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Outgrowth-Regulating Actions of Glutamate in Isolated Hippocampal Pyramidal Neurons

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The present study examined the effects of glutamate on the outgrowth of dendrites and axons in isolated hippocampal pyramidal-like neurons in cell culture. During the first day of culture the survival and outgrowth of these neurons was unaffected by high concentrations (up to 1 mM) of glutamate, quisqualic acid (QA), kainic acid (KA), and *N*-methyl-D-aspartic acid. Beginning on day 2 of culture high levels of glutamate, KA and QA were toxic to the majority of pyramidal neurons, while subtoxic levels of these agents caused a well-defined, dose-dependent, sequence of effects on dendritic outgrowth. At increasing concentrations of glutamate, QA, and KA, the following events were observed: (1) dendritic outgrowth rates were reduced, while axonal elongation rates were unaffected; (2) dendritic length was reduced, while axons continued to grow; (3) dendrites regressed dramatically, and axonal outgrowth rate was reduced. These dendrite-specific effects of glutamate were apparently mediated at the growth cones since focal application of glutamate to individual dendritic growth cones resulted in suppression of growth cone activity and a regression of the dendrite; axons were unaffected by focal glutamate application. Pharmacological tests using glutamate receptor agonists and antagonists demonstrated that receptors of the KA/QA type mediated the glutamate effects on outgrowth and survival. The calcium channel blocker Co^{2+} prevented both glutamate neurotoxicity and glutamate-induced dendritic regression. Ionophore A23187 and elevations in extracellular K^+ levels each caused a dose-dependent series of outgrowth and survival responses similar to those caused by glutamate. Taken together, these results indicate that activation of glutamate receptors leads to the opening of voltage-dependent calcium channels; the resulting increases in calcium influx lead to the observed alterations in dendritic outgrowth and neuronal survival.

Glutamate is believed to be a major excitatory neurotransmitter in the mammalian CNS (Fonnum, 1984). In the hippocampus, glutamatergic afferents provide prominent input to pyramidal neuron dendrites (Isaacson and Pribram, 1975). Glutamate action on pyramidal neurons may mediate fundamental brain processes such as learning and memory (see Lynch, 1986, for review). Somewhat paradoxically, glutamate may also be involved in the degeneration of pyramidal neurons seen in several diseases including epilepsy (Schwartz et al., 1984), Huntington's disease (Coyle and Schwartz, 1976), Alzheimer's disease (Geddes et al., 1986; Maragos et al., 1987) and stroke (Rothman, 1984; Simon et al., 1984b). It is clear that high levels of glutamate and related excitatory amino acids (EAAs) including kainic acid (KA), quisqualic acid (QA), and *N*-methyl-D-aspartic acid (NMDA) can cause a striking neurodegeneration of specific neurons, including hippocampal pyramidal neurons (Olney, 1978, 1983; Coyle et al., 1981; Choi et al., 1987).

Recent work in several systems has suggested a novel role for neurotransmitters: alteration of neurite outgrowth. Studies in the snail *Helisoma* showed that neurotransmitters such as serotonin and dopamine can suppress neurite outgrowth *in vitro* (Haydon et al., 1984, 1985; McCobb and Kater, 1986; Mattson and Kater, 1987) and during normal development *in vivo* (Goldberg et al., 1986). In vertebrates, preliminary reports have appeared which suggest that neurotransmitters might serve similar roles in regulating neuronal outgrowth (Frosch et al., 1986; Lankford et al., 1986; Mattson et al., 1987). The possibility that nontoxic levels of glutamate might affect neuronal outgrowth has not been tested, but evidence is available which is consistent with the possibility that nontoxic levels of glutamate might affect brain cytoarchitecture. Thus, reductions in the extent of the dendritic arbors of pyramidal neurons are seen in neurodegenerative disorders in which glutamate is implicated (Mehraein et al., 1975; Paul and Scheibel, 1986). In addition, recent studies showed an association between long-term potentiation of synaptic (glutamate) transmission in the hippocampus and alterations in the structure of dendritic spines of pyramidal neurons (Lee et al., 1980; Chang and Greenough, 1984; Lynch, 1986). The present study represents an initial test of the hypothesis that glutamate can exert the kinds of graded effects on neuronal outgrowth and survival which are consistent with a role for this neurotransmitter in regulating the formation, modulation, and degeneration of brain cytoarchitecture.

The cellular mechanisms underlying the outgrowth and survival responses of neurons to neurotransmitters are not known in detail, but an important role for calcium has been proposed in each case. For example, there is evidence that glutamate-induced calcium entry mediates morphological changes in synapses underlying memory processes (Lynch, 1986). In addition, one model for the cellular basis of EAA toxicity implicates Ca^{2+} entry through ion channels activated by the EAA (e.g., Berdi-

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chevsky et al., 1983; Retz and Coyle, 1984). In molluscan neurons, the cellular mechanism of action of the neurotransmitters mediating outgrowth responses has been more directly addressed and clearly involves neurotransmitter-induced calcium influx from the cell exterior (Cohan et al., 1987; Mattson and Kater, 1987; Mattson et al., 1988).

The present study employed isolated hippocampal pyramidal neurons to directly test the possible differential effects of glutamate on axonal and dendritic outgrowth, as well as the receptors and ionic mechanisms mediating the outgrowth responses. These neurons provided a particularly useful model for such studies because the axon and dendrites can be unambiguously identified by morphological and immunocytological criteria (Caceres et al., 1984, 1986; M. P. Mattson, unpublished observations). The results show that glutamate can act locally on dendritic growth cones to selectively reduce the length of the dendrites; these effects are mediated via KA and/or QA receptors linked to calcium influx through voltage-dependent channels.

Materials and Methods

Hippocampal cultures. The culture methods employed were similar to those of Banker and Cowan (1977, 1979). Hippocampi were obtained from 18-d-old rat fetuses. At this stage of development, pyramidal-like neurons constitute approximately 60% of the total neuronal population and gliosis is minimal (Banker and Cowan, 1977; M. P. Mattson, personal observations). Hippocampi were incubated for 15 min in a solution of 2 mg/ml trypsin in Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution buffered with 10 mM HEPES (BSS; Sigma; approximately 3 hippocampi/ml). The hippocampi were then rinsed once in BSS, followed by a 5 min incubation in a solution of 2 mg trypsin inhibitor (Sigma)/ml of BSS, and a final rinse in BSS. Cells were then dissociated by trituration through the narrowed bore of a fire-polished pasteur pipette and were distributed to 35 mm, polylysine-coated, plastic culture dishes (Corning) containing 2 ml of Eagle's Minimum Essential Medium (MEM; GIBCO) buffered with 10 mM sodium bicarbonate and supplemented 10% with fetal bovine serum (Sigma). The fetal bovine serum provides a factor(s) which promotes cell attachment (G. A. Banker, personal communication; M. P. Mattson, unpublished observations). The dissociated neurons were plated at a density of approximately 50 neurons/mm^2 of growth substrate. This plating density provided cultures in which the majority of neurons grew in physical isolation from other cells. After 4 hr of incubation at 37°C in a humidified 5% CO_2/95% room air atmosphere, the attached cells were washed once with 3 ml of MEM, and then 2 ml of MEM containing the N2 supplements of Bottenstein and Sato (1979) was added; cultures were maintained in this medium at 37°C in a humidified 5% CO_2/95% room air atmosphere.

Chemicals. Glutamate, KA, QA, NMDA, D-gamma-glutamylglycine (DGG), DL-2-amino-5-phosphonovaleric acid (APV), acetylcholine, serotonin, dopamine, and $CoCl_2$ (Sigma) were prepared as stocks in MEM and were added to cultures in 22 or 220 μl volumes. Ionophore A23187 (Sigma) was dissolved in dimethylsulfoxide and was added to cultures in 10 μl volumes. Equivalent volumes of vehicle were added to control cultures and did not affect neuronal outgrowth or survival. For experiments in which treatments with Co^{2+} or receptor antagonists and glutamate were combined, Co^{2+} or antagonists were added to the cultures 15 min prior to addition of glutamate.

Assessments of neuronal outgrowth and survival. Neurons maintained in culture for up to 5 d were used for all experiments in the present study. Pyramidal neurons were identified by morphological criteria (Banker and Cowan, 1977; Fig. 1). These neurons project one long and branching axon and several short, straight dendrites. In cultured hippocampal pyramidal neurons, the single, long process has been positively identified as the axon by its immunoreactivity to tau antibody and lack of immunoreactivity to MAP2 antibody; dendrites stain heavily with MAP2 antibody and lack tau (Caceres et al., 1984, 1986). In preliminary experiments we confirmed this relationship between axonal and dendritic morphology and compartmentalization of MAP2 and tau in pyramidal neurons under the conditions of the present study.

For morphological analyses, neurons were visualized and photographed with a phase-contrast Nikon Diaphot inverted microscope. Individual, isolated neurons were located and relocated by their positions with respect to scratches etched into the outer, bottom surface of the culture dish. Elongation rates of axons and dendrites were determined from tracings of projected negatives of sequential photographs taken at a magnification of 200×. Only neurons which had established a characteristic pyramidal-like morphology and which were free from contact with other cells were selected for analyses. Two or three pyramidal neurons per culture dish were assessed, and the experiments were repeated at least twice. In the experiments involving long-term (3 d) exposure to glutamate, the analyses of dendritic and axonal outgrowth were done by a person blind to the experimental treatment history of the cultures. Axon and dendrite lengths before and after treatment were determined from measurements made on tracings of projected photographic negatives.

For the experiments in which glutamate was applied locally to axonal and dendritic growth cones, the cultures were maintained in HEPES-buffered BSS modified to contain 1 mM $MgCl_2$ and 5 mM $CaCl_2$ (HBSS); the temperature was held at 30°C with an infrared incubator lamp (Opti-Quip, Highland Mills, NY). Pyramidal neurons were located in the dish, and their relative positions were diagrammed to allow relocation; neurons were examined and photographed at a magnification of 400×. Micropipettes with tip diameters of 5–7 μm, held and maneuvered with a micromanipulator, were connected via polyethylene tubing to a 6 ml syringe which was used to adjust the flow rate. The standard protocol for these experiments involved initial applications (5–10 min pulses) of control HBSS to axonal and dendritic growth cones followed by a 10–30 min wait period; glutamate was then applied to the growth cones in 5–10 min pulses.

For assessments of cell survival, viability was determined 24 hr following experimental treatment. Photographs for survival counts were taken at a magnification of 100×. Four microscope fields were assessed/culture dish (30–60 neurons/field), and experiments were repeated at least once. The number of surviving neurons was calculated as a percentage of the initial number of neurons present prior to experimental treatment; values were expressed as a percentage of the number of neurons surviving in control cultures. In general, neurons which died during the 24 hr interval were absent the next day. Remaining, nonviable neurons were identified by morphological criteria; viable neurons had phase-bright somas and intact processes, while nonviable neurons had fragmented neurites. The results of initial experiments showed that viability counts, according to these morphological criteria, agreed within 5% with counts of Trypan blue (0.4% solution in 0.15 M saline) excluding neurons. Statistical comparisons were done using Student's *t* test, and values are expressed as mean ± SEM.

