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Long-Term Potentiation and Activity-Dependent Retinotopic Sharpening in the Regenerating Retinotectal Projection of Goldfish: Common Sensitive Period and Sensitivity to NMDA Blockers

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The regenerating retinotectal projection in goldfish goes through an activity-driven refinement that appears to involve the elimination of inappropriate branches from early arbors. Retinotopically appropriate branches may be stabilized because the normally correlated firing of neighboring ganglion cells causes summation of their postsynaptic responses and increases their effectiveness by a Hebbian mechanism. In this study, I report that the regenerating projection has an increased capacity for long-term potentiation (LTP) that may be related to the activity-driven sharpening. In the normal projection, field potentials, reflecting currents from EPSPs elicited by optic nerve shock, are large (>4 mV) and very stable. In newly regenerated projections, field potentials are initially small (<1 mV), but a train of 20 stimuli at 0.1 Hz results in a large (100–200%) increase in amplitude that is stable for at least 8 hr, and in 3 cases overnight. The capacity for potentiation is greatest from 20 to 40 d postcrush, the time just after arrival of the optic fibers, and during the period of retinotopic sharpening. A greater-than-normal capacity for potentiation persists for many months. Topical application of NMDA receptor blockers AP5 or AP7 at 25 μM prevents potentiation without decrementing ongoing responses. The closely related agent AP6, which is not an NMDA receptor blocker, does not prevent potentiation. In addition, infusion of the NMDA receptor blockers AP5 or AP7 into the tectal ventricle (4 μl/d of 500 μM solution) for 2–3 weeks during regeneration prevented retinotopic sharpening, as assessed by electrophysiological mapping. At each tectal point, responsive areas in the visual field were enlarged to 28° vs 11–12° in control regenerates and normals. This was comparable to data from fish regenerating with activity blocked with intraocular tetrodotoxin or synchronized by stroboscopic illumination and indicates uncorrected errors in targeting of regenerated arbors (Schmidt, 1985). The results support the involvement of NMDA receptors in sharpening and suggest that the initial step in stabilizing appropriate branches may be a long-lasting increase in synaptic gain.

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During visual development and regeneration, many of the fine details of circuitry appear to be established by the selective stabilization of appropriate synapses from an initially more diffuse set of connections, a process that requires normal patterns of visual activity. Features whose development is activity driven include the segregation of ocular dominance patches both in developing mammalian visual cortex (Stryker and Harris, 1986) and in dually innervated tecta of fish and frog (Meyer, 1982; Boss and Schmidt, 1984; Reh and Constantine-Paton, 1985), the segregation of eye-specific afferents onto separate lamina of the lateral geniculate nucleus (LGN; Shatz and Stryker, 1988), the segregation of receptive field types in LGN (Dubin et al., 1986), and the refinement of retinotopic maps both in LGN of cat (Archer et al., 1982) and in tectum of goldfish (Meyer, 1983; Schmidt and Edwards, 1983; Cook and Rankin, 1986). In several cases, the emergence of precise connections appears to require the coincident activation of appropriate afferents to stabilize their inputs onto the common postsynaptic cells, as the sharpening or segregation may be blocked by the synchronization of all inputs as well as by blocking activity in all afferents (Stryker and Strickland, 1984; Schmidt and Eisele, 1985; Cook and Rankin, 1986). Normally coincident activation may derive from correlation between the activity of neighboring, but not distant ganglion cells of the same type, both in their visually driven activity and in their spontaneous activity (Willshaw and von der Malsburg, 1976; Arnett, 1978; Ginsberg et al., 1984; Mastronarde, 1989).

This apparent requirement for coincident activation of converging inputs bears a formal similarity to associative conditioning, one of the model systems for studies of learning and memory. The prevailing view of associative learning is that it involves a change in synaptic circuits, either an increased strength in transmission at existing synapses or an increased number of synapses from the specific inputs (Hebb, 1949; Carew et al., 1981; Thompson et al., 1983; Lynch and Baudry, 1984). A leading model of associative learning is the long-term potentiation (LTP) of various inputs to hippocampus, which involves an immediate increase in the gain of the participating synapses. The potentiations can be triggered either by a direct associative pairing of weak and strong inputs or simply by a sufficiently strong train of stimuli to a single set of inputs (Abraham et al., 1986; Sastry et al., 1986; Gustafsson et al., 1987). In the latter case, the inputs are said to display cooperativity in bringing about a sufficient postsynaptic depolarization (via spatial summation of EPSPs) to activate NMDA receptor gated channels

which then trigger the potentiation of synaptic responses (Harris et al., 1984; Kelso et al., 1986).

Recently, several studies have supported a role for NMDA receptors in the development of the visual system. In cat visual cortex, Kleinschmidt et al. (1987) reported that infusion of the NMDA receptor antagonist AP5 prevented the changes in ocular dominance produced by monocular deprivation. In dually innervated tectum of the 3-eyed frog, Cline et al. (1987) reported that AP5 desegregated eye-specific stripes. In addition, Komatsu et al. (1988) reported a capacity for LTP in cat visual cortex that was present only during the critical period, although they did not test whether the LTP was sensitive to NMDA receptor antagonists.

In this study, I report that the regenerating retinotectal projection displays a remarkably increased capacity for LTP during the period when it is undergoing an activity-driven sharpening of its retinotopic map. This LTP, like that in hippocampus, requires strong spatial summation, and it involves the activation of NMDA receptors, since it can be blocked by low concentrations of AP5 and AP7. In addition, I also report that the sharpening of the map is sensitive to NMDA receptor blockers chronically infused from an osmotic minipump during the time of sharpening. The results show that AP5 and AP7 (but not AP6) specifically block sharpening in the regenerating projection without disrupting the existing sharp retinotopic map in the nonregenerating projection. Thus, the enhanced capacity for LTP and the retinotopic sharpening share a common time course and sensitivity to NMDA receptor blockers. Abstracts of this work appeared previously (Schmidt, 1987, 1988).

Materials and Methods

Crushing of the optic nerve. Common goldfish (11–15 cm in length) were purchased from Grassyforks Fisheries (Martinsville, IN) and kept at 20°C. Following anesthesia by immersion in a 0.1% solution of tricaine methanesulfonate (TMS, Crescent Res. Chem., Paradise Valley, AR), the nerve was exposed and crushed in the orbit under visual control using fine curved forceps. The sheath and retinal artery were left intact, but a complete break in the white myelin of the nerve could be seen through the translucent sheath. The fish were then revived and divided into 2 groups. In the first group, a minipump was implanted into each fish for chronic infusion of NMDA receptor blockers or control solutions to test for effects on the sharpening of the map (Table 1). Fish in the second group were used at various time points after nerve crush to study potentiation of field potential responses in tectum.

Implantation of minipumps. At 20–22 d postcrush, the fish were again anesthetized for implantation of the minipumps (Schmidt and Shashoua, 1988). The dorsal cranium was opened to expose the tecta, and the tapered tip of a cannula (made from PE tubing) was inserted through the tectal commissure for infusion of Ringer's solutions into the tectal ventricle. The cannula was fixed in place to prevent any motion of the tip (by flanges where it penetrated the cranial wall and by dental acrylic over the cranium) as previously described (Schmidt and Shashoua, 1988). To ensure the immediate onset of action for the AP5, AP6, or AP7, 5 μl of the same solution was applied to the tectal surface before resealing of the cranium with cyanoacrylate glue. An osmotic minipump (Model 2002, capacity 220 μl, Alzet Corp., Palo Alto, CA) was inserted into the end of the cannula (about 1 cm above the fish's head) and remained tethered to the fish's head as it swam (Fig. 1). The pump was weighed before and after filling to determine the starting volume. After removal, the remaining volume was carefully removed and measured with a blunt-tipped Hamilton microliter syringe, and the difference was taken as the amount delivered over the 2–3 weeks that the pump was in place. The pumps averaged from 2.3 to 5.3 μl/d delivered to the tectal ventricles. Since the fluid cavities in and around the brain total about 150–200 μl, a substantial dilution of the solution (about 50×) must have occurred. These fluid volumes consist of 3 parts: cerebrospinal fluid (CSF), extracellular fluid (ECF), and extradural fluid (EDF). The EDF volume is by far the largest as the fish's brain is recessed several mm from the cranium, and it contains at least 150 μl in these fish, measured by extraction with glass capillary tubing. The ECF and CSF together are assumed to be about ⅓ of the total brain weight of 75 mg, or about 25 μl.

Tectal unit recordings and mappings. The fish were reanesthetized and the tectum reexposed by cutting away the dorsal cranial plate. The fish was placed in an eye-in-water apparatus designed so that the eye looks out onto a water-filled hemisphere for mapping visual units recorded from the tectal neuropil as previously described (Schmidt and Edwards, 1983). Briefly, these units were the arbors of retinal ganglion cells and were recorded with platinum-tipped, Wood's metal-filled pipettes. Routinely several units were recorded at each point in tectum, and the combined responsive area of the visual field, termed the multiunit receptive field, was mapped to determine the degree of convergence. When these areas were circular, the diameter was taken as a measure of their extent. When the areas were elongated, the average of the long and short axes was used. Occasionally, single units could be discriminated by spike height and were mapped separately. Magnification factors, the number of micrometers on the tectal surface that correspond to 1° of visual angle in the map, were computed for the 2 dimensions of each map using linear regression, as previously described (Schmidt and Edwards, 1983).

