured embryonic hippocampal neurons (Barbin *et al*., 1993). Thus, these studies have not revealed any simple relationship between neural activity and dendritic growth. Rather, this relationship seems to depend on many factors including the manner in which activity is affected, the degree of maturation of the neurons, and the specific molecular mechanisms that are involved in the growth.

In the present study, tetanus toxin was used to block neural activity. This approach may differ from other studies using TTX or receptor antagonists. Tetanus toxin strongly reduced synaptic transmission and entirely blocked neural network activity (Groc, Gustafsson, Hanse, unpublished, Groc *et al*., 2001). Nevertheless, there will then remain some synaptic activity that might be sufficient to mediate some trophic actions. For example, GABA can act on cultured hippocampal neurons to express the chloride transporter KCC2 even after a strong reduction of GABA release (Ganguly *et al*., 2001).

A second condition that may affect the relationship between neural activity and neuronal growth is at which developmental stage activity is blocked. The question then arises to what extent the presently observed differential effect on the apical and the basal dendritic tree can be explained by this condition. It is well established that the apical dendritic arbor differentiates earlier than the basal one (Tyzio *et al*., 1999) (see also, Pokorny & Yamamoto, 1981; Gaiarsa *et al*., 1992; Lopez-Gallardo & Prada, 2001). Assuming that the activity-dependent requirement for branching occurs at a stage that precedes branching itself, before birth in the case of the apical dendrite and after birth in the case of the basal dendrite, tetanus toxin injected at P1 will be efficient in suppressing the branching of the basal, but not the apical, dendrites. If so, injection of tetanus toxin before birth should suppress branching of both apical and basal dendrites, and injection of tetanus toxin at later stages should not have any effect on their branching. These predictions remain to be tested.

A third condition that may affect the relationship between neural activity and neuronal growth is the specific molecular mechanisms that mediate the dendritic growth. The question then arises as to whether there may be such differences between the apical and the basal dendrites. In the adult hippocampus, basal and apical dendrites respond differently to glucocorticoid-induced dendritic alteration (Woolley *et al*., 1990). Furthermore, apical and basal dendrites of cultured neocortical cells are regulated differently by neurotrophins and by neural activity. Thus, cortical cells treated with brain derived neurotrophic factor (BDNF) exhibited a dramatic increase in the number of primary basal, but not apical, dendritic branches, and blockade of electrical and synaptic activity increased the basal dendritic tree only (McAllister *et al*., 1996). The difference between apical and basal dendritic responsiveness to activity blockade or neurotrophins may come from differential expression of key molecules. Examples of such molecules that promote dendritic growth include neurotrophin receptors (McAllister *et al*., 1996), the CPG15 molecule (Nedivi *et al*., 1998), Rho family members (e.g. Rac1), as well as the type I cell-surface protein, Notch (reviewed in Redmond & Ghosh, 2001). It is thus possible that the apical and basal dendritic growth *in vivo* rely on different molecular mechanism(s).

Our results suggest that it is the initiation of new dendritic branches, rather than the elongation of branches that is activity-dependent. This result agrees with that of an *in vivo* study in Xenopus larva, where synaptic activity was found to differentially regulate branch initiation and elongation *via* Rac and RhoA factors, respectively (Li *et al*., 2002). Moreover, synaptic activity may keep down the expression of other signals, such as the calcium/calmodulin-dependent protein kinase II, that block initiation of dendritic branching (Wu & Cline, 1998; Zou & Cline, 1999).

In conclusion, blockade of neural activity in the CA1 area during the first postnatal week severely impaired the generation of new basal dendritic branches whilst having no effect on the elongation of those branches, or on the growth of the apical dendritic tree and axonal arbour. Thus, the present study establishes one important role *in vivo* for the physiological spontaneous neural activity in the developing hippocampus.

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