Results

Characterization of the effects of glutamate on neuronal survival

Previous studies of glutamate neurotoxicity *in vitro* (e.g., Rothman, 1984, 1985; Choi et al., 1987) were done in high-density cultures of interacting neurons; under those conditions, morphological analyses of axonal and dendritic outgrowth were not possible. The present study employed isolated pyramidal-like hippocampal neurons with morphologically distinct axons and dendrites in order to examine the possible effects of nontoxic levels of glutamate on axonal and dendritic outgrowth.

During the first day in culture, neurons were insensitive to glutamate neurotoxicity (Table 1). In contrast, pyramidal neurons which had been in culture for 2 or more days became sensitive to glutamate neurotoxicity. Indeed, 58 and 85% of 2- and 4-d-old pyramidal neurons, respectively, were killed by 1 mM glutamate (Table 1). Toxicity was characterized by somal swelling and process fragmentation within 2 hr of exposure; the axons and dendrites degenerated in place, and only moderate process regression was observed (Fig. 1*C*). These toxic effects were quite specific for pyramidal neurons since the viability of bipolar neurons, stellate neurons, and glial cells was not significantly altered by millimolar concentrations of glutamate (over 100 nonpyramidal neurons and 50 glial cells examined in 10

cultures). These results indicated that isolated pyramidal neurons in culture are selectively vulnerable to glutamate toxicity and that within the population of pyramidal neurons there are glutamate-sensitive and -insensitive subpopulations.

Previous work demonstrated that a brief (5 min) pulse of millimolar levels of glutamate was toxic to cultured cortical neurons (Choi et al., 1987). Similarly, we found that a 10 min pulse of 1 mM glutamate was sufficient to induce rapid cell death ($n = 4$ cultures). Thus, glutamate apparently triggers a series of cellular events which cannot be readily reversed by simply removing glutamate.

A slower manifestation of toxicity occurred over a 24 hr period and was seen in approximately 30% of the pyramidal neurons exposed to 100 μM glutamate (Table 1). This slower cell death was characterized morphologically by pronounced reduction in dendritic length (and modest reductions in axonal length) commencing within 2 hr of exposure and terminating with somal detachment from the growth substrate ($n = 38$ neurons). Somal swelling and process fragmentation were not observed in response to 100 μM glutamate. A pronounced reduction in dendritic length was also seen in the neurons that survived exposure to 100 μM glutamate (Fig. 1*B*). Lower levels of glutamate (≤50 μM) did not significantly affect pyramidal cell survival over a 24 hr period (Table 1) but did cause modest reductions in dendritic arbors (Figs. 1*A*; 3–5).

At this point it had to be considered that the reduction in dendritic length caused by glutamate was an early event in a slower form of neurodegeneration. We tested this possibility by examining the outgrowth of neurons exposed to 50 μM glutamate for a 3 d period (Fig. 2). Under these conditions, pyramidal neuron survival in control and glutamate-treated cultures were similar at 87% ($n = 108$) and 85% ($n = 196$) of the initial number of neurons, respectively. Examination of neurons exposed to 50 μM glutamate revealed a reduction in dendritic length compared to controls (Fig. 2). Ratios of axonal to dendritic lengths in neurons exposed to 50 μM glutamate were greatly increased (fivefold) compared to control neurons (Fig. 2). On day 5 in culture the ratio of axonal to dendritic length of control neurons was 1.0 ± 0.2 ($n = 8$), while the ratio in neurons exposed to 50 μM glutamate was 4.8 ± 0.9 ($n = 10$; $p < 0.001$). The change in axon/dendrite ratio could be entirely accounted for by the reductions in dendritic extent since the average axonal lengths of control and glutamate-treated neurons were not significantly different (157 ± 33 and 188 ± 22 μm, respectively). These results provided the first evidence that nontoxic levels of glutamate can selectively affect the outgrowth of dendrites versus the axon. We pursued this phenomenon by further characterizing the effects of glutamate on axonal and dendritic outgrowth.

Table 1. Effects of EAAs, DGG, and calcium-altering agents on pyramidal neuron survival

Treatment	24 hr survival of pyramidal neurons (% control) Day 1	Day 2	Day 4
Glutamate			
50 μM	102 ± 6.1	99 ± 5.4	93 ± 7.1
100 μM	97 ± 6.3	67 ± 8.4[a]	66 ± 5.0[a]
1 mM	89 ± 7.4	42 ± 8.9[a]	15 ± 1.4[a]
5 mM DGG	N.A.	90 ± 7.4	96 ± 8.4
5 mM DGG + 1 mM glutamate	N.A.	89 ± 9.6[b]	93 ± 4.1[b]
1 mM NMDA	101 ± 6.9	95 ± 5.4	100 ± 4.3
100 μM KA	91 ± 1.9	61 ± 0.5[a]	42 ± 7.5[a]
1 mM KA	92 ± 3.2	51 ± 5.6[a]	26 ± 11.1[a]
100 μM QA	N.A.	N.A.	57 ± 4.7[a]
1 mM QA	N.A.	N.A.	33 ± 5.0[a]
100 μM Co^{2+}	N.A.	N.A.	102 ± 4.8
100 μM Co^{2+} +1 mM glutamate	N.A.	N.A.	83 ± 5.8[b]
10 μM A23187	N.A.	N.A.	2 ± 1.6[a]

Viable pyramidal neurons of the indicated ages (days in culture) were counted before and 24 hr after exposure to the given agents. Values are expressed as a percentage of the pyramidal neurons surviving over 24 hr periods in control cultures (average survival in control cultures was 92 ± 5.3%, $n = 8$ cultures). N.A., not assessed.

[a] $p < 0.05$–0.0001 compared with controls.

[b] $p < 0.01$–0.001 compared with the value of neurons exposed to 1 mM glutamate.

Characterization of the effects of glutamate on dendritic and axonal outgrowth

The development with time in culture of dendritic outgrowth sensitivity to glutamate was examined. During the first 24 hr in culture, the outgrowth of axons and dendrites was unaffected by 50 μM glutamate (Fig. 3). In contrast, significant changes in the outgrowth of dendrites, but not axons, were observed to occur in 2- or 4-d-old neurons exposed to 50 μM glutamate. Dendritic elongation ceased in 2-d-old neurons exposed to 50 μM glutamate, while dendrites decreased in length in 4-d-old neurons (Fig. 3). The effects of glutamate on dendritic outgrowth were evident within the first 2 hr of exposure; in 4-d-old neurons, dendritic regression continued for at least 20 hr (Fig. 4). The cytoarchitectural changes caused by glutamate were apparently specific for this neurotransmitter since ACh, serotonin, and dopamine at concentrations up to 100 μM had no discernible effects on axonal or dendritic outgrowth ($n = 7$–12 neurons assessed). We next examined the dose dependency of glutamate effects on outgrowth in the sensitive 4-d-old pyramidal neurons. A stereotyped sequence of events was observed in neurons exposed to increasing concentrations of glutamate (Figs. 1, 5). In response to 10 μM glutamate, the outgrowth of dendrites was significantly reduced and ceased in most instances within 6 hr of exposure (13 of 15 dendrites). In contrast, axonal elongation continued at the pretreatment rate (Fig. 5). Exposure to 50 μM glutamate caused a significant and steady reduction in dendritic length over a 20 hr period; axonal elongation rates were unaffected by 50 μM glutamate (Figs. 4, 5). Glutamate at 100 μM caused a pronounced reduction in dendritic lengths and also suppressed axonal elongation (Figs. 1 and 5).

Several attempts were made to reverse the reduction in dendritic length caused by glutamate. Following exposure to glutamate (50 μM) for time periods of 10 min to 2 hr, cultures were washed two–five times in fresh culture medium. Even the brief 10 min pulse of glutamate caused a progressive reduction in dendritic length which continued for at least one hr after washing ($n = 22$ neurons). After washing the cells, dendritic regression ceased within 4 hr in all ($n = 12$) neurons previously exposed to 50 μM glutamate and in 7 of 10 neurons exposed to 100 μM glutamate. However, renewed outgrowth from these dendrites was not observed during a subsequent 20 hr period despite the fact that axons continued to elongate at constant rates during this period ($n = 19$ neurons). Taken together, these results indicate that subtoxic levels of glutamate have predictable and