Potentiation of field potentials. The method was similar to that described by Schmidt (1979) and Schmidt et al. (1983). Fish were anesthetized in TMS, the cranium was opened to expose the tecta, and the eye was removed to expose the stump of the optic nerve. The fish was placed in an apparatus that was chilled by ice in an outer chamber. The fish was maintained on dilute (0.005%) TMS that was prechilled by flowing through coils in an ice chest. The temperature of the fish was monitored via a miniature thermometer in the fish's gills and was kept within the range of 12–14°C. Fish at this temperature remain viable for more than 48 hr. A concentric bipolar suction electrode filled with Ringer's solution was placed over the nerve stump, and it held the nerve securely, kept it moist and viable, and reliably and reproducibly stimulated the nerve without need for any adjustments. Field potentials in tectum were elicited by supramaximal stimulation of the optic nerve fibers. For regenerating optic nerves, strengths of 100 V × 1.0 msec were routinely used to ensure activation of all fine regenerating fibers. This was always found to be several times greater than the supramaximal value. DC recordings were made with a Ringer's-filled pipette (2–4 μm tip diameter) from various depths in the tectum relative to a distant ground electrode consisting of a chlorided silver wire inserted behind the cerebellum. Recorded traces could be digitized at 3.2 samples/msec, stored and displayed using an LSI 11/23 microcomputer and a Tektronix digital plotter, or viewed on the screen of a storage oscilloscope. Amplitudes of maximal negative responses recorded from the main retinal recipient layer (Superficial gray and white, SFGS, depth of 125 μm) were measured from the traces. Single traces rather than averaged traces were routinely used to avoid repeated stimulation that, particularly at early postcrush timepoints, could result in potentiation of the responses. NMDA receptor blockers were topically applied to the surface of the tectum in Ringer's solution.

Optomotor testing. To assess whether the NMDA blockers depressed postsynaptic activity, the optomotor response was used as a quantifiable measure of performance in a task known to be mediated by the optic tectum (Springer et al., 1977). Fish were tested in an optomotor testing apparatus similar to that employed by Springer et al. (1977). It consisted of a cylindrical glass tank, 30 cm in diameter, surrounded by a rotating (90°/sec) striped drum, 42 cm in diameter. Alternating black and white vertical stripes were 1.9 cm wide, corresponding to 2.6° of visual angle from the center and substantially more from the edge of the glass tank where the fish usually tracked them.

Each fish went through the following test procedure 3 times. The fish was acclimated for 5 min in the tank with no drum motion. This was followed by 2 min of clockwise (CW) spin with no measurement and 3 min timed with a stopwatch for the number of seconds spent swimming with the stripes. The drum direction was then reversed to counter clockwise (CCW), and 2 min was allowed without measurement, followed by 3 min timed for swimming with the stripes.

The first test was the control test. Then the fish was anesthetized by immersion in ice water and had 2 tiny holes drilled in its cranium over the tecta, the first to allow the insertion of the needle of a Hamilton microliter syringe and the second (located several mm away) to vent any pressure. The first injection was a sham injection, which was followed by a second test 1 hr later. Finally, the fish was injected intra-

cranially with an NMDA receptor blocker (or with kynurenate) through the same hole and tested a third time 1 hr later. The numbers from the CW and CCW timings were then combined to give the total percent of time swimming with the stripes and compared with controls, sham injections, and blank runs (times spent swimming CW with no drum motion).

Results

Long-term potentiation of postsynaptic responses

The immature retinotectal projection formed during regeneration has a greatly enhanced capacity for large and stable increases in the gain of synaptic transmission that can be evoked by short trains of supramaximal stimuli to the optic nerve. This conclusion was reached by recording the field potentials elicited by optic nerve shock in 40 fish at various times after nerve crush and comparing them with 5 normal fish tested in the same manner. The results were very reproducible; potentiations were elicited in 30 of 32 cases attempted. The 2 fish that did not potentiate were recorded in late summer when regeneration as well as the general physiology of the fish is seasonally altered (Schmidt et al., 1988). One fish was discarded (after showing some potentiation) because of a technical failure, and 7 fish were controls. Because the increase is elicited by strong trains of activity, is stable for many hours without decrement, and involves the activation of NMDA receptors, the term "long-term potentiation" (LTP) will be used to describe it, as it has been used to describe a similar phenomenon in cat visual cortex (Komatsu et al., 1988).

The phenomenon of LTP. The basic phenomenon is illustrated in Figures 2 and 3, sets of recordings from the optic terminal layer in tecta at 33 and 39 d after nerve crush. At these early times, the field potentials prior to potentiation were very small (approximately 1 mV), occurred at long latencies (due to the unmyelinated state of the optic fibers), and fatigued very rapidly when the stimulation was repeated at rates as low as 1 Hz, as previously reported (Schmidt et al., 1983). The extracellularly recorded field potentials, even though they are long latency in these developing projections, reflect primarily the monosynaptic EPSPs in the tectal neurons (Schmidt, 1979; Langdon and Freeman, 1986). The long latency is due to the slow conduction velocity of the regenerating axons, as can be seen in the fiber volley that remains under high Mg^{2+}/low Ca^{2+} conditions when the postsynaptic responses are gone (Fig. 3). In favorable cases and as the fibers mature, one can see 3 separate presynaptic

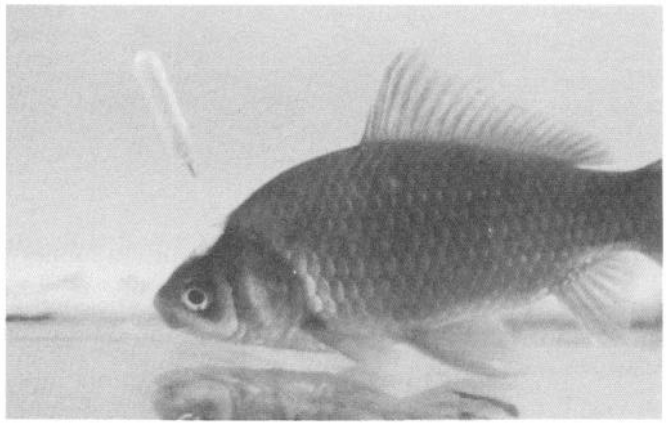

Figure 1. Fish swimming with the osmotic minipump attached to the cannula emerging from the cranium. The fish carried these pumps for an average of 16 d (see Table 1).

Table 1. Multiunit receptive field sizes

Fish/drug	Conc.[a] in pump (mM)	Days of infusion	Flow rate (μl/day)	Day of recording	*n* (RFs)	MURF[b] size (deg.)
Experimental fish						
1. AP5	10	21–45	2.3	58	13	28.1
2. AP5	1	21–35	4.1	53	22	26.4
3. AP7	1	21–33	3.8	63	26	29.3
4. AP7	1	21–33	3.8	65	32	32.5
5. AP7						
Regen. side	0.5	21–35	3.1	91	15	27.4
Normal side	0.5	21–35	3.1	91	28	10.8
6. AP5	0.5	21–34	4.3	105	28	28.6
7. AP5	0.5	21–43	4.1	107	18	24.6
Avg. NMDA receptor blocked		21–37	3.6	83	23	28.1 (0.9 SE)
Control fish						
1. AP6	1.0 mM	20–31	4.5	69	39	10.7
2. AP6	0.5 mM	21–38	5.3	97	33	11.1
3. AP5	0.1 mM	20–32	5.2	53	12	10.9
4. Ringer's	–	21–39	4.7	40	29	10.6
5. GAR	0.25 mg/ml	21–33	4.7	40	39	12.2
6. GAR	0.25 mg/ml	21–33	4.7	43	23	13.1
7. GAR	0.25 mg/ml	21–34	4.6	54	17	12.2
8. Anti-S-100	0.25 mg/ml	21–36	3.3	53	27	12.2
Avg. control		21–35	4.6	56	27	11.6 (0.3 SE)

[a] The total volume of the fluids in and around the brain is about 200 μl, so that the dilution factor is about 50×.

[b] MURF, multiunit receptive field.

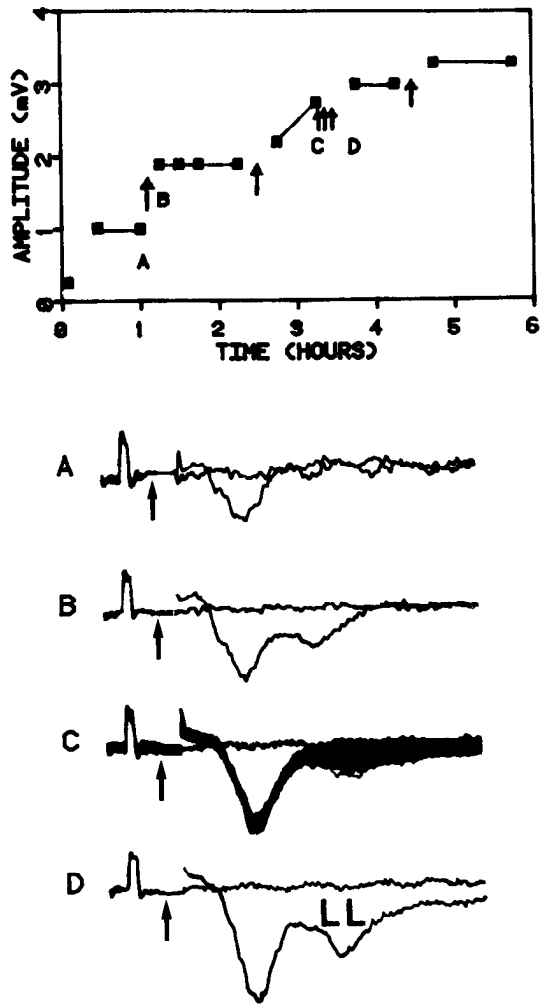

Figure 2. Potentiation of postsynaptic responses as demonstrated by field potential recordings. Field potentials elicited by optic nerve stimulation were recorded from the optic terminal layer (125 μm depth) in the tectum at 33 d postcrush. At the top, the amplitude of the responses is plotted vs time. Below are sample responses from times marked by the letters in the plot (blank trials are included to show the baseline). The first response in this case was atypically small; potentiating trains were administered after the responses were stable for ½ hr. In the plot, *arrows* show the times of the potentiating trains of stimuli (20 stimuli at 0.1 Hz). Test stimuli were given at 15–30-min intervals to avoid causing potentiation. Sixty traces were superimposed in *C* below to demonstrate that the increased gain was stable and that the responses became resistant to fatigue. Traces are 100 msec long. Negative is downward in all traces. *LL,* The long latency (probably polysynaptic) component. The calibration pulse is 1 mV and 2 msec long. The stimulus artifact was electronically suppressed, and an *arrow* marks the time of stimulation in each trace.

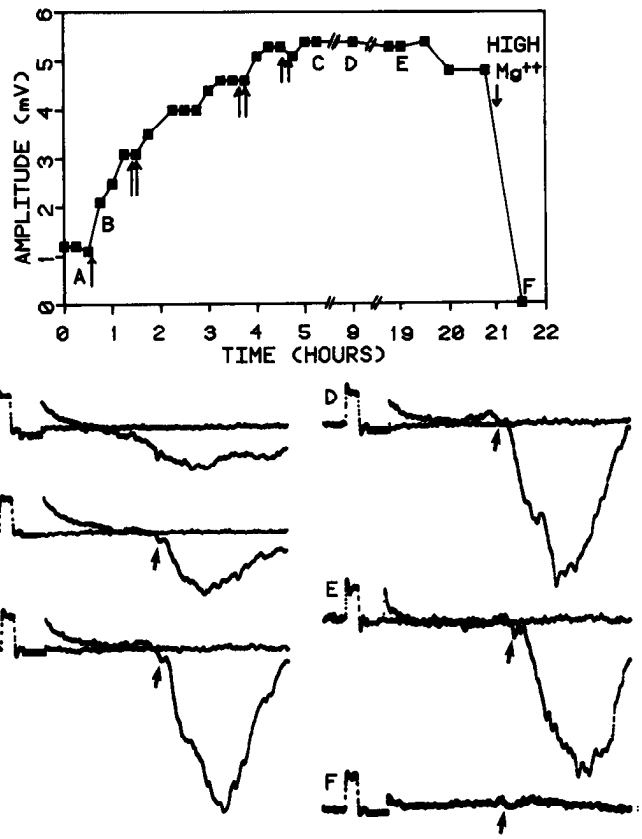

Figure 3. Potentiation of postsynaptic responses as demonstrated by field potential recordings in a fish at 39 d postcrush. *Top,* Amplitude of the response is plotted vs time. *Bottom,* Sample responses from times marked by the letters in the plot. Conventions are as in Figure 2, except that traces are 50 msec long. The calibration pulses are 1 mV × 2 msec. The stimulus artifact was electronically suppressed. Note the breaks in the time axis in the upper plot as the potentiation was tracked into the second day. The *arrow* labeled *high* Mg^{2+} denotes the time of topical application of Ringer's with 0 mM Ca^{2+} and 40 mM Mg^{2+} (isoosmotic replacement of Na^{+}) briefly to the tectum to block postsynaptic responses and leave the presynaptic fiber volleys (*arrows*). Two traces were superimposed here to show the reproducibility of the presynaptic fiber volley. Note the long latency indicating the slow conduction velocity in these regenerating fibers (<0.5 m/sec).

fiber volleys (Schmidt, 1979; Schmidt et al., 1983; Langdon et al., 1988), the other 2 being smaller and slower than the main presynaptic volley. Even though supramaximal stimuli elicited very small postsynaptic potentials, administering 20 stimuli at 0.1 Hz resulted in an increased amplitude of the response partly present at 15 min and gradually developing over the next hour (Figs. 2, 3). With repeated potentiation, a longer latency component often appeared in each response (Fig. 2). The source of this component is not completely established, but it probably represents either a very slow conducting group of optic fibers or (more likely) a recurrent polysynaptic excitation (King and Schmidt, 1989). It is typically much more labile and variable to repeated stimulation, even after potentiation (Fig. *2C*). Administering additional trains of stimuli results in further increases in synaptic gain for the shorter (definitely monosynaptic) response and also for the longer latency response, but the increases were smaller percentage increases. Following potentiation, the monosynaptic response is also much more resistant to fatigue. The increase in gain was routinely stable for 8 hr, and in 3 cases tested was stable overnight (Fig. 3).

Following potentiation, the responses of even very early regenerated projections were large and stable enough to permit a laminar analysis of the sources and sinks of synaptic transmission. One of these cases is shown in Figure 4. The maximum negative responses were found at 100–150 μm depth and reversed to positive-going potentials at 300–400 μm as previously reported in normal projections (Schmidt, 1979) and in regenerating projections (Schmidt et al., 1983). The synaptic sinks were also distributed roughly normally, with the earliest sink (caused by the fastest presynaptic fiber volley) occurring most

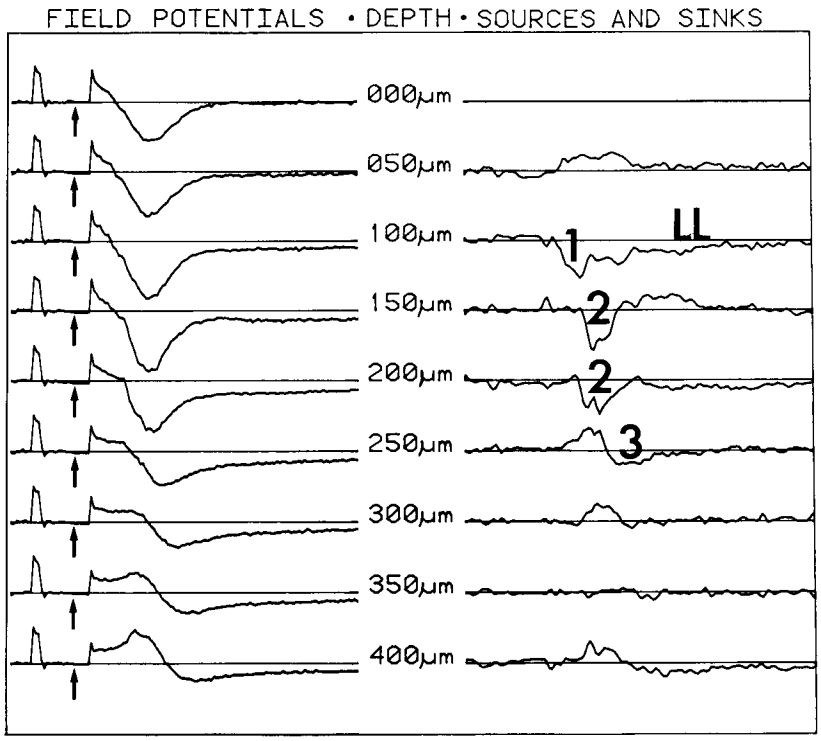

Figure 4. Traces of field potentials (*left*) and of calculated sources and sinks of synaptic current (*right*) elicited by optic nerve shock in a goldfish tectum 22 d after nerve crush. *Arrows* mark the time of nerve stimulation (the artifact was electronically suppressed). Depths of recording are shown next to each trace, which is an average of 10 responses. Negative is downward. On the right are the sources and sinks of synaptic current calculated by a second differencing technique. Sinks, reflecting current going into the cells at excitatory synapses, downward in each trace, are labeled *1*, *2*, and *3* to correspond to the 3 fiber groups in the optic nerve. *LL*, The long latency response (probably polysynaptic). The calibration pulse at the beginning of each trace is 1 mV in amplitude and 2 msec in duration. Total trace length of 80 msec.

superficial at 100 μm ahead of a second sink at 150 and 200 μm. A third small sink occurring later was present somewhat deeper at 250–300 μm as in normals. The long latency component in the field potentials was not as prominent in this case as it was in Figure 2, but an extended synaptic sink can be seen at 100 μm, reflecting this long latency response. In general, the sinks for this longer latency (possibly polysynaptic) component were located at the same depths as those of the monosynaptic response.

Requirements for eliciting LTP. The requirement for eliciting LTP seems to be a train of strong stimulations that elicit consistently strong postsynaptic responses. First, submaximal instead of supramaximal stimulation (the same trains at 0.1 Hz but response amplitude at ⅓ maximum) did not result in any increase in gain, even though a subsequent supramaximal train in the same projection elicited a 67% increase. Second, for the train to be successful in eliciting potentiation, the response to each of the individual stimuli of the train had to follow substantially undecremented. In 6 cases where the postsynaptic response fell to ⅔ or less of the starting amplitude, there were no increases in gain; instead most cases exhibited transient synaptic depressions lasting about 30 min. The rates of stimulation which the projection could follow and which then elicited potentiations were limited by this fatigue factor. In general, 0.1 Hz worked for all projections tested (28 cases) and was adopted as the standard for comparison. Trains at 0.2 and 0.4 Hz also worked well (4 cases), but 1 Hz gave variable results (6 cases). In 3 of the 6 cases there was no increase and sometimes a transient depression lasting about ½ hr; in the other 3 cases (generally more mature projections at longer times postcrush) there were substantial increases of 30–90%. At 3–5 Hz in all 3 cases tested, there was a transient synaptic depression (10–25%). At the low end of the frequency scale, administering the same train of 20 stimuli at 0.01 Hz still resulted in a 67% increase where the faster trains had failed. These data show a requirement for strong postsynaptic activation and, along with the sensitivity to NMDA receptor blockers (see below), support the requirement for substantial postsynaptic depolarization to trigger potentiation. The low frequencies that had to be used to avoid the fatigue precluded temporal summation, but triggering the potentiation nevertheless required repeated activation, albeit at intervals as long as 10 sec.