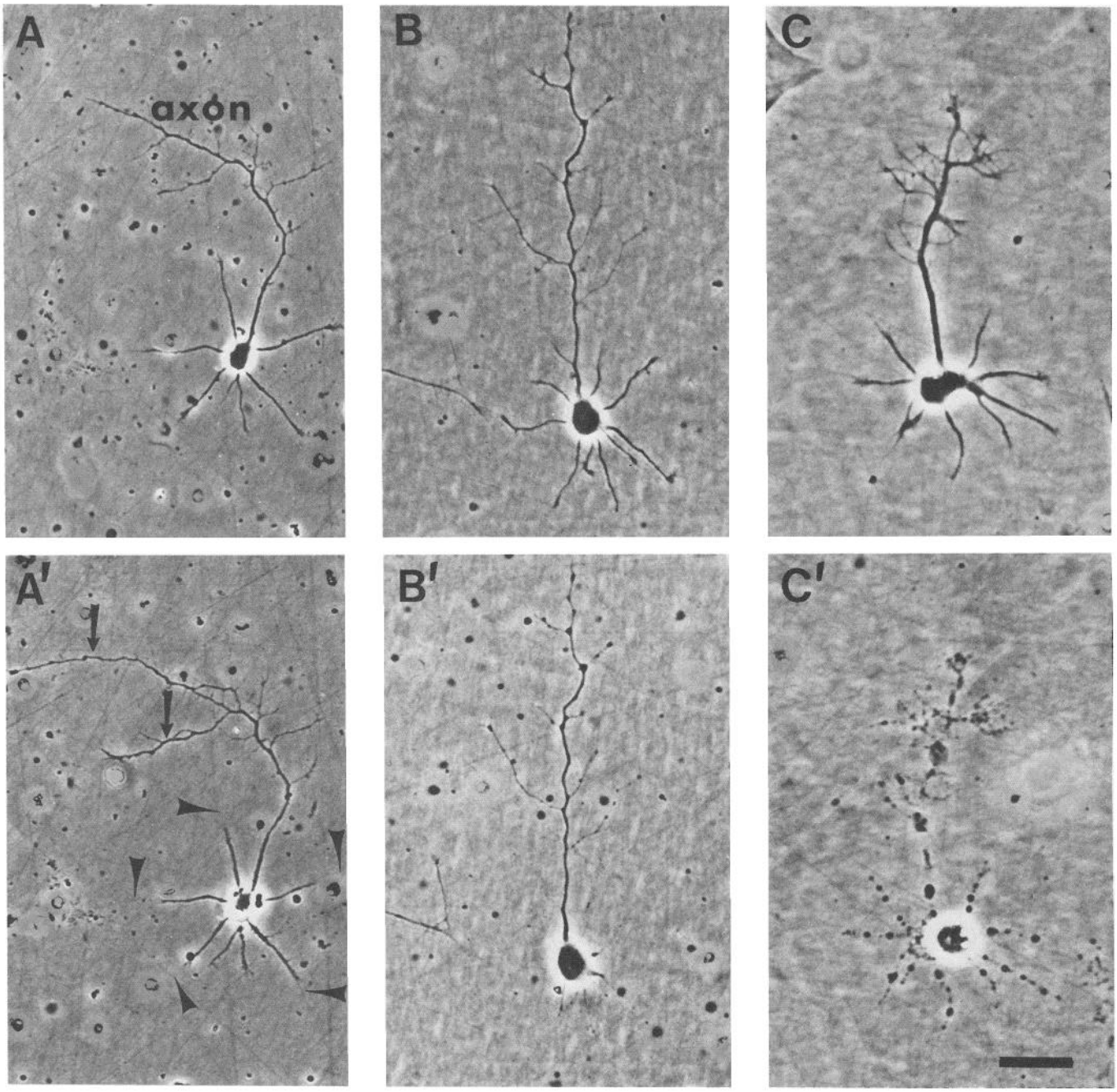

Figure 1. Graded effects of glutamate on the outgrowth and survival of hippocampal pyramidal neurons. *A–C,* Photographs of 4-d-old neurons immediately prior to (*A–C*) and 20 hr (*A', B'*) or 2 hr (*C'*) hr after exposure to glutamate at concentrations of 50 μM (*A, A'*), 100 μM (*B, B'*), or 1 mM (*C, C'*). *Arrows* and *arrowheads* in *A'* point to the locations of axonal and dendritic tips, respectively, prior to glutamate exposure (*A*). See text for details. Scale bar, 25 μm.

lasting effects on dendritic outgrowth without significantly altering axonal outgrowth.

Local control of dendritic growth cones by glutamate

In identified *Helisoma* neurons, neurotransmitters can act directly on individual growth cones to inhibit the activity of filopodia as well as neurite elongation (Haydon et al., 1984; Mattson and Kater, 1987). In order to more closely examine the effects of glutamate on the outgrowth of individual dendrites, we used a micropipette to apply glutamate locally to individual dendritic growth cones while monitoring outgrowth (Fig. 6). Controls in these experiments consisted of applicaiton of medium without glutamate as well as the application of glutamate to axonal growth cones. Control medium released locally within 5–10 μm of axonal or dendritic growth cones had no detectable effects on filopodial activity or neurite elongation (Fig. 6). For glutamate application, the micropipette contained 500 μM glutamate, but the actual concentration of glutamate reaching the growth cones was undoubtedly less due to dilution in the incubation medium. When glutamate was released onto axonal growth cones, both elongation and growth cone motility continued. In contrast to the axons, the growth cone motility and

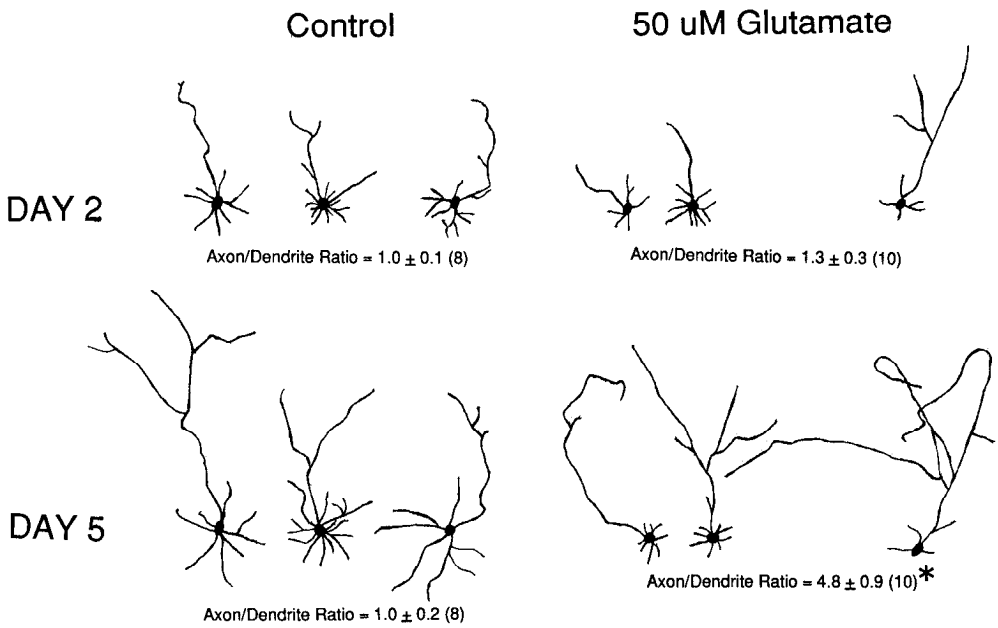

Figure 2. Long-term effects of glutamate on dendritic outgrowth. On day 2 of culture, neurons were photographed and then exposed to control medium or medium containing 50 μM glutamate. The same neurons were photographed 3 d later. Tracings are of representative control and glutamate-treated neurons (total numbers of neurons assessed are indicated in parentheses). Values for ratios of axonal to dendritic lengths represent the mean and SEM; on day 5 the ratio for control neurons is significantly greater than that for glutamate-treated neurons (* $p < 0.001$). On day 5 the average axonal lengths of control and glutamate-treated neurons were not significantly different (157 ± 33 and 188 ± 22 μm, respectively).

elongation of dendrites were negatively affected by glutamate (Fig. 6). The dendrites to which glutamate was focally applied were rapidly reduced in length and filopodia were absent; dendrites further from the site of glutamate release were relatively unaffected. These specific, local effects of glutamate on dendritic versus axonal growth cones were seen in all 27 pyramidal neurons examined.

Glutamate receptors affecting neuronal outgrowth and survival

In order to establish that the effects of glutamate on neuronal outgrowth and survival were receptor-mediated, we employed the general glutamate receptor antagonist DGG as well as the NMDA receptor-specific antagonist APV (Watkins and Evans, 1981; Meldrum, 1985). DGG at 5mM prevented the negative effects of glutamate on dendritic outgrowth (Figs. 7 and 8). Indeed, in the presence of DGG, dendrites continued to elongate at pretreatment levels even in the presence of a glutamate concentration (100 μM) which would otherwise have caused a pronounced reduction in dendritic lengths (compare Figs. 1 and 8). Furthermore, the toxic effect of 1 mM glutamate was prevented by 5 mM DGG (Table 1). However, under the latter conditions dendritic outgrowth ceased in 15 of 18 dendrites examined,

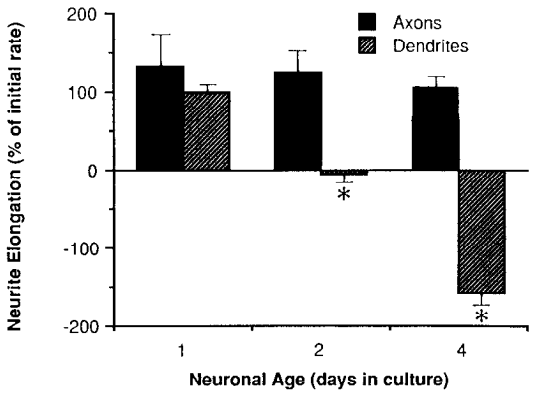

Figure 3. Age-dependent effects of glutamate upon neuronal outgrowth. Rates of axonal and dendritic neurite elongation of neurons grown for the indicated times in culture were determined during a 2 hr incubation prior to addition of 50 μM glutamate and for 6 hr following treatment. Values are expressed as the percentage change in outgrowth rate after exposure to glutamate and represent the mean and SEM of determinations made on from 4 to 6 axons and from 8 to 15 dendrites (2 or 3 separate cultures). Negative values indicate a reduction in neurite length. *$p < 0.05$–0.001 compared with axons or control dendrites. The elongation rates of control axons and dendrites (μm/hr) were as follows: day 1–axons, 5.1 ± 0.2; dendrites, 2.8 ± 0.2; day 2—axons, 6.0 ± 0.3; dendrites, 2.2 ± 0.1; day 4–axons, 4.8 ± 0.3; dendrites, 1.1 ± 0.1.

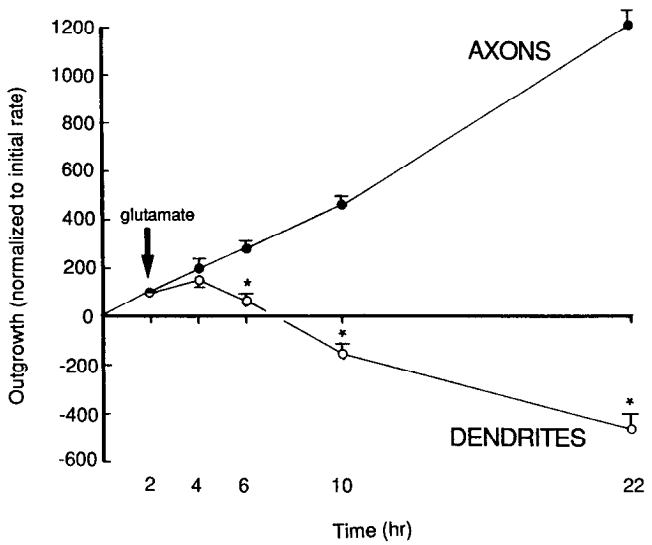

Figure 4. Time course of glutamate effects on axonal and dendritic outgrowth. Elongation rates of axons and dendrites were determined for 2 hr prior to addition of 50 μM glutamate to establish initial rates of elongation (axons averaged 4.9 ± 0.7 μm/hr and dendrites 1.3 ± 0.5 μm/hr). Values for outgrowth after exposure to glutamate (*arrow*) are normalized to the initial rates of elongation and are expressed as cumulative percentage of the initial rate. The negative slope indicates a reduction in dendritic length. Points represent the mean and SEM of determinations made on 3–7 axons and 9–19 dendrites. Where error bars are not seen the points obscure the SEM. *$p < 0.01$–0.001 compared with the initial rate.