Control regenerating cases. Only the test stimuli at 15–30-min intervals were given in 7 control cases at similar times

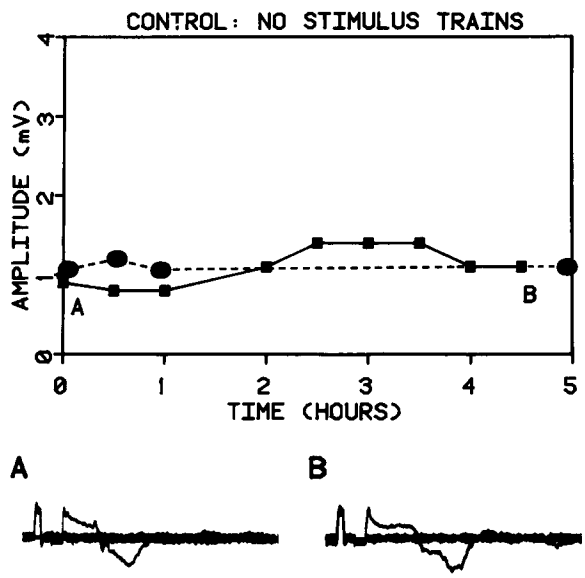

Figure 5. Lack of potentiation in control cases given test stimuli but no trains of stimuli. *Top,* Response amplitudes are plotted for 2 cases (*filled ovals* and *squares*). Representative traces are shown below (*A* and *B*). In the other case, monitoring continued up to 8 h but showed no increase in gain. Conventions as in Figure 2.

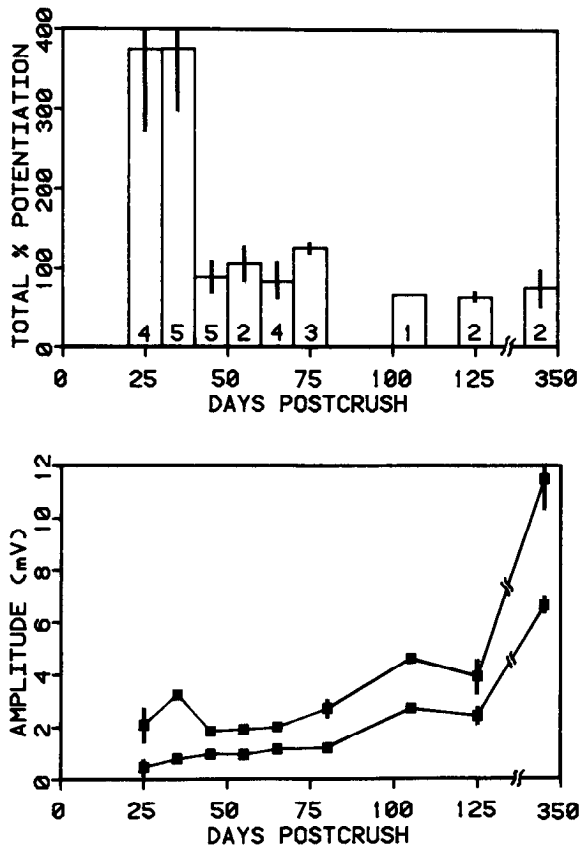

Figure 6. Plots showing the time course of the increased capacity for potentiation during regeneration (*top*), and the absolute size of the field potentials before and after potentiation sessions (*bottom*). The greatest total percent potentiation occurred in fish between 20–30 and 30–40 d postcrush, but substantial potentiations occurred even at times longer than 300 d. The number of fish whose results were averaged is shown on each bar. The standard error is shown for each point where it is greater than the size of the symbol itself.

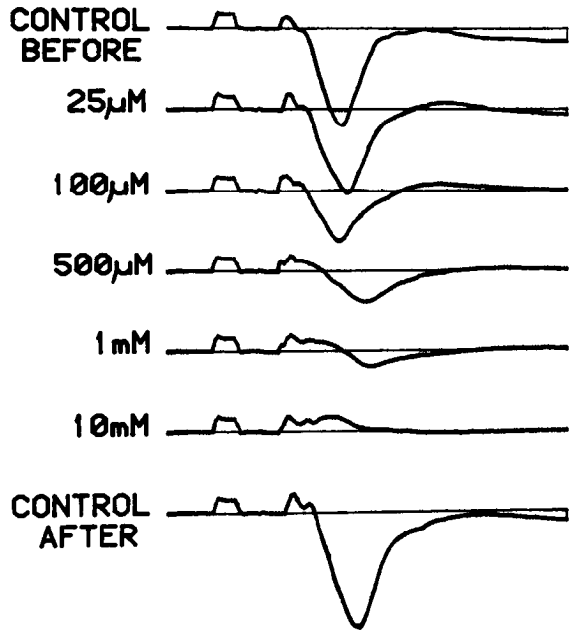

Figure 7. Effects of different doses of AP5 on the amplitude of the field potentials in a normal tectum. All recordings were made at 125 μm depth in 1 fish. Increasingly higher concentrations were applied topically to the tectal surface, followed by a washout with repeated changes of Ringer's. The equilibration time between increasing doses was 15 min, but 2 hr for the washout.

postcrush, and the response amplitude was monitored for 5–8 hr. These cases (Fig. 5) did not show the increases in synaptic gain that were elicited by trains of stimuli in the experimental series (Figs. 2, 3), although small slow changes sometimes occurred. The average amplitude of the responses at the end of the period in these controls was 121.2% of that at the begining (n = 7, SEM = 7.6%). The average increases were not significantly different when test stimuli were given at 15-min vs 30-min intervals (10.5%, SEM = 6.5%, n = 3 vs 29.5%, SEM = 11.4%, n = 4). However, the responses of the more immature projections (2 cases at 20–40 d postcrush) showed gradual increases in gain to the test stimuli alone (24 and 53%). However, these increases were far smaller than the enormous increases (average of 375%, smallest 130%) that the trains produced at this time (see the time course below). These small increases can best be explained by the assumption that the early projections are so sensitive to convergent activity that they are slightly potentiated by the test stimuli alone. In order to elicit strong potentiations, however, repeated stimulation is required at rates substantially faster than the test interval of 15–30 min.

Lack of LTP in normals. Normal fish (5 cases) were tested for comparison with the regenerates using the same standard trains of 20 stimuli at 0.1 Hz. After 5–8-hr periods and multiple attempts at potentiation, the average increase was only 2.9% (SEM = 4.6%). The largest was only 20%. Potentiations of 10–20% were reported by Lewis and Teyler (1986) at train frequencies of 1–5 Hz, but not below 1 Hz. Thus, the normal projection has little capacity for potentiation even though the responses are generally much larger.

Time course of enhanced capability for potentiation. Regenerating projections at various time points after nerve crush were assayed for their ability to show LTP over day-long sessions to standard trains of stimuli. In general, the largest potentiations were obtained between 20 and 40 d postcrush, although substantial potentiations, larger than in normal fish, were obtained at longer times up to a year (Fig. 6).

Field potentials can first be recorded around 20 d in fish regenerating at 20°C, and they are much less than 1 mV at that time (Schmidt et al., 1983). The responses from 4 fish at 20–30 d postcrush in this study averaged 0.47 mV but potentiated an average of 375% over several hours to reach an average of 2.09 mV. Responses from fish between 30 and 40 d postcrush started at 0.78 mV and grew to 3.22 mV, but also averaged a 375% increase. From 40 to 70 d, the responses started at or near 1 mV and the percent increase was generally much smaller (Fig. 5). However, substantial increases of 75% were seen up to 320 d, the longest time tested. This is well beyond the point when the field potentials seem to return to normal (which was previously reported as around 160 d by Schmidt et al., 1983), and it reflects a continued plasticity of the regenerated projection.