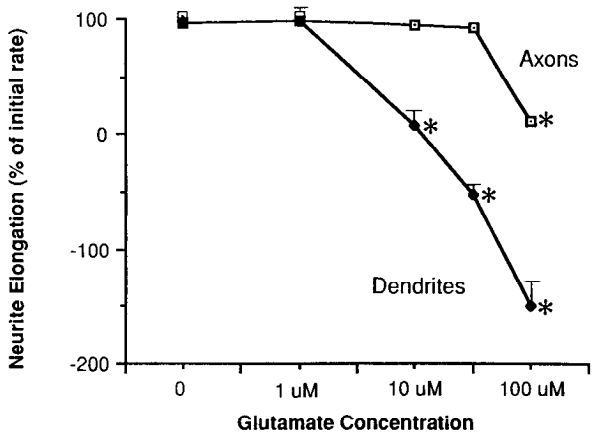

Figure 5. Dose-dependent effects of glutamate on dendritic and axonal outgrowth. Dendritic and axonal outgrowth rates were quantified in 4-d-old pyramidal neurons for a 2 hr period prior to exposure to glutamate and for three 2 hr intervals after exposure. Values are the post-treatment outgrowth rates expressed as a percentage of the pretreatment rate (mean and SEM of determinations made on from 4 to 6 axons and from 9 to 15 dendrites; neurons were from 2 or 3 separate cultures). Where error bars are not seen, the data points obscure the SEMs. Dendrites and axons of neurons in control cultures continued to elongate at rates of 1.2 ± 0.1 and 4.3 ± 0.2 μm/hr, respectively, during the course of the experiments. $*p < 0.01$–0.001 compared with control (100%) rates.

suggesting that some degree of glutamate receptor activation was occurring. The NMDA receptor-specific antagonist APV at concentrations up to 1 mM did not reduce the effects of glutamate on dendritic outgrowth (50 μM glutamate, $n = 10$ neurons) or cell survival (1 mM glutamate, 8 fields examined). These results suggested that dendritic glutamate receptors, not of the NMDA-preferring type, mediated each of the graded changes in neuroarchitecture induced by increasing concentrations of glutamate.

There are currently three recognized classes of receptors for EAAs, namely, those which prefer NMDA, QA, and KA (see Coyle et al., 1981; Watkins and Evans, 1981, for reviews). In order to further examine which of these receptor types mediated the outgrowth-regulating effects of glutamate, the elongation rates of axons and dendrites were quantified in neurons exposed to increasing concentrations of NMDA, KA, or QA (Fig. 9). NMDA at concentrations up to 1 mM had no significant effects on axonal or dendritic outgrowth rates (Fig. 9), nor did NMDA affect cell survival (Table 1). In contrast, KA and QA each produced dose-dependent graded effects on neuronal architecture similar to those observed with glutamate (Fig. 9). Thus, KA (1 μM) and QA (10 μM) both suppressed dendritic outgrowth but had no significant effect on axonal elongation rates. Ten-fold higher concentrations of these EAAs caused a pronounced reduction in dendritic lengths accompanied by a suppression of axonal elongation (Fig. 9), and were toxic over a 24 hr period to 40–60% of the pyramidal neurons (Table 1). Further increases in the levels of KA (100 μM) and QA (1 mM) were toxic to 60–75% of the pyramidal neurons (Table 1). These results are consistent with the presence of KA/QA type EAA receptors on the dendrites of isolated hippocampal pyramidal neurons whose activation leads either to cell death at high concentrations or to a selective inhibition of dendritic outgrowth at nontoxic concentrations.

Calcium involvement in the effects of glutamate on outgrowth and survival

Neurotransmitter-induced influx of calcium from the cell exterior mediates the suppresive effects of serotonin on neurite outgrowth from identified molluscan neurons (Cohan et al., 1987; Mattson and Kater, 1987). One proposed cellular mechanism whereby EAAs exert their toxic effects is by depolarization-induced calcium influx (Coyle et al., 1981; Berdichevsky et al., 1983; Mayer and Westbrook, 1987). In order to test the possibility that depolarization-induced calcium influx was involved in the graded effects of EAAs on pyramidal cell outgrowth and survival, we employed agents known for their negative or positive effects on calcium influx as well as elevated extracellular K^+. Co^{2+} (100 μM), which is expected to block voltage-dependent calcium channels (Hagiwara and Byerly, 1981; Miller, 1987), alone had no significant effects on dendritic or axonal outgrowth rates (Fig. 10) or cell survival (Table 1). When neurons were exposed to 100 μM Co^{2+} in combination with glutamate at a concentration which normally caused reductions in dendritic length (50 μM), dendritic outgrowth rates were unaffected. Furthermore, 100 μM Co^{2+} prevented dendritic regression in neurons exposed to 100 μM glutamate, although a slowing of elongation was observed (Fig. 10). Finally, while 85% of the pyramidal neurons were killed by 1 mM glutamate, only 17% of the pyramidal cell population was killed when exposed to 1 mM glutamate plus 100 μM Co^{2+} (Table 1). Taken together, these data indicated that the effects of glutamate on both neuronal outgrowth and survival were linked to calcium influx through voltage-dependent plasma membrane channels.

If depolarization-induced calcium influx was indeed responsible for the effects of glutamate on dendritic outgrowth and cell survival, then elevation of extracellular K^+ should also affect outgrowth and survival. Indeed, raising the level of K^+ in the culture medium to 50–100 mM caused a pronounced reduction in the lengths of both axons and dendrites over a 20 hr period in all 34 neurons examined. Higher levels of extracellular K^+ (200 mM) were toxic over a 20 hr period to all 155 pyramidal neurons examined. In one experiment, 100 μM Co^{2+} was added to the cultures prior to elevation of K^+ to 100 mM; under these conditions both axons and dendrites continued to grow in all

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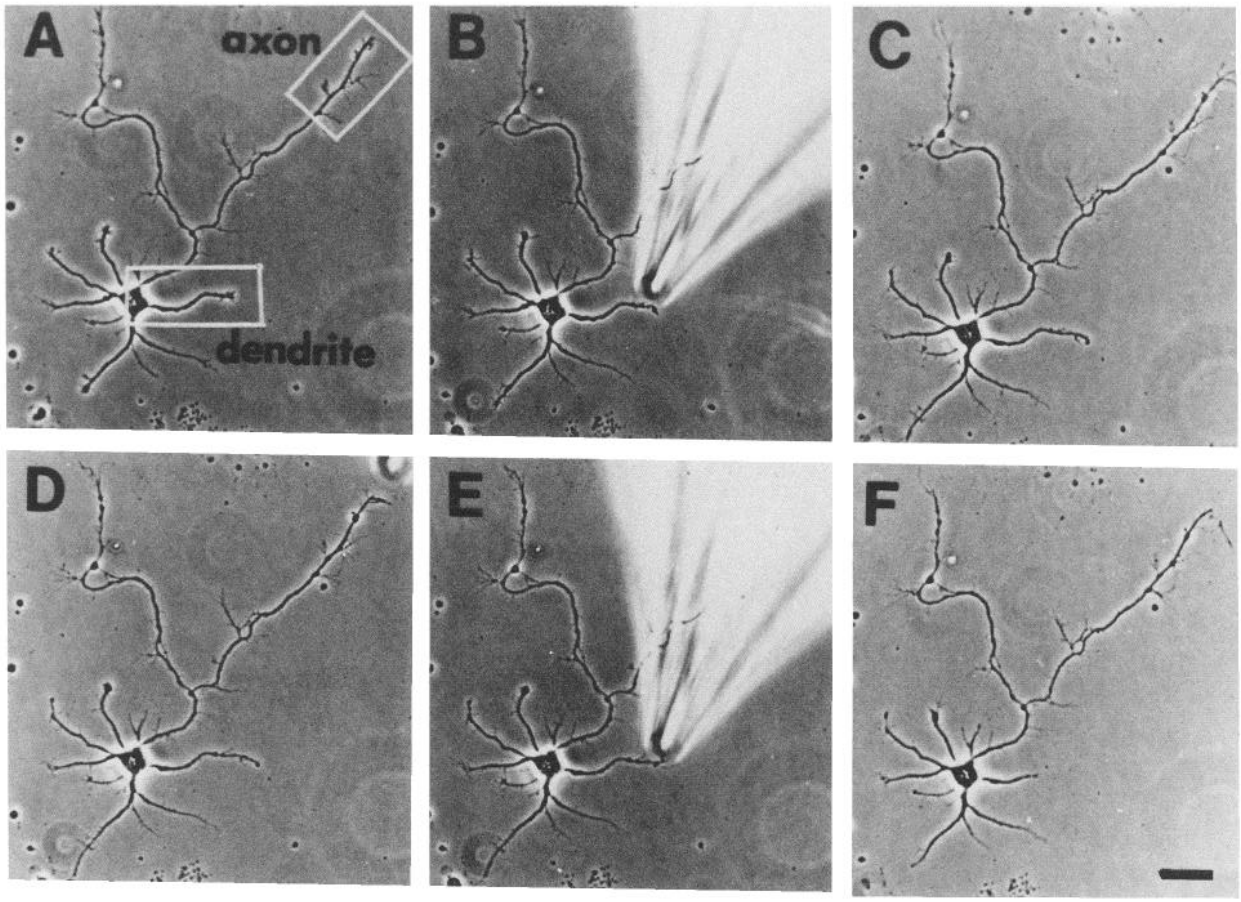
Figure 6. Effects of focal application of glutamate to individual growth cones. *A–F,* Micrographs of a pyramidal neuron taken at different points in a typical experiment. The lower two sets of micrographs are magnifications of the axon (*A′, C′, D′,* and *F′*) and dendrite (*A″, C″, D″,* and *F″*) boxed in *A* (letters correspond to those in the upper micrographs (*A, C, D,* and *F*). *A, A′,* and *A″,* Pyramidal neuron with a long branched axon and several short dendrites prior to experimental treatment ($t = 0$). *B,* Control medium is released onto the dendritic growth cone for 10 min; the axonal growth cone is also exposed to control medium for 10 min (not shown). *C, C′, C″,* $t = 30$ min; note that both the dendrite and axon have elongated and possess active growth cones (i.e., filopodia are present). *D, D′, D″,* $t = 35$ min; glutamate (500 μM concentration in the pipette) is applied to the axon for 10 min. Both the dendritic and axonal growth cones remain active. *E,* $t = 45$ min; glutamate is released onto the dendritic growth cone for 10 min. *F, F′, F″,* $t = 90$ min; note that the dendrite exposed to glutamate has regressed dramatically and is devoid of filopodia (*F″*), while the axonal growth cone exposed to glutamate remains active (*F′*). These local, dendrite-specific effects of glutamate were seen in all 27 neurons examined. Scale bar, 25 μm in *A–F;* 10 μm in *A′–F′* and A″–F″.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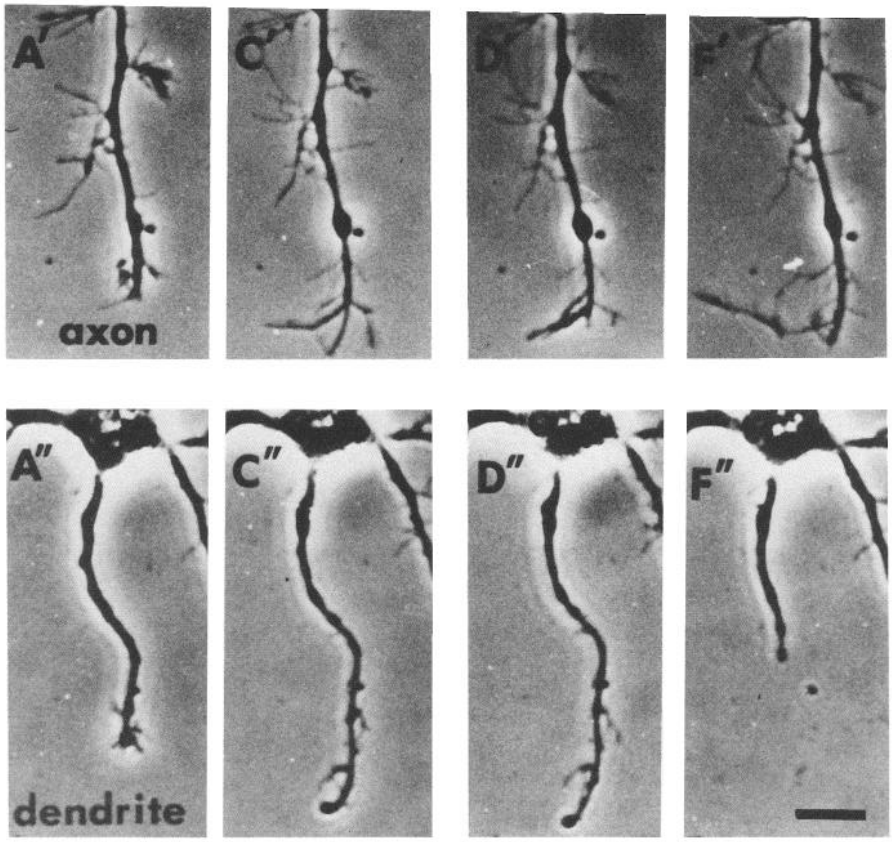
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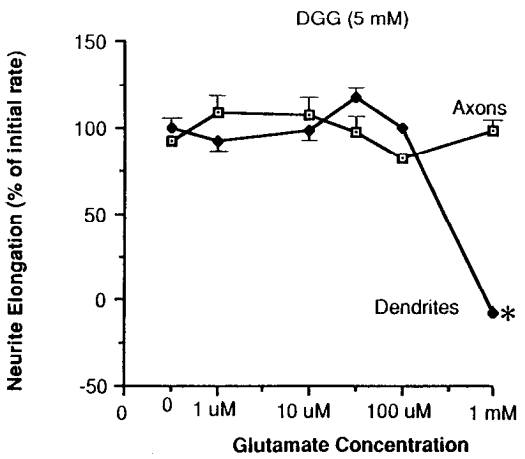

Figure 7. Effects of DGG on glutamate-affected neuronal outgrowth. Dendritic and axonal outgrowth rates were quantified in 4-d-old pyramidal neurons for a 2 hr period prior to exposure to the glutamate receptor antagonist DGG (control) or DGG plus the indicated concentrations of glutamate; the effects of treatments were determined in three 2 hr periods after exposure. Values are the posttreatment outgrowth rates expressed as a percentage of the initial pretreatment rate (mean and SEM of determinations made on from 3 to 6 axons and from 8 to 14 dendrites). Where error bars are not seen, the data points obscure the SEMs. $*p < 0.001$ compared with control.

11 neurons examined. Apparently, depolarization-induced calcium influx can have negative effects on both axonal and dendritic outgrowth; however dendrites are selectively affected by glutamate presumably because they possess functional glutamate receptors.

Our final set of experiments employed the calcium ionophore A23187 to test a possible causal relationship between increases in calcium influx and the graded effects of EAAs on neuroarchitecture. High levels of A23187 (≥ 1 μM) were detrimental to neuronal survival. Cell death was seen in approximately 20% of pyramidal neurons exposed to 1 μM A23187 (Table 1); this death did not involve somal swelling or process fragmentation. A further increase of A23187 concentration to 10 μM was acutely toxic, rapidly killing essentially all neurons in the cultures within 30 min of exposure (Table 1). Furthermore, glia and nonpyramidal neurons were also killed by this high level of ionophore (all of 30 glia and 65 nonpyramidal neurons examined), suggesting a common susceptibility of brain cells to calcium influx. When neurons were exposed to nontoxic levels of A23187 at increasing concentrations, we observed graded effects on neuroarchitecture similar to those induced by EAAs (Fig. 11). However, in neurons exposed to A23187 the differential sensitivities of dendrites and axons observed in response to EAAs were much less pronounced (Fig. 11). Thus, elongation rates of axons and dendrites exposed to 500 nM A23187 were suppressed to 59 and 33% of the initial rates, respectively (Fig. 11). Increasing the A23187 concentration to 1 μM caused modest reductions in axonal lengths and a more marked regression of dendrites (Fig. 11). These experiments with A23187 indicate that graded increases in calcium influx are sufficient to cause, at low levels, a reduction in dendritic length and, at high levels, cell death.

Discussion

The present study was an initial test of the hypothesis that the neurotransmitter glutamate can exert specific, localized, and graded effects on the outgrowth and survival of hippocampal pyramidal neurons (Fig. 12). Dendritic length was selectively reduced by nontoxic levels of glutamate. These results suggest that excitotoxicity may be the result of the overactivity of a process which at lower levels of activation is involved in regulating dendritic geometry. While several investigators have proposed that overactivity of glutamate may be involved in the neurodegeneration seen in several significant brain diseases (Coyle and Schwartz, 1976; Schwartz et al., 1984; Wieloch, 1985; Maragos et al., 1987), the results of the present study represent the first direct demonstration of specific effects of lower levels of glutamate on dendritic outgrowth. The findings presented and discussed here are consistent with the possibility that, in addition to its roles in information coding, glutamate may be involved in regulating brain cytoarchitecture during normal development and adult plasticity.

Glutamate and dendritic outgrowth

Only within the last five years have direct tests been made of the hypothesis that neurotransmitters can alter neuronal outgrowth and synaptogenesis (Kater and Mattson, 1988). Neurotransmitters were first shown to affect neuronal outgrowth in identified molluscan neurons in isolated cell culture (Haydon et al., 1984, 1985; McCobb and Kater, 1986). These findings *in vitro* led the way for recent experiments which demonstrated a role for serotonin in regulating neuroarchitecture during normal embryonic development (Goldberg et al., 1986). In addition, Jones et al. (1986) recently reported that glutamate stimulated neurite sprouting in neurons from intact *Helisoma* buccal ganglia. Preliminary reports have also appeared which indicate that ACh and dopamine can influence neurite outgrowth in cultured vertebrate central neurons (Frosch et al., 1986; Lankford et al., 1986). The results of the present study provide the first demonstration that the outgrowth of axons and dendrites in mammalian brain neurons can be differentially affected by a neurotransmitter.

The experiments of the present study took advantage of the substantial knowledge available on the structure, neurotransmitter pharmacology, electrophysiology, intracellular mechanisms, and pathology of the hippocampus. It therefore seems important to consider the findings of the present study in the context of the *in situ* anatomy and physiology of the hippocampus. Within the hippocampus glutamatergic afferents to pyramidal neurons are believed to be localized to distal regions of the dendrites (Isaacson and Pribram, 1975; Monaghan and Cotman, 1982; Collinridge et al., 1983a, b; Harris and Cotman, 1983; Sawada et al., 1983; Foster and Fagg, 1984; Greenamyre et al., 1984; Geddes et al., 1986). The observation in the present study of a pronounced sensitivity of dendritic versus axonal outgrowth to glutamate is consistent with a localization of functional glutamate receptors on dendrites but not the axon. Since glutamate provides the major excitatory input to pyramidal neuron dendrites *in situ* (Isaacson and Pribram, 1975; Collinridge et al., 1983a, b), it is reasonable to consider that incoming glutamate-releasing axons might regulate the dendritic morphologies of the pyramidal neurons. In the present study we used as a model of this developmental scenario isolated pyramidal neurons whose dendritic growth cones were exposed to a micropipette releasing glutamate. In this case the micropipette was our representation of a glutamate-releasing axon. The results of these experiments demonstrated that, indeed, glutamate can locally affect the outgrowth of individual dendritic growth cones. In fact, the dendritic growth cones to which glutamate was applied regressed