Involvement of NMDA receptors. In 6 regenerating fish, the NMDA receptor blocker AP5 or AP7 (25 or 50 μM) was topically applied to the tectal surface before the potentiating trains were administered in order to test whether NMDA receptors must be activated to trigger the potentiations. We determined in previous tests that a concentration of 25–50 μM did not greatly decrement the field potentials. Figure 7 shows the results of

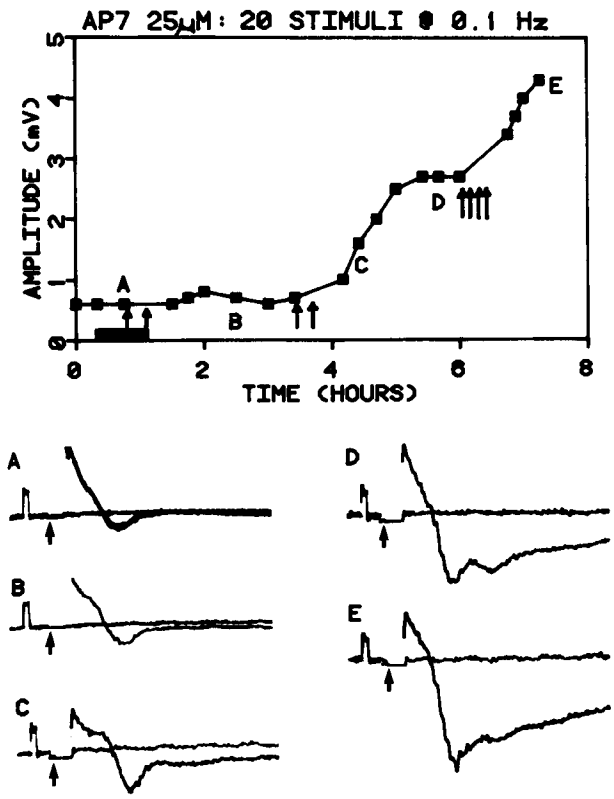

Figure 8. Experiment showing that AP7 blocks potentiation in a regenerating fish. Conventions are as in Figure 2. *Top,* Amplitude of the responses is plotted vs time. *Bottom,* Sample responses from times marked by the letters in the plot. *Arrows* in the plot show the times of the potentiating trains of stimuli (20 stimuli at 0.1 Hz). The first time that these trains were applied, the tectal NMDA receptors were blocked by topical application of AP7 (*striped bar*). These 2 trains elicited no consistent increase in the synaptic gain (compare *A* and *B*). Two hours after washout of AP7, the same trains of stimuli resulted in very large increases in gain (*C* and *D*). Further trains of stimuli elicited further gains (*E*).

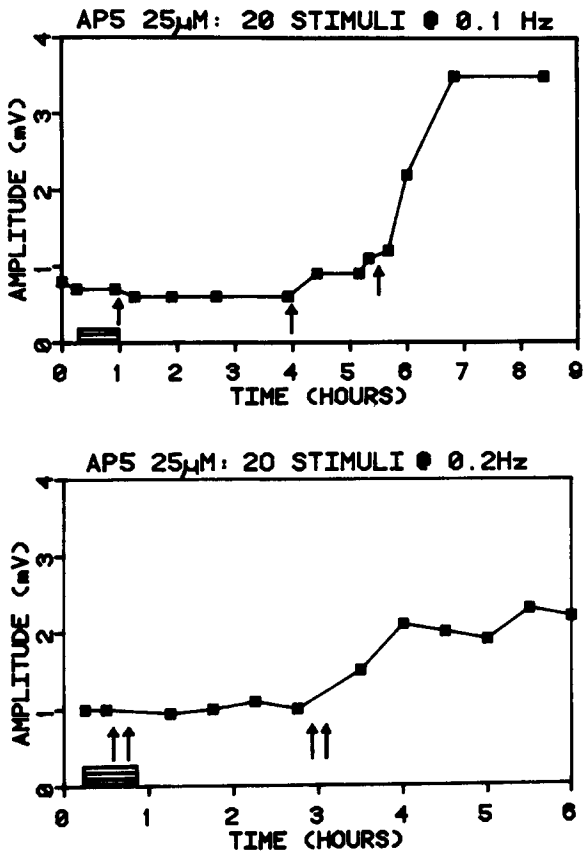

Figure 9. Plots of results from 2 experiments showing that AP5 also blocks the potentiation of retinotectal synapses. Conventions are as in Figure 8.

application of successively higher concentrations of AP5 to the normal tectum. These experiments showed that both AP5 and AP7 equilibrated within 5–10 min. The 25-μM concentration in this case decrements the response by 15%. This is similar to results reported in hippocampus and indicates that most of the transmission is mediated by non-NMDA receptors. Concentrations in the mM range, however, strongly decreased the amplitude of the field potentials, probably by blocking non-NMDA receptors.

In the regenerating projections, the AP5 or AP7 did not measurably reduce the responses (Figs. 8, 9). The average amplitude after application was 102% of that before application (SEM = 2.7%, $n = 6$). In addition, when the standard trains of stimuli were given, no potentiations resulted. The change in amplitude in these fish varied from −14% to +17%, with an average of +2.6% (SEM = 4.3, $n = 6$). This group included fish at 34, 34, and 35 d, during the period of maximum capacity for potentiation, and they too averaged only a 2.3% increase. In order to demonstrate that these same projections were capable of large potentiations, the AP5 or AP7 was washed out by repeated topical application of Ringer's solution over a period of 1.5–2 hr so that the same trains could be given without the blockers present. The trains without blockers present produced potentiations averaging 103.8% (SEM = 35.9%, $n = 6$) in the same fish. This demonstrated that the earlier lack of potentiation was due to the presence of the NMDA receptor blockers and not to an inability of the projection to undergo potentiation.

The closely related compound AP6, which differs by one methyl group from both AP5 and AP7, is not an NMDA receptor blocker. It was topically applied under the same conditions in 2 cases as a control for any possible nonspecific effects of these drugs. AP6 did not affect the amplitude of the responses, nor did its presence during the train of stimuli block the subsequent potentiation (Fig. 10). Following 2 sets of trains with AP6 present, the response grew by a total of 116%. In the second fish, the response grew by 67% following a single train. Clearly, the potentiation was not blocked by AP6, and this fact suggests that the effect of AP5 or AP7 in blocking potentiation is likely to be due to the block of NMDA receptors.

NMDA receptors and sharpening of the map

The infusion of either AP5 or AP7 into the tectal ventricles during regeneration substantially prevented the sharpening of retinotopic precision. This result is based on recordings from 7 fish chronically infused with either AP5 or AP7 compared with 8 others infused with control solutions delivered identically from minipumps attached to the fish's head (Fig. 1). The minipumps produced a steady infusion beginning from 21 d postcrush and lasting 11–24 d (average of 15 d, Table 1). Four experimental fish received AP5 at concentrations of 10, 1, 0.5, and 0.5 mM and 3 fish received AP7 at concentrations of 1, 1, and 0.5 mM,

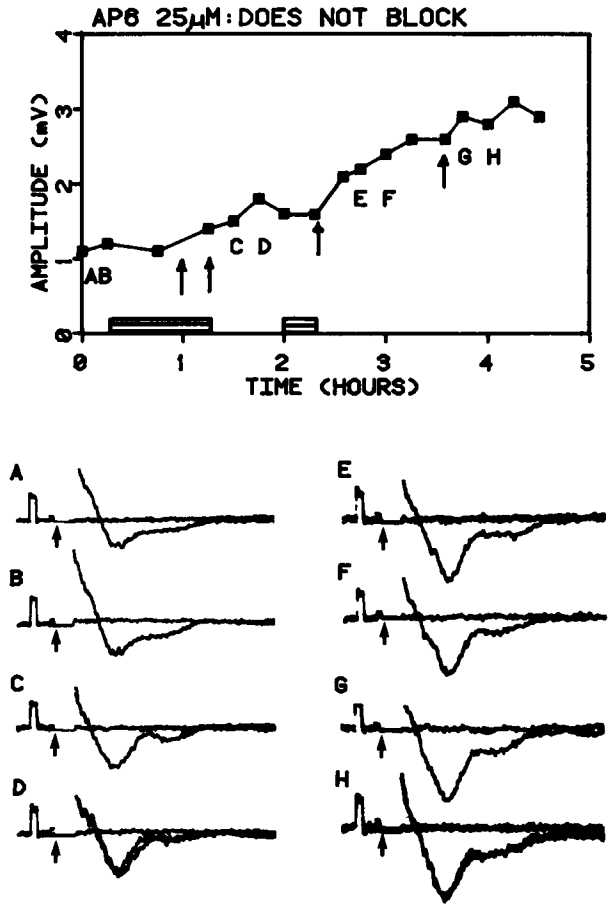

Figure 10. Results of an experiment showing that AP6, unlike AP5 and AP7, does not block potentiation of retinotectal synapses. *Top,* Amplitude of the responses is plotted vs time. *Bottom,* Sample responses from times marked by the letters in the plot. *Arrows* in the plot show the times of the potentiating trains of stimuli (20 stimuli at 0.1 Hz). Note that the increased gains from *A* through *F* were caused by trains administered while AP6 was present in tectum. Other conventions are as in Figure 8.

respectively. The estimated dilution factor of 50× means that the effective concentrations ranged from approximately 200 μM to 10 μM. In all experimental fish, the general effect on the maps recorded from 53 to 107 d postcrush were the same. The overall organization of the retinotopic map was normal, but the multiunit receptive fields were greatly enlarged (Table 1; compare Figs. 11 and 12, right, with Figs. 12, left, and 13); that is, multiunit activity recorded at each tectal site could be driven from a much wider than normal area of visual field.

Retinotopic organization of the map. The organization of the retinotopic map is such that the nasal part of the visual field is represented on rostral tectum, temporal field on caudal tectum, dorsal field on medial tectum, and ventral field on lateral tectum, much of which curves under and is out of view from the dorsal aspect. A precise retinotopic organization can be seen in maps of normal projections (Fig. 12, left), and a rough retinotopic map can be seen in the unsharpened maps of the experimental fish. As the electrode position in tectum was moved systematically more caudally, the visually responsive areas in visual field were centered progressively more temporally. The magnification factor (MF) that mathematically expresses this relationship can be calculated as the number of micrometers on tectal surface representing each degree in the visual field, and these factors were not affected either by the infusion process or by the block of NMDA receptors. In the rostrocaudal direction, the MFs averaged 21.4 (1.6 SEM) in the NMDA-blocked projections vs 20.0 (1.1 SEM) for the control infusions. In the mediolateral direction, the MFs were 22.7 (1.3 SEM) for the NMDA-blocked projections vs 23.1 (1.3 SEM) for the control infusions. These figures were not significantly different from each other or from those of control regenerate fish of this size (20.2 and 24.7, respectively). Thus, the reestablishment of the rough retinotopic map, which normally occurs by 35 d, was not prevented by the infusion of the NMDA blockers.