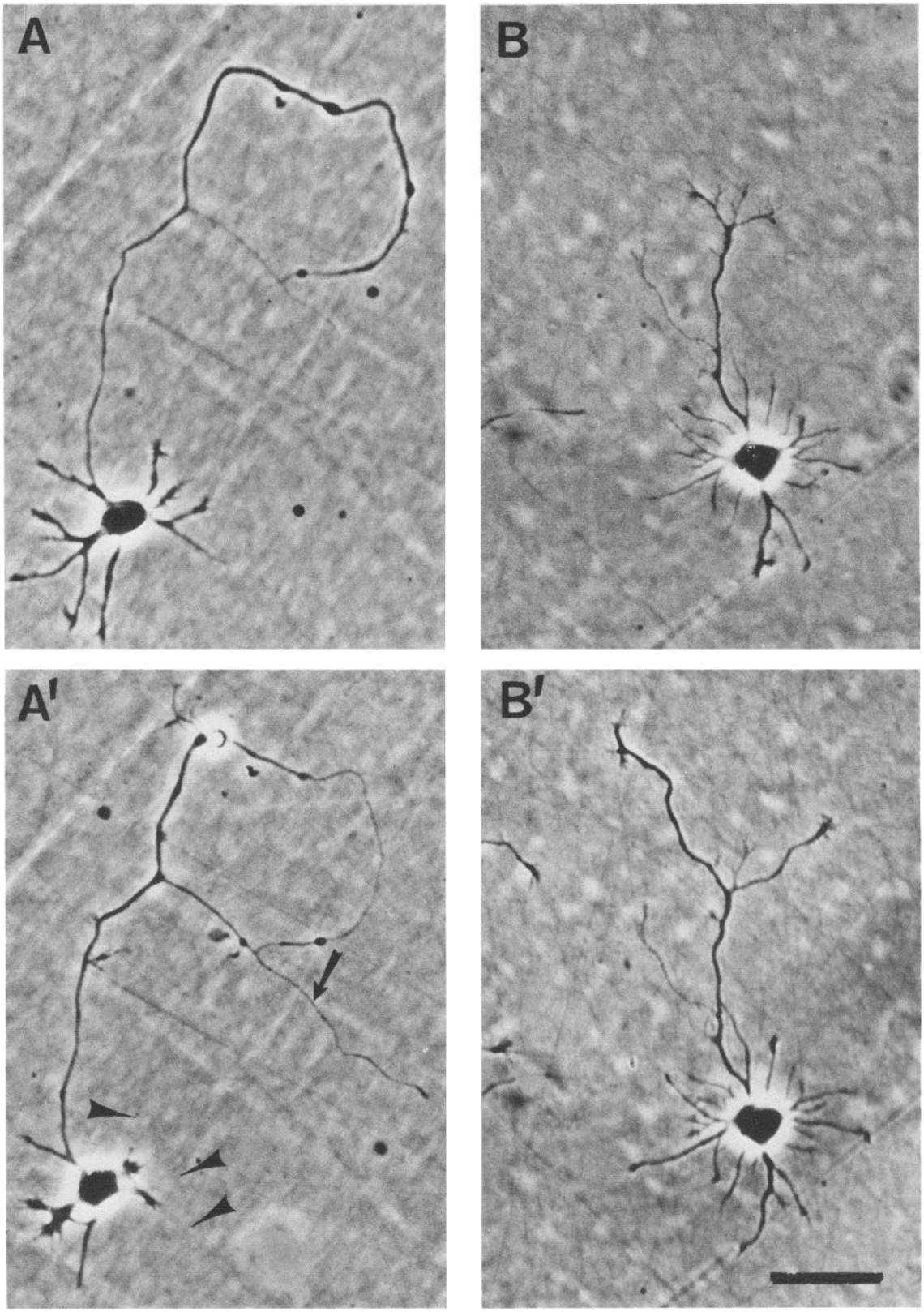

Figure 8. Glutamate receptor antagonist DGG prevents dendritic pruning. *A, A',* Four-day-old neuron before (*A*) and 20 hr after exposure to 50 μM glutamate (*A'*); note that dendrites are reduced in length while the axon has continued to elongate. *Arrows* and *arrowheads* in *A'* demarcate the location of axonal and dendritic tips, respectively, prior to glutamate exposure (*A*). *B and B',* Neuron before (*B*) and 20 hr after exposure to 5 mM DGG plus 50 μM glutamate; note that both the axon and dendrites have continued to elongate. Scale bar, 25 μm.

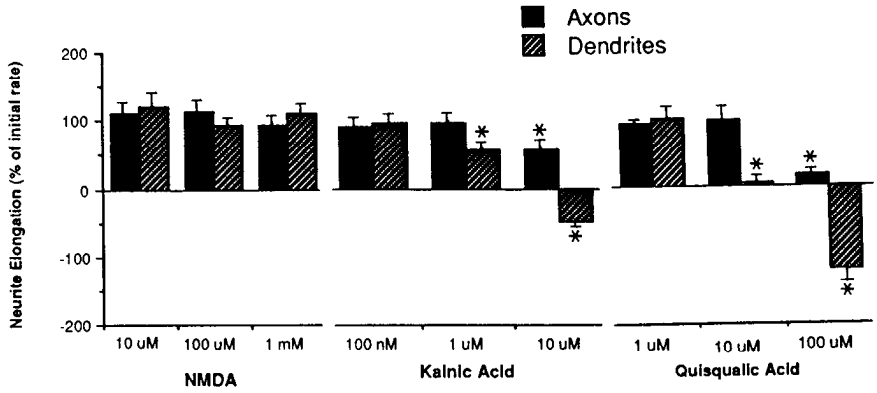

Figure 9. Specificity and dose dependence of the effects of excitatory amino acid transmitters on dendritic and axonal outgrowth. Axonal and dendritic outgrowth rates of 4-d-old pyramidal-like neurons were quantified for a 2 hr period prior to exposure to the indicated EAAs and for three 2 hr periods after exposure. Values are posttreatment outgrowth rates expressed as a percentage of the initial pretreatment rate (mean and SEM of determinations made on from 3 to 7 axons and from 8 to 15 dendrites). *$p < 0.05$–0.001 compared with control (100%) rates.

dramatically, while the outgrowth of distant dendrites was relatively unaffected. The exact concentration of glutamate reaching the growth cones under these conditions is not known, but a comparison of the degrees of regression of dendrites exposed to bath-applied glutamate suggests that the level was approximately 50–100 μM. While it is not clear whether these results *in vitro* represent mechanisms functional *in vivo,* it is clear that nontoxic levels of glutamate can exert localized, specific effects on pyramidal neuron dendritic morphology.

Other neurotransmitters are also likely to influence the dendritic architecture of pyramidal neurons. In *Helisoma* it has been shown that different neurotransmitters can interact to regulate neuronal outgrowth. McCobb and Kater (1986) demonstrated that ACh, which suppresses electrical activity in specific *Helisoma* neurons, was able to prevent the suppression of neurite outgrowth normally caused by the depolarizing neurotransmitter serotonin. It is well-known that GABA acts to suppress electrical activity in hippocampal pyramidal neurons (Isaacson and Pribram, 1975). It is therefore possible that GABA might modify the negative effects of glutamate on dendritic outgrowth.

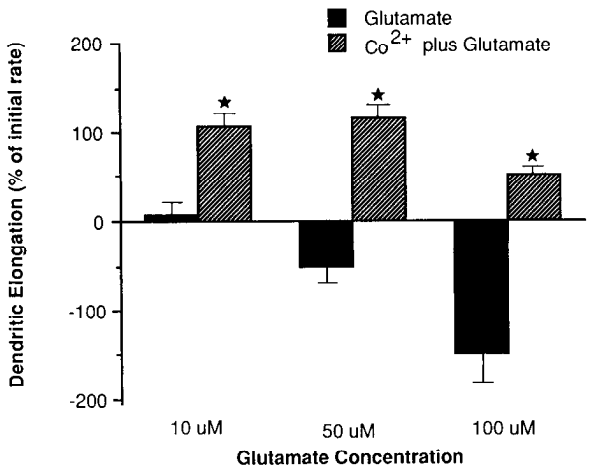

Figure 10. The calcium channel blocker Co^{2+} reduces the effects of glutamate on dendritic outgrowth. Dendritic elongation rates of 4-d-old neurons were quantified for a 2 hr period prior to exposure to the indicated agents and for three 2 hr periods after exposure. The concentration of Co^{2+} was 100 μM (this level of Co^{2+} alone did not alter axonal or dendritic elongation rates, $n = 8$ neurons). Values represent the mean and SEM of determinations made on 4 or 5 axons and 11 to 15 dendrites. *$p < 0.01$–0.001 compared with the values for neurons exposed to glutamate alone.

The results of preliminary experiments in this laboratory suggest that, indeed, GABA plus the potentiator of GABA inhibition diazepam can prevent the dendritic pruning normally seen in response to glutamate (Mattson et al., 1987). The cumulative effects of different neurotransmitters acting upon the same cell may therefore be involved in determining the overall shape of the neuron. Clearly, such interactive regulation of neuronal outgrowth would contribute to the tremendous complexity of neuronal form seen in the brain.

Glutamate receptors mediating cytoarchitectural changes

Past studies of synaptic potentials in adult rat hippocampus indicated the presence of at least 3 major receptor types, including KA-, QA-, and NMDA-preferring receptors (Collinridge et al., 1983a, b; Dingledine, 1983; Fagni et al., 1983; Sawada et al., 1983). In contrast, central neurons of immature rats may have reduced levels of functional glutamate receptors (Coyle et al., 1981; Olney, 1983). In the present study we found that the outgrowth and survival of embryonic pyramidal neurons was sensitive to KA and QA but not to NMDA. These results suggest that NMDA receptors are not expressed or are not functional in isolated embryonic pyramidal neurons, at least during the early stages of outgrowth in cultures studied here. Recently, Rothman (1985) reported that NMDA was toxic to all neurons in high-density, long-term (weeks) cultures initiated from E18

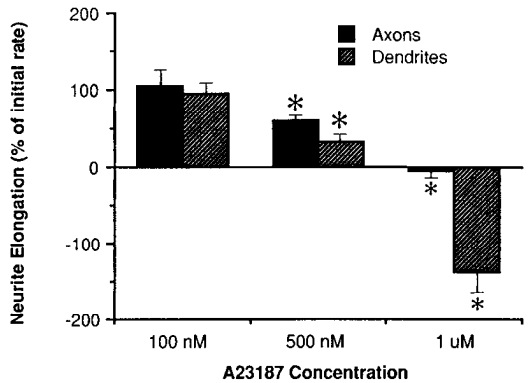

Figure 11. Dose-dependent effects of calcium ionophore A23187 on neuroarchitecture. Axonal and dendritic outgrowth rates of 4-d-old neurons were quantified for a 2 hr period prior to exposure to A23187 at the indicated concentrations and for three 2 hr periods after exposure. Values represent the mean and SEM of determinations made on 4 or 5 axons and 9 to 14 dendrites. *$p < 0.05$–0.001 compared with control (100%) values.

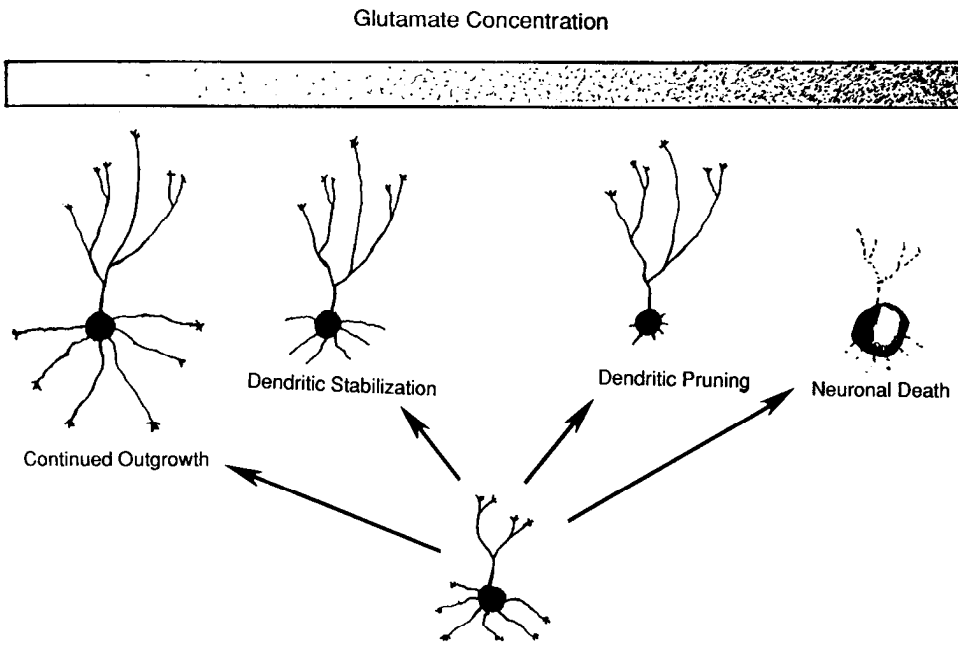

Figure 12. Regulation of hippocampal pyramidal neuron dendritic architecture by glutamate. A growing neuron may encounter different levels of glutamate within the proximate environment. Very low levels do not alter the course of outgrowth (*left*); moderate levels lead to dendritic stabilization, while further increases in glutamate concentration cause a reduction in dendritic length. At very high levels, glutamate is toxic. See text for details.

rat fetuses. The insensitivity of neurons in the present study to NMDA may be due to either their lack of contact with other cells or their relative immaturity. It is possible that neurons grown in isolation do not express functional NMDA receptors and that cell interactions occurring in high-density cultures may induce the expression of this class of EAA receptors. On the other hand, it is also reasonable to consider that NMDA receptors may be expressed with time as the result of a programmed scenario of differentiation. In any case, the information derived from the present and past studies is consistent with the possibility that non-NMDA glutamate receptors may be involved in regulating dendritic outgrowth during embryonic development, while all 3 cases of receptors have the potential to modify neuroarchitecture in the adult brain.