Lack of sharpening in fish infused with NMDA receptor blockers. The maps of regenerated projections of the AP5- and AP7-infused fish (Figs. 11 and 12, right) differed markedly from those of control regenerating projections that were identically infused (Fig. 13) and from uninfused control regenerates recorded 39–105 d postcrush in that the multiunit receptive fields were consistently several times larger than controls (see Table 1). The results were similar to those obtained from regenerating projections whose activity was either blocked by tetrodotoxin (TTX) or synchronized by stroboscopic illumination (Schmidt and Edwards, 1983; Schmidt and Eisele, 1985).

The enlarged multiunit receptive fields appear to reflect an abnormal convergence onto each tectal site of ganglion cell arbors from a wide area of retina. In previous studies, this was confirmed by recording single ganglion cells both from the tectum (axonal arbors) and from the retina (somas) to show that these were of normal size (Schmidt and Edwards, 1983; Eisele and Schmidt, 1988). This can also be seen in the lower half of Figure 11, where responses from different regions of these enlarged multiunit areas are shown. In those cases where single units were recorded or could be isolated by spike height, they always had normal receptive fields for ganglion cells. The single unit receptive fields were 7–12° in diameter, responded to light on, light off, or both, and did not habituate to repeated stimulation. Several single-unit responses from different parts of larger multiunit fields can be seen in Figure 11. These include the entry (off) responses of the first and second examples on the right and the exit (on) responses of both cases in the last example, where single large spikes can be discerned.

Lack of effect of NMDA receptor blockers on normal map. In 1 fish, the intact projection from the left eye to the right tectum (Fig. 12, left) was recorded at the same time as the regenerated projection (Fig. 11, right). The regenerating projection failed to sharpen as expected and multiunit receptive fields averaged 27.4° (right side). However, the intact projection, which experienced the same concentration of NMDA receptor blockers, was not rendered abnormal, indicating that the processes of retinotopic sharpening are much more dependent on NMDA receptors than those of retinotopic maintenance. This finding parallels results reported previously for the effects of blocking activity with TTX (Schmidt and Edwards, 1983) and synchronizing activity with strobe illumination (Schmidt and Eisele, 1985).

Normal sharpening in control infused fish. In 2 fish infused with similar concentrations of AP6, which is not an NMDA receptor blocker, the map regenerated normally, with multiunit receptive fields averaging 11.1 and 10.7°. One of these maps is shown in Figure 13. Since AP6 is very similar chemically to AP5 and AP7, the effects caused by the latter should result from their block of NMDA receptors. Other control fish, infused with either too low a concentration of AP5 (0.1 mM), Ringer's so-

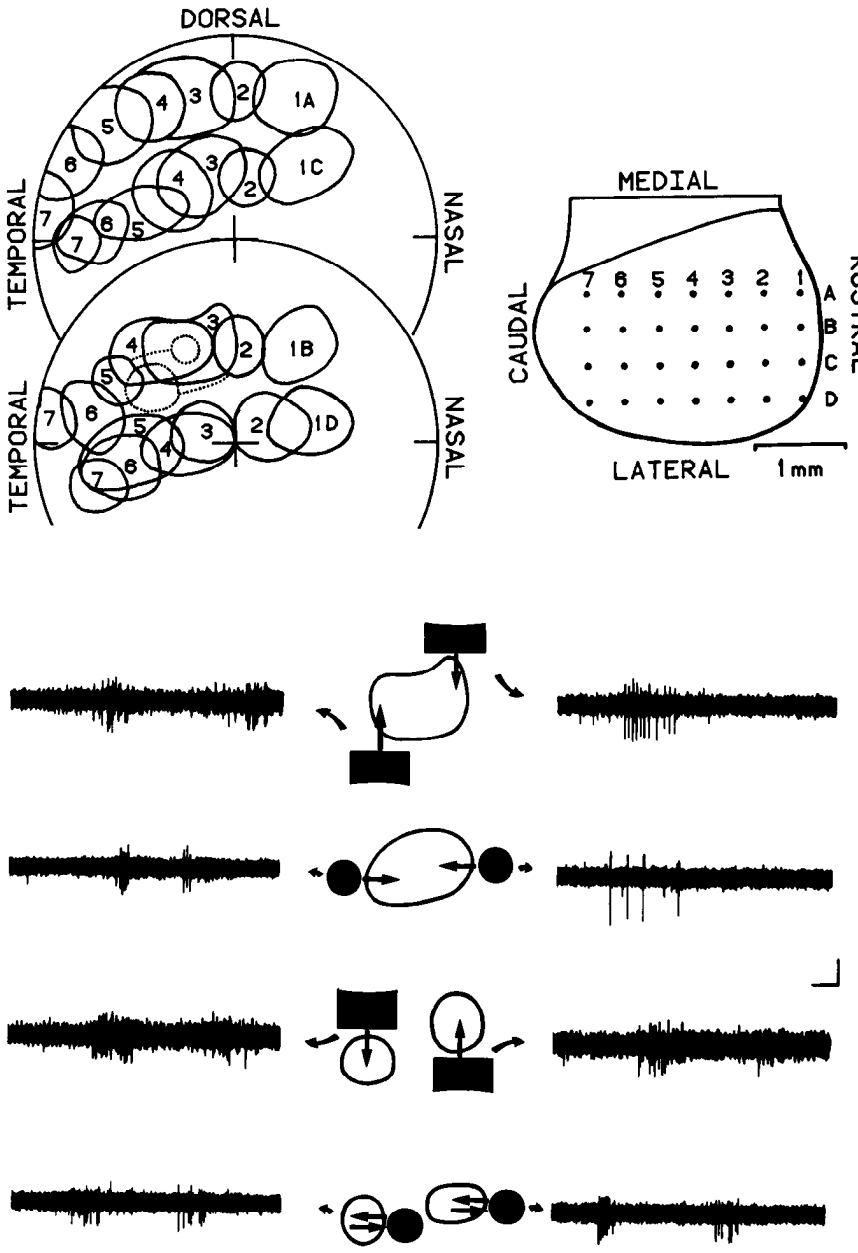

Figure 11. Map of a retinotectal projection regenerated during infusion of AP5. The recording was made 105 d postcrush, and the infusion from the minipump lasted from 21 to 34 d postcrush (Fish 6, Table 1). At the right is a drawing of the tectal surface viewed from above. Each point in the tectal array is an electrode penetration. The receptive fields recorded at these points, although enlarged, fall into an orderly array in the visual field similar to the array seen on the tectal surface and numbered accordingly. For clarity, 2 separate representations of the hemispherical visual field are presented, each with 2 rows of receptive fields. The contours within the visual field define the boundaries of the multiunit receptive fields. Two points had spatially separate multiunit receptive fields; these were B2 and B5. For convenience, the drawing of the tectum has been inverted about 1 axis so that the arrays are oriented in the same direction. Below are shown some responses recorded during stimulus presentations in the multiunit receptive fields. From top to bottom, these receptive fields are B3, D5, and B2 from the map above and a second example of a split receptive field from a separate fish at bottom. *Straight arrows* show the movements of the visual stimuli, either *black rectangular cards* or *circular spots,* into and out of the responsive areas. *Curved arrows* associate these stimuli with the responses shown in the traces on either side. Note that the different amplitudes of the spikes and the spatially separated areas from which responses are evoked indicate that separate ganglion cell arbors are being recorded at the same point in tectum. Scale bar, 20 μV and 100 msec.

lution alone, or inert antibodies in Ringer's, averaged 11.9°. These were all indistinguishable from those of previously reported control regenerates (11.9, 0.4 SEM vs 11.6 for infused controls here). Thus, there was no general effect of the minipump implantation and infusion procedure on the sharpening of retinotopic precision, and the effect in the AP5- and AP7-infused fish appeared to be due to the block of NMDA receptors.

Behavioral tests in presence of NMDA blockers. In order to test whether NMDA blockers substantially decrease postsynaptic activity in the tectum, fish were tested for an optomotor response that is known to be tectally mediated (Springer et al., 1977). Fish were tested in the optomotor drum 1 hr following a single injection of NMDA blocker equivalent to the amount infused by the minipumps over a 24-hr period (4 μl of 1 mM AP5). With no drum motion, fish swam an average of only 26 out of 360 sec. When the drum was rotated, control fish swam with the moving stripes an average of 281 out of 360 sec. Sham-injected fish swam with the stripes an average of 257 sec, and fish injected with AP5 swam an average of 247 sec with the moving stripes. None of these differences were statistically significant, and, in particular, the performance after AP5 was not different from that after the sham injections ($p = 0.73$, $n = 8$). In 3 further fish, a higher dose of AP5 was injected (4 μl × 10 mM), but this also did not affect performance (250 and 244 sec of swimming with the stripes for AP5- and sham-injected fish, respectively). In the field potential experiments, AP5 or AP7 solutions applied topically to the tectum equilibrated within 10 min, so that the hour allowed here should have been more than adequate.