Selective neuronal sensitivity to excitatory amino acids

In the present study we found that isolated bipolar and stellate neurons were resistant to the toxic effects of glutamate, KA, and QA, while nearly all pyramidal neurons were killed by these EAAs. It is unclear why approximately 15% of the pyramidal neurons were resistant to glutamate. One possibility is that the insensitive and sensitive neurons were at different developmental stages. Indeed, a small percentage of the cells taken from the hippocampi of 18 d embryos undergo at least one cell division and differentiate as pyramidal-like neurons in culture (M. P. Mattson, unpublished observation). The cellular basis of differential sensitivity to glutamate may be related to differences in EAA receptors or in intrinsic differences in the cellular mechanisms mediating cell death. Our results tend to favor the former possibility. Thus, we found that manipulations expected to bypass the glutamate receptor step in the excitotoxic pathway (excess calcium influx induced by A23187 or high levels of extracellular K^+) were toxic to all pyramidal neurons, suggesting that perhaps all neurons can be killed by excess depolarization. However, Choi (1987) found that mouse neocortical neurons resistant to glutamate neurotoxicity nevertheless exhibited robust electophysiological responses to glutamate similar to those seen in vulnerable neurons. The latter result is consistent with intrinsic differences in susceptibility to overexcitation. It will clearly be of interest to examine further the mechanisms underlying selective neuronal vulnerability to EAA toxicity.

Cellular mechanism of glutamate action

Several lines of evidence have implicated calcium as a mediator of the effects of neurotransmitters and electrical activity on neuronal outgrowth (Cohan et al., 1987; Mattson and Kater, 1987, Mattson et al., 1987) and survival (Coyle et al., 1981; Berdichevsky et al., 1983; Coyle et al., 1981; Donaldson et al., 1983; Mayer and Westbrook, 1987). Studies in invertebrate central neurons demonstrated that serotonin and electrical activity can suppress neurite outgrowth by causing calcium influx (Cohan et al., 1987; Mattson and Kater, 1987). Serotonin apparently induced calcium influx by activating voltage-dependent calcium channels. In the case of neurotoxicity, calcium involvement has been proposed based on studies in which EAAs caused increased calcium uptake in brain regions (Griffiths et al., 1984; Simon et al., 1984a) and cultured central neurons (Retz and Coyle, 1984) susceptible to EAA toxicity. In addition, manipulations expected to reduce calcium influx prevented glutamate toxicity in cortical neurons (Choi, 1987), neuroblastoma cells (Murphy et al., 1986), and hippocampal pyramidal neurons (present study). Recently, Kudo and coworkers (Kudo and Ogura, 1986; Kudo et al., 1987) used the calcium indicator dye Fura 2 to directly demonstrate glutamate-induced increases in intracellular calcium levels in hippocampal neurons. In the present study, we found that Co^{2+} prevented both glutamate neurotoxicity and the effects of subtoxic levels of glutamate on dendritic outgrowth. We also found that high levels of A23187 were toxic and that nontoxic levels of A23187 could reduce the lengths of dendrites and axons. Clearly, our findings add further support for the involvement of calcium in neurotransmitter effects on neuronal outgrowth and survival. However, calcium influx may not be the only mechanism involved. Thus, Rothman (1985) reported that the acute neurotoxicity of EAAs is mediated by a calcium-independent mechanism related to passive chloride influx, while Choi (1987) provided evidence that a calcium-independent

mechanism mediates acute (min) EAA-induced cell death in cerebral neurons, while a calcium-dependent mechanism is involved in more chronic (hr) toxicity.

The mechanism by which glutamate induces calcium influx is probably mediated by membrane depolarization leading to activation of voltage-dependent calcium channels. We found that elevating the level of extracellular K^+ resulted in a dose-dependent regression of both dendrites and the axon. Very high levels of K^+ were toxic. The facts that glutamate selectively affected dendritic outgrowth while elevated K^+ affected both axons and dendrites suggests that activated glutamate receptors cause local membrane depolarizations which lead to local increases in calcium influx and therefore localized outgrowth responses. Direct demonstrations of this possibility will require local measurements of intracellular calcium levels in axons and dendrites of pyramidal neurons exposed to glutamate.

A comparison of the effects of A23187 on axonal and dendritic outgrowth reveals that dendrites are more sensitive to this calcium-elevating agent than are axons (Fig. 11). Since A23187 is likely to promote calcium influx similarly in axons and dendrites, these results indicate that there may be differences in cytoskeletal elements or regulatory molecules responsive to calcium in these two processes. It is well known that axons and dendrites differ in certain cytoskeletal-related proteins such as microtubule-associated proteins (Caceres et al., 1984, 1986). Thus, it is possible that these proteins, and/or other yet to be identified proteins which may be involved in neurite outgrowth, may have differential sensitivities to a calcium stimulus. Potential factors which merit consideration in future studies include calcium binding proteins such as calmodulin (Means et al., 1982) and calcium-dependent proteinases, which are known to degrade cytoskeletal elements (Nixon et al., 1986).

An hypothesis for glutamate regulation of hippocampal cytoarchitecture

The results of the present study demonstrate that different levels of excitatory glutamatergic input to pyramidal cell dendrites modify their structure in a predictable and progressive manner (Fig. 12). While it is unclear to what extent our results *in vitro* extend to the intact developing and functioning brain, several previous findings *in situ* merit discussion in light of our findings. The progression of dendritic outgrowth and stabilization during development has been well documented. In addition, there is considerable evidence that dendritic architecture undergoes significant changes throughout adult life (Beull and Coleman, 1979; Purves et al., 1986). Furthermore, structural correlates of brain function have also been found; plastic structural changes in synapses on hippocampal neuron dendrites occur during the long-term potentiation of synaptic transmission believed to underlie learning and memory (Desmond and Levy, 1983; Lynch, 1986). Finally, *in situ* data are consistent with the hypothesis that glutamate overactivity can lead to neurodegeneration (Maragos et al., 1987).

It is striking that the scenario of glutamate effects on neuroarchitecture observed in isolated hippocampal neurons in the present study is similar to the kinds of graded neurodegenerative changes seen *in situ* in several brain pathologies in which hippocampal pyramidal neurons are selectively vulnerable. Indeed, a comparison of our summary Figure 12 with Golgi-stained hippocampus of patients with increasing severities of epilepsy (Paul and Scheibel, 1986) or Alzheimer's disease (Mehraein et al., 1975) reveals a similar progression of alterations in neuroarchitecture. Furthermore, our observations of refractoriness of axonal outgrowth to EAAs in the present study is entirely consistent with the axon-sparing nature of EAA-induced lesions observed *in situ* (Schwartz et al., 1983). Several investigators have proposed that the degeneration of neurons in these pathological states may be mediated by EAAs (Schwartz et al., 1984; Maragos et al., 1987). Of further interest in relation to the etiology of neurodegeneration is our finding that the dendritic pruning caused by glutamate was not readily reversible. If this characteristic of the dendrite-pruning effect of subtoxic levels of glutamate in cultured neurons is maintained *in vivo,* then it is likely that even relatively acute exposures of pyramidal neurons to glutamate would cause a long-lasting effect on neuronal architecture.

The results of the present study demonstrate that, in addition to its role in interneuronal electrophysiological communication, glutamate can act as a regulator of neuronal form. These initial findings indicate that neurotransmitters have the potential for modifying neuroarchitecture in normal development and adult plasticity, as well as in neurodegenerative disorders. Under normal conditions of brain function, it is clear that neurotransmitters such as glutamate act within finely tuned ranges which allow for the expression of the normal behavioral repertoire of the organism and associated changes in neuronal cytoarchitecture. However, if neurotransmitter activity lies beyond the normal range, abnormal changes in dendritic architecture and even cell death can result. It should therefore be considered that several neurodegenerative disorders including Alzheimer's disease, epilepsy, and stroke may involve alterations of neurotransmitter action which under normal conditions allow for the proper maintenance and modulation of neuronal cytoarchitecture.

References

Banker, G. A., and W. M. Cowan (1977) Rat hippocampal neurons in dispersed cell culture. Brain Res. *126:* 397–425.

Banker, G. A., and W. M. Cowan (1979) Further observations on hippocampal neurons in dispersed cell culture. J. Comp. Neurol. *187:* 469–494.

Berdichevsky, E., N. Riveros, S. Sanchez-Armass, and F. Orrego (1983) Kainate, N-methylaspartate and other excitatory amino acids increase calcium influx into rat brain cortex cells *in vitro.* Neurosci. Lett. *36:* 75–80.

Beull, S. J., and P. D. Coleman (1979) Dendritic growth in the aged human brain and failure of growth in senile dementia. Science *206:* 854–856.

Bottenstein, J. E., and G. H. Sato (1979) Growth of a rat neruoblastoma cell line in serum-free supplemented medium. Proc. Natl. Acad. Sci. USA *76:*514–519.

Caceres, A., G. A. Banker, O. Steward, L. Binder, and M. Payne (1984) MAP2 is localized to the dendrites of hippocampal neurons which develop in culture. Dev. Brain Res. *13:* 314–318.

Caceres, A., G. A. Banker, and L. Binder (1986) Immunocytochemical localization of tubulin and microtubule-associated protein 2 during the development of hippocampal neurons in culture. J. Neurosci. *6:* 714–722.

Chang, F. L. F., and W. T. Greenough (1984) Transient and enduring morphological correlates of synaptic activity and efficacy in the rat hippocampal slice. Brain Res. *309:* 35–46.

Choi, D. W. (1987) Ionic dependence of glutamate neurotoxicity. J. Neurosci. *7:* 369–379.

Choi, D. W., M. Maulucci-Gedde, and A. R. Kriegstein (1987) Glutamate neurotoxicity in cortical cell culture. J. Neurosci *7:*357–368.

Cohan, C. S., J. A. Connor, and S. B. Kater (1987) Electrically and chemically mediated increases in intracellular calcium in neuronal growth cones. J. Neurosci. *7:* 3588–3599.