Injections of kynurenic acid, an antagonist of both NMDA and non-NMDA excitatory amino acid receptors, made by this same method did cause decreased behavioral performance, showing that the drugs did have access to the tectum. Ten microliters of 10 mM kynurenic acid caused a significant 25% (SEM

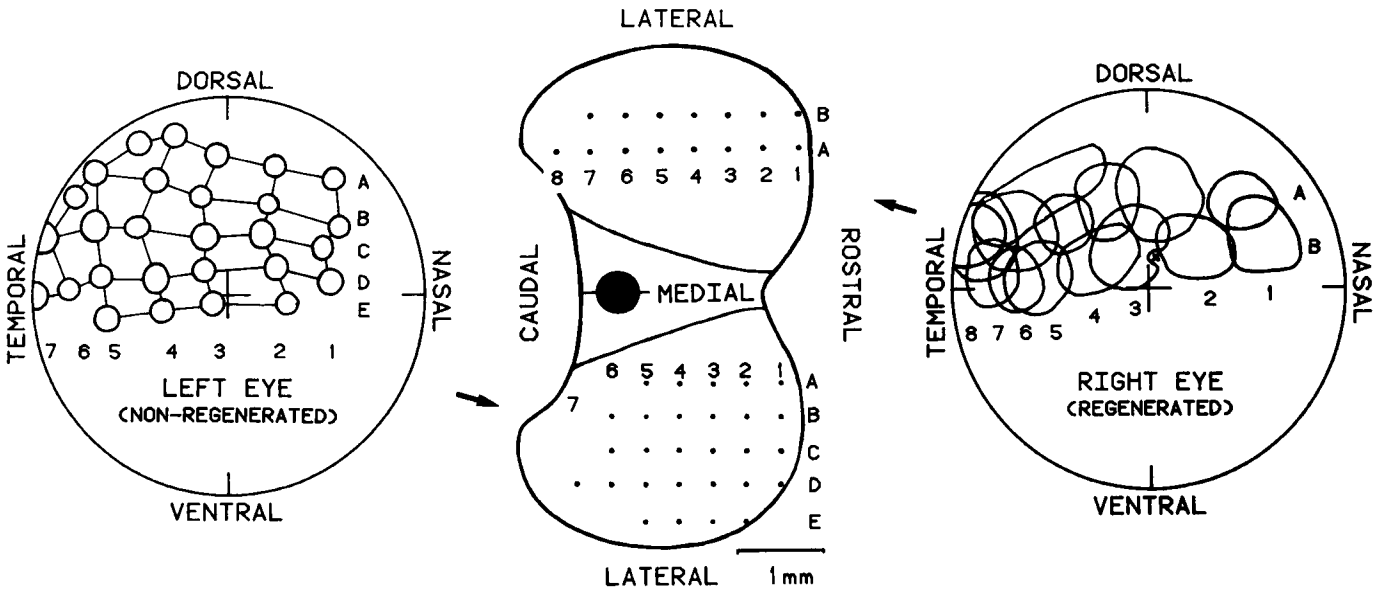

Figure 12. Maps of 2 retinotectal projections, 1 regenerating and 1 not regenerating, recorded from 1 fish infused with AP7 from 21 to 35 d postcrush (Fish 5, Table 1). On the left is the orderly map of the nonregenerating projection of the left eye to the right tectum, which was not affected by the infused AP7. On the opposite tectum of this fish, the regenerating projection from the right eye (right) was prevented from sharpening by the infused AP7 (average multiunit receptive field size of 27.4°). Conventions are as in Figure 11, except that the visual field of the right eye was reversed so that the array would directly correspond to that on the tectum. *Large blackened circle* at the midline shows the point of insertion of the cannula through the tectal commissure.

= 6.8%, $n = 10$) reduction in the time swimming with the stripes compared to the previous sham injections in these fish. A rapid equilibration was expected, since topically applied AP5 and AP7 in the field potential experiments was found to equilibrate in less than 10 min and to wash out in less than 2 hr (Fig. 7). Thus, the behavioral performance on the tectally mediated optomotor response suggested that no substantial decrement of tectal activity was produced by AP5 concentrations as high as the highest expected from the minipump infusions. This result was in agreement with the lack of effect of low to moderate concentrations of AP5 on the field potentials elicited by optic nerve shock.

Discussion

The major findings of this study are (1) that strong low-frequency activation of the immature regenerating optic fibers induces pronounced LTP, (2) that the period of greatest potentiation coincides with the activity-dependent retinotopic sharpening of the map, (3) that the potentiations are blocked by low concentrations of NMDA receptor blockers, (4) that chronic infusion of low concentrations of these same blockers prevents retinotopic sharpening during regeneration but does not unsharpen mature maps, and finally (5) that these concentrations of blockers do not hinder performance in a tectally mediated behavioral task, the optomotor response.

Characteristics of the LTP

The LTP was most consistently elicited by trains of 20 stimuli at a frequency of 0.1 Hz, in contrast to frequencies of 100 Hz commonly used in hippocampus. The requirement for low frequencies seems to stem from the inability of the immature fibers to follow at frequencies of even 1 Hz (Schmidt et al., 1983); this was verified here in monitoring the field potentials during the

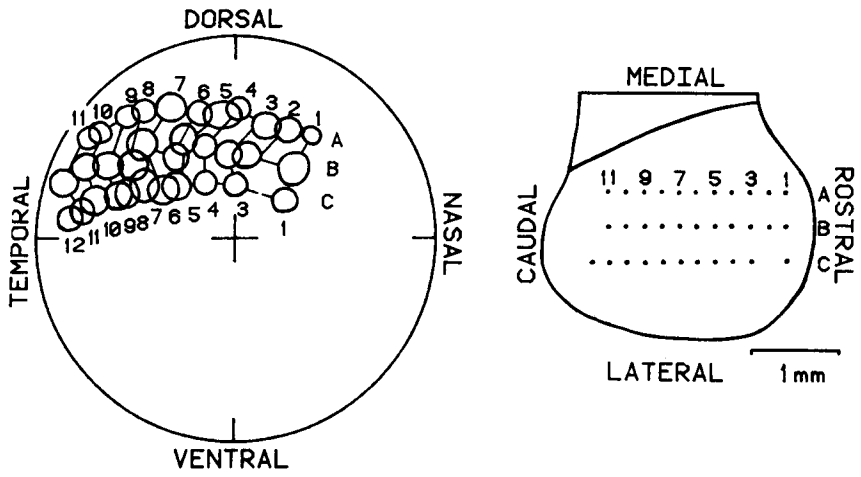

Figure 13. Regenerated retinotectal projection recorded at 97 d postcrush in a fish infused with AP6 from 21 to 38 d (Control Fish 2). The map is retinotopic and multiunit receptive fields are of normal size. Conventions are as in Figure 11.

trains of stimuli. This inability probably reflects the properties of the immature retinotectal synapses rather than conduction down the axon, since after potentiation the postsynaptic potentials can generally follow to higher frequencies. The low frequency of stimulation precludes temporal summation of postsynaptic responses. However, LTP in hippocampus can also be elicited by single shocks if sufficient depolarization is assured either by blocking inhibition with picrotoxin (Abraham et al., 1986) or pairing presynaptic activation with intracellular injections of depolarizing current (Gustafsson et al., 1987) or by pairing with trains of stimuli to another input (Sastry et al., 1986). The reason why the tectal cells might be sufficiently depolarized or disinhibited to allow their NMDA receptors to open, although not established at present, may be related to changes occurring during regeneration (Brink and Meyer, 1986; see below). Thus, the finding here that strong but isolated stimuli can trigger potentiation is not greatly different from observations elsewhere.

Large and long-lasting calcium transients could be evoked by repeated applications of glutamate several seconds apart (Connor et al., 1988), and calcium entry through NMDA receptors is thought to be the trigger for LTP (Lynch et al., 1983; Malenka et al., 1988). These channels, which have a high permeability for calcium ions (MacDermott et al., 1986), are fully conducting only when transmitter is bound to the receptor site and the postsynaptic cell is sufficiently depolarized to relieve a magnesium block of the channel (Nowak et al., 1984).

Eliciting potentiation in the immature retinotectal projection requires strong stimulation, generally supramaximal stimulation which assures that all optic fibers are activated for maximum spatial summation postsynaptically. Cases in which submaximal stimulation was used, or in which the frequency was too high for the postsynaptic response to follow, failed to show potentiation. In fact, in the latter case, transient synaptic depression was frequently noted. These results parallel findings in hippocampus. However, there is a paradox in the fact that potentiation was easily elicited in immature projections where the size of the postsynaptic responses measured in the field potentials was much smaller than in the mature projection (<1 mV vs 4–6 mV). With such small optic responses, how could sufficient depolarization be achieved? The answer may be that denervation causes a depolarization in tectal cells. Brink and Meyer (1986) reported that cells in denervated tectum fire in spontaneous bursts, beginning 2 d after nerve crush and continuing until reinnervation occurs as established by recording responses to optic nerve stimulation.

The increase in synaptic gain did not appear immediately after the stimulus train was over, but developed over the next 15–45 min. This rise was not tracked carefully because test stimuli could only be given at 15-min intervals to avoid potentiation from the test stimuli alone. However, the time course was clearly slower than in hippocampus, where the increase appears within 5 min with similar potentiating trains (20 stimuli at 0.1 Hz, Gustafsson et al., 1987). This was expected since hippocampal slices are maintained at 32°C vs the 12° for these fish, and a Q_{10} of 2.5 would make the process more than 6 times slower. Thus, 5 min at 32°C would be equivalent to 30 min at 12°C.

Block of LTP by NMDA receptor blockers

The transmitter at the retinotectal synapses is unknown but may be an excitatory amino acid since 1 mM kynurenic acid blocks field potentials from optic nerve shock (Langdon and Freeman, 1986), and this sensitivity to such blockers was confirmed in preliminary observations in our laboratory. In addition, I have shown here that high (1–10-mM) concentrations of AP5, which are also nonspecific, also block retinotectal synaptic transmission (Fig. 7). [Nicotinic acetylcholine receptors, on the other hand, have been localized to the presynaptic terminals of retinal axons (Henley et al., 1986).] In addition, Kageyama and Meyer (1987) showed that antibodies to glutamate immunostained optic fibers and terminals in tectum and some ganglion cell bodies in retina. In this study, low concentrations of either AP5 or AP7, which should be specific for NMDA receptors, blocked the potentiation normally elicited by the trains of stimuli but did not substantially decrement the amplitude of the postsynaptic responses. This finding was the same as previously reported for several projections in hippocampus (Harris et al., 1984; Morris et al., 1986; Errington et al., 1987; Gustafsson et al., 1987) with the exception of the mossy fiber projection where potentiation occurs even in the presence of these blockers (Harris and Cotman, 1986). The concentrations used here (25–50 μM) were slightly lower than those generally used in hippocampus (50–100 μM), showing the strong sensitivity of LTP induction in tectum to NMDA blockers. A selective action at NMDA receptors was also suggested by the lack of effect of AP6, which is chemically similar to AP5 and AP7 but does not block NMDA receptors.

Period of enhanced capacity for LTP

The time period during regeneration when the projection was most strongly potentiated corresponded to the period when the pattern of activity sharpens the retinotopic map. A sharp map can be recorded electrophysiologically by 35 d postcrush (Schmidt and Edwards, 1983), although anatomical studies suggest that sharpening takes somewhat longer, around 70 d (Cook and Rankin, 1986). The sensitive period for disruption of sharpening by either intraocular TTX or strobe illumination assessed electrophysiologically shows a maximum sensitivity between 20 and 50 d (Eisele and Schmidt, 1988). The maximum potentiations were obtained between 20 and 40 d. Likewise, there was some sensitivity to activity manipulations out to 120 d and some capacity for potentiation continues somewhat longer, up to a year. In contrast, nonregenerating projections could barely be potentiated at all (3% increase) and were not disrupted by infusion of NMDA receptor blockers (this study), TTX block of activity (Schmidt and Edwards, 1983), or synchronization of activity by strobe illumination (Schmidt and Eisele, 1985). Thus, there is a strong parallel between the enhanced capacity for LTP and the retinotopic sharpening that occurs through an activity-dependent mechanism. Another parallel exists in the expression of several growth-associated proteins during regeneration, among them GAP 48 (also called GAP 43 or F1), which peak around 2–3 weeks, decline strongly around 40 d, and then decline more slowly thereafter (Benowitz and Schmidt, 1987). GAP 43 is of particular interest because it is phosphorylated during LTP in hippocampus (Lovinger et al., 1985), and it would be of interest to know whether that is the case in tectum as well.

Sharpening of the map and NMDA receptors

The NMDA receptor blockers infused into tectum produced effects on the regenerating retinotectal map that were similar to those found earlier in cases where all optic afferent activity was blocked with tetrodotoxin (Schmidt and Edwards, 1983) or where

visual activity was synchronized by exposure to stroboscopic illumination (Schmidt and Eisele, 1985; Eisele and Schmidt, 1988). The regenerating optic fibers formed a rough retinotopic map, but the multiunit receptive fields recorded at each point were much larger than normal, approximately 28° vs 11°. In addition, the NMDA receptor blockers, like TTX and strobe illumination, had no effect on the nonregenerating projection in the same fish. The effects of both TTX and strobe on multiunit receptive field size could be attributed to uncorrected errors in targeting of optic axons that produced abnormal convergence, as single ganglion cells had normal receptive fields (Schmidt and Edwards, 1983; Eisele and Schmidt, 1988). Current results were consistent with this interpretation as well, since single units isolated from the multiunit responses had normal receptive fields. Projections prevented from sharpening by TTX or strobe treatments, like those reported here, are stable for several months. They slowly sharpen, however, after a year under normal visual conditions (Schmidt and Eisele, 1985).

Anatomical studies show that the initial retinotopic map formed during regeneration is not very precise (Meyer, 1983; Cook and Rankin, 1986), owing in large part to the formation by each axon of many side branches over a large area of tectum (newt: Fujisawa et al., 1982; goldfish: Schmidt et al., 1988). These widespread branches are then retracted and smaller compact arbors begin to appear during the time when the electrophysiologically recorded map is sharpening (Schmidt et al., 1988). One of the factors affecting this retraction is the pattern of activity in the optic fibers, as the branches of the arbors are not as compact following regeneration under strobe, which can account for the lack of sharpening in the map (Schmidt, 1989). The inappropriate branches of the optic arbors make synapses in the inappropriate tectal areas as assessed both electrophysiologically with field potentials (Matsumoto et al., 1987) and anatomically with HRP labeling in the EM (Kageyama and Meyer, 1988).

The present results link the activity-dependent sharpening of the retinotopic map with LTP in 2 ways: (1) both are prevented by low concentrations of NMDA receptor blockers, and (2) the most sensitive period for disrupting sharpening corresponds to the time of greatest capacity for LTP. Since both LTP and sharpening require synchronous activation of inputs, and since the NMDA receptors are well suited for detecting such coincident activity owing to their conditional requirements for opening, it seems reasonable to suggest that NMDA receptor activation may trigger both changes. Specifically, LTP might serve as a short-term change in synaptic efficacy that could lead to the changes in anatomical connectivity during the retinotopic sharpening. Recent anatomical results from the developing frog retinotectal projection also suggest that NMDA blockers disrupt retinotopic sharpening in tectum (Cline and Constantine-Paton, 1988).

Parallels with the formation of ocular dominance stripes

In cat visual cortex, there is also an enhanced capacity for LTP of both the geniculocortical and polysynaptic responses that coincides with the height of the sensitive period for the binocular competition (Komatsu et al., 1988). Binocular competition between afferents from the 2 eyes is known to be mediated by activity (Stryker and Harris, 1986), in particular the pattern of activity (Stryker and Strickland, 1984). Kleinschmidt et al. (1987) showed that infusion of the NMDA receptor blocker AP5 prevented the effects of monocular deprivation so that the open eye was unable to command more territory at the expense of the closed (and presumably less active) eye.

Anatomical evidence that NMDA receptor blockers affect the competition between eyes comes from studies of dually innervated frog tectum where the afferents form eye-specific stripes reminiscent of ocular dominance columns. In both fish (Meyer, 1982; Boss and Schmidt, 1984) and frog (Reh and Constantine-Paton, 1985), the segregation into eye-specific stripes is prevented by blocking activity binocularly with TTX injections. In addition, Cline et al. (1987) showed that chronic application of the NMDA receptor blocker AP5 causes elimination of the stripes in developing frog tectum.

Why is there greater capacity for LTP?

The cause of the enhanced capacity for potentiation associated with development or regrowth of these visual projections is unknown but several possibilities are evident. The first is that there may be more NMDA receptors at the synapses during this time. Tsumoto et al. (1987) suggest that a greater portion of geniculocortical synaptic transmission is mediated by NMDA receptors in kittens than in adults. The field potentials in this study, however, did not show any evidence for this, since field potentials were not smaller after blocking of NMDA receptors in the regenerating retinotectal projection (Figs. 8, 9). Although I did not systematically test rapid trains of stimuli that might be considered more natural, the records indicated that the responses could not follow such stimulation. In addition, the behavioral results argue that NMDA responses are not necessary for generating functional responses as assessed by the tectally mediated optomotor behavior. These results also argue against the interpretation that NMDA blockers prevent sharpening merely by lowering the level of postsynaptic activation (Miller et al., 1987), although the fact that high doses of kynurenate attenuated but did not completely block the optomotor behavior makes this conclusion less than certain.

A second possible reason for the enhanced capacity for potentiation could be a lower level of inhibition. Even mature visual cortex shows strong LTP if inhibition is blocked with bicuculine (Artola and Singer, 1987). This shift in the excitatory/inhibitory balance could occur in tectum if optic axons reestablish their connections with primary tectal neurons before those with inhibitory interneurons. A lower level of activity in the inhibitory neurons, as well as fewer GABA receptors, could account for a lower threshold for Hebbian changes in connectivity (Bear et al., 1988).

Finally, there may be differences in other molecular components that respond to the entry of calcium through the NMDA receptors, such as protein kinase C, calcium-calmodulin–dependent kinase, or GAP 43. LTP in hippocampus requires C kinase activation (Kauer et al., 1988) and results in C kinase–mediated phosphorylation of GAP 43 (Lovinger et al., 1985) which is expressed both during development and during the sensitive period of regeneration in this projection (Benowitz and Schmidt, 1987).

Available evidence suggests a complex interaction between the post- and presynaptic elements both to signal the increase in synaptic efficacy that is often thought to reflect increased transmitter release (Errington et al., 1987) and to trigger the outright anatomical changes in the presynaptic arbors (Fujisawa et al., 1982; Cline et al., 1987; Schmidt et al., 1988). The nature of these signals remains to be investigated.

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