Collinridge, G. I., S. J. Kehl, and H. McLennan (1983a) The antagonism of amino acid-induced excitations of rat hippocampal CA_1 neurons *in vitro.* J. Physiol. (Lond.) *334:* 19–31.

Collinridge, G. L., S. J. Kehl, and H. McLennan (1983b) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol. (Lond.) *334:* 34–46.

Coyle, J. T., and R. Schwartz (1976) Lesion of striatal neurons with kainic acid provides a model for Huntington's chorea. Nature *263:* 244–246.

Coyle, J. T., S. J. Bird, R. H. Evans, R. L. Gulley, J. V. Nadler, W. J. Nicklas, and J. W. Olney (1981) Excitatory amino acid neurotoxins: Selectivity, specificity, and mechanism of action. Neurosci. Res. Prog. Bull. *19:* 331–427.

Desmond, N., and W. B. Levy (1983) Synaptic correlates of associative potentiation/depression: An ultrastructural study in the hippocampus. Brain Res. *265:* 21–31.

Dingledine, R. (1983) N-methylaspartate activates voltage-dependent calcium conductance in rat hippocampal pyramidal cells. J. Physiol. (Lond.) *343:* 385–405.

Donaldson, P. L., I. Duce, and P. Usherwood (1983) Calcium accumulation precedes the degenerative effects of L-glutamate on locust muscle fibre. Brain Res. *274:* 261–265.

Fagni, L., M. Baudry, and G. Lynch (1983) Classification and properties of acidic amino acid receptors in hippocampus. I. Electrophysiological studies of an apparent desensitization and interaction with drugs which block transmission. J. Neurosci. *3:* 1538–1546.

Fonnum, F. (1984) Glutamate: A neurotransmitter in mammalian brain. J. Neurochem. *42:* 1–11.

Foster, A. C., and G. E. Fagg (1984) Acidic amino acid binding sites in mammalian neuronal membranes: Their characteristics and relationship to synaptic receptors. Brain Res. Rev. *7:* 103–164.

Frosch, M. P., M. D. Phillips, E. Aizenman, D. L. Tauck, and S. A. Lipton (1986) Nicotinic cholinergic blocking agents enhance process outgrowth by solitary tract retinal ganglion cells in culture. Soc. Neurosci. Abstr. *12:* 1505.

Geddes, J. W., J. Chang-Chiu, S. M. Cooper, I. T. Lott, and C. W. Cotman (1986) Density and distribution of NMDA receptors in the human hippocampus in Alzheimer's disease. Brain Res. *399:* 156–161.

Goldberg, J. I., P. Natvig, and S. B. Kater (1986) Embryonic and postembryonic development of the serotonin phenotype in *Helisoma.* Soc. Neurosci. Abstr. *12:* 1163.

Greenamyre, T., A. B. Young, and J. B. Penney (1984) Quantitative autoradiographic distribution of the L-[^{3}-H] glutamate-binding sites in rat central nervous system. J. Neurosci. *4:* 2133–2144.

Griffiths, T., M. C. Evans, and B. S. Meldrum (1984) Status epilepticus: The reversibility of calcium loading and acute neuronal pathological changes in the rat hippocampus. Neuroscience *12:* 557–567.

Hagiwara, S., and L. Byerly (1981) Calcium channel. Annu. Rev. Neurosci. *4:* 69–125.

Harris, F. W., and C. W. Cotman (1983) Effects of acidic amino acid antagonists on paired-pulse potentiation at the lateral perforant path. Exp. Brain Res. *52:* 455–460.

Haydon, P. G., D. P. McCobb, and S. B. Kater (1984) Serotonin selectively inhibits growth cone dynamics and synaptogenesis of specific identified neurons of *Helisoma.* Science *226:* 561–564.

Haydon, P. G., C. S. Cohan, D. P. McCobb, H. R. Miller, and S. B. Kater (1985) Neuron-specific growth cone properties as seen in identified neurons of *Helisoma.* J. Neurosci. Res. *13:*135–147.

Isaacson, R. L., and K. H. Pribram, Eds. (1975) *The Hippocampus,* Vols. 1 and 2, Plenum, New York.

Jones, P. G., S. J. Rosser, and A. G. M. Bulloch (1986) Glutamate enhancement of neurite outgrowth in *Helisoma* neurons. Soc. Neurosci. Abstr. *12:* 509.

Kater, S. B., and M. P. Mattson (1988) Extrinsic and intrinsic regulators of neuronal outgrowth and synaptogenesis in identified *Helisoma* neurons in isolated cell culture. In *Cell Culture Approaches to Invertebrate Neurosciences,* D. J. Beadle and S. B. Kater, eds., Academic, London.

Kudo, Y., and A. Ogura (1986) Glutamate-induced increase in intracellular Ca^{2+} concentration in isolated hippocampal neurons. Br. J. Pharmacol. *89:* 191–198.

Kudo, Y., K. Ito, H. Miyakawa, Y. Izumi, A. Ogura, and H. Kato (1987) Cytoplasmic calcium elevation in hippocampal granule cell induced by perforant path stimulation and L-glutamate application. Brain Res. *407:* 168–172.

Lankford, K. L., F. G. DeMello, and W. L. Klein (1986) Transient D1 receptor mediates dopamine inhibition of growth cone motility and neurite outgrowth in a subset of vertebrate CNS neurons. Soc. Neurosci. Abstr. *12:* 1116.

Lee, K., F. Schottler, M. Oliver, and G. Lynch (1980) Brief bursts of high frequency stimulation produce two types of structural changes in rat hippocampus. J. Neurophysiol. *44:* 247–258.

Lynch, G. (1986) *Synapses, Circuits, and the Beginnings of Memory,* MIT Press, Cambridge, MA.

Maragos, W. F., J. T. Greenamyre, J. B. Penney, and A. B. Young (1987) Glutamate dysfunction in Alzheimer's disease: An hypothesis. Trends Neurosci. *10:* 65–68.

Mattson, M. P., and S. B. Kater (1987) Calcium regulation of neurite elongation and growth cone motility. J. Neurosci. *7:* 4034–4043.

Mattson, M. P., P. Dou, and S. B. Kater (1987) Pruning of hippocampal pyramidal neuron dendritic architecture *in vitro* by glutamate and a protective effect of GABA plus diazepam. Soc. Neurosci. Abstr. *13:* 367.

Mattson, M. P., A. Taylor-Hunter, and S. B. Kater (1988) Neurite outgrowth in individual neurons of a neuronal population is differentially regulated by calcium and cyclic AMP. J. Neurosci. *8:* 1704–1711.

Mayer, M. L., and G. L. Westbrook (1987) Cellular mechanisms underlying excitotoxicity. Trends Neurosci. *10:* 59–61.

McCobb, D. P., and S. B. Kater (1986) Serotonin inhibition of growth cone motility is blocked by acetylcholine. Soc. Neurosci. Abstr. *12:* 1117.

Means, A. R., J. S. Tash, and J. G. Chafouleas (1982) Physiological implications of the presence, distribution, and regulation of calmodulin in eukaryotic cells. Physiol. Rev. *62:* 1–39.

Mehraein, P., M. Yamada, and E. Tarnowska-Dziduszko (1975) Quantitative study on dendrites and dendritic spines in Alzheimer's disease and senile dimentia. Adv. Neurol. *12:* 453–458.

Meldrum, B. (1985) Possible therapeutic applications of antagonists of excitatory amino acid neurotransmitters. Clin. Sci. *68:* 113–132.

Miller, R. J. (1987) Multiple calcium channels and neuronal function. Science *235:* 46–52.

Monaghan, D. T., and C. W. Cotman (1982) The distribution of ^{3}H-kainic acid binding sites in rat CNS as determined by autoradiography. Brain Res. *252:* 91–100.

Murphy, T. H., R. L. Schnaar, K. Sandberg, A. Malouf, and J. T. Coyle (1986) Excitotoxin mediated lysis of neuroblastoma cells in culture is decreased by quabain and calcium antagonists. Soc. Neurosci. Abstr. *12:* 343.

Nixon, R. A., R. Quackenbush, and A. Vitto (1986) Multiple calcium-activated neutral proteinases (CANP) in mouse retinal ganglion cell neurons: Specificities for endogenous neuronal substrates and comparison to purified brain CANP. J. Neurosci. *6:* 1252–1263.

Olney, J. W. (1978) Neurotoxicity of excitatory amino acids. In *Kainic Acid as a Tool in Neurobiology,* E. G. McGeer, J. W. Olncy, and P. L. McGeer, eds., pp. 95–121, Raven, New York.

Olney, J. W. (1983) Excitotoxins: An overview. In *Excitotoxins,* K. Fuxe, P. Roberts, and R. Schwartz, eds., pp. 82–96, MacMillan, London.

Paul, L. A., and A. B. Scheibel (1986) Structural substrates of epilepsy. Adv. Neurol. *44:* 775–786.

Purves, D., R. D. Hadley, and J. Voyvodic (1986) Dynamic changes in the dendritic geometry of individual neurons visualized over periods of up to three months in the superior cervical ganglion of living mice. J. Neurosci. *6:* 1051–1060.

Retz, K. C., and J. Coyle (1984) The differential effects of excitatory amino acids on uptake of $^{45}CaCl_2$ by slices from mouse striatum. Neuropharmacology *23:* 89–94.

Rothman, S. M. (1985) The neurotoxicity of excitatory amino acids is produced by passive chloride influx. J. Neurosci. *5:* 1483–1489.

Rothman, S. (1984) Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. J. Neurosci. *4:* 1884–1891.

Sawada, S., S. Takada, and C. Yamamoto (1983) Selective activation of synapses near the tip of drug-ejecting microelectrode, and effects of antagonists of excitatory amino acids in the hippocampus. Brain Res. *267:* 156–160.

Schwartz, R., W. O. Whetsell, and R. M. Mangano (1983) Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. Science *229:* 315–318.

Schwartz, R., A. C. Foster, E. D. French, W. O. Whetsell, and C. Kohler

(1984) Excitotoxic models for neurodegenerative disorders. Life Sci. *35:* 19–32.

Simon, R. P., T. Griffiths, M. C. Evans, J. H. Swan, and B. S. Meldrum (1984a) Calcium overload in selectively vulnerable neurons of the hippocampus during and after ischemia: An electron microscopy study in the rat. J. Cereb. Blood Flow Metab. *4:* 350–361.

Simon, R. P., J. H. Swan, T. Griffiths, and B. S. Meldrum (1984b) Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. Science *226:* 850–852.

Watkins, J. C., and R. H. Evans (1981) Excitatory amino acid transmitters. Annu. Rev. Pharmacol. Toxicol. *21:* 165–204.

Wieloch, T. (1985) Neurochemical correlates to selective neuronal vulnerability. Prog. Brain Res. *63:* 69–85.