Retinal axons with and without their somata, growing to and arborizing in the tectum of *Xenopus* embryos: a time-lapse video study of single fibres *in vivo*

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**Summary**

Time-lapse video recordings were made of individual retinal ganglion cell fibres growing to and terminating in the optic tectum of *Xenopus* embryos. The fibres were stained by inserting a crystal of the lipophilic fluorescent dye, Dil, into the developing retina. Growth cones were observed in the optic tract and tectum using 20 ms flashes of light to induce fluorescence approximately once every minute. Fluorescent images were captured with a SIT camera, processed and saved on a time-lapse video recorder. The main conclusions from observing normal growing fibres are as follows. (1) Axons in the optic tract grow at a steady rate directly toward their targets without retracting or branching. (2) As axons approach the tectum they slow down and their growth cones become more complex. (3) Most terminal branches in the tectum are formed by back branching rather than by bifurcation of leading growth cones.

In a second experiment, labelled growing axons were separated from their cell bodies by removing the retina. Such isolated axons continued to grow for up to 3 h *in vivo* and were capable of recognizing the tectum and arborizing there. This result shows that growth cones must contain the machinery needed to sense and respond to their specific pathways and targets.

Key words: *Xenopus*, retinal axon, time lapse, CNS, fibre, tectum, *in vivo*.

**Introduction**

The dynamic events of neural development, such as the movements of growth cones and the formation of axonal arbors, if they could be visualized, might provide insight into the nature of the forces that underlie them. In the 1930s and 40s Speidel (1933, 1941) looked at growing sensory nerve fibres in the transparent amphibian tail. He saw amoeboid growth cones, similar to those Harrison (1910) observed on growing neurites in culture, on the tips of extending fibres. By focusing on the same fibres for prolonged periods, sometimes months, Speidel saw how branches formed and retracted, and how neurites were extremely sensitive to a variety of chemical, mechanical and nutritive stimuli. With the advent of anterograde tracers for neural pathways, it became possible to take ‘snapshots’ of connections forming in the brains of carefully staged embryos. From this we learned that axons usually grow unerringly to their appropriate targets along distinctive pathways (Crossland, Cowan, Rogers & Kelly, 1974; Lance-Jones & Landmesser, 1981; Holt & Harris, 1983; Raper, Bastiani & Goodman, 1983), that they may wait at certain places in the pathway before they continue with their final projections and arborizations (Hollyday, 1983; Myers, Eisen & Westerfield, 1986; Shatz & Luskin, 1987) and that some of the initial arbors may be retracted during a period of refinement and of competition between young terminals (Innocenti, Fiore & Caminiti, 1977; Land & Lund, 1979; LeVay & Stryker, 1979). This refinement may be associated with cell death in the projecting population of neurones in the vertebrate CNS (Hamburger, 1975; Cowan, 1979).

With vital fluorescent dyes it has become possible to watch particular axons or groups of them in single animals at various stages of development. Techniques
employing these dyes have been used to look at a number of neurodevelopmental processes. For example, Kater & Hadley (1982) studied the early stages of nerve regeneration in the snail; O’Rourke & Fraser (1985) visualized a refinement process in the retinotectal projection of *Xenopus*; Purves, Hadley & Voyvodic (1986) showed that dendritic arbors of rat sympathetic neurones change dramatically over time; and Myers et al. (1986) observed axonal growth in identified motoneurones in zebrafish.

In this paper, we report the use of a fluorescent lipophilic vital dye and high sensitivity video equipment to make the first time-lapse recordings of CNS axons growing in vivo. We use the rapidly developing retinotectal system of embryonic *Xenopus laevis*. Here axons grow from the chiasm to the optic tectum in less than 12 h. A few hours later they start to form terminal arbors. From time-lapse recordings of normal fibres we present three basic findings: first, axons grow at a relatively steady rate along the optic tract toward the tectum without branching, pausing or retracting; second, they slow down as they approach the target and third, terminal arbors are formed by a back-branching process rather than iterative bifurcations of leading growth cones.

Stimulated by observations in tissue culture that growth cones remain motile for some time after they are separated from their parent cell bodies (Shaw & Bray, 1977), we separated labelled axons from their somata by removing the retina. Such isolated axons continue to grow for up to 3 h in vivo and display normal pathfinding behaviour. They follow a normal trajectory at a usual speed in the optic tract and, if they reach the target, they slow down and begin to arborize. Thus, target recognition factors and the cellular machinery that controls the growth cone’s response to them must work locally in the growing axon. The soma need not be continuously involved.

**Materials and methods**

*Xenopus* embryos from stages 24 to 29/30 (staging according to Nieuwkoop & Faber, 1956) were anaesthetized with MS222 (tricaine methane sulphonate, Sandoz) and immobilized by being wedged into a depression in a Sylguard dish and held down with a metal weight. A small hole was made in the epidermal tissue over the right eye through which a few crystals of Dil (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, Molecular Probes) were inserted (see Honig & Hume, 1986, for use of this dye). An attempt was made to imbed the crystals in the primordial neural retina. The animals were labelled at these early stages, usually before axonogenesis began (Holt, 1984) so that we could observe the first labelled axons in the tract with the assurance that the labelling procedure had not damaged these axons. The animals were then allowed to recover.

Labelled embryos were prepared for video microscopy between stages 35/36 and 40. It is during these stages that the first retinal axons grow up the optic tract and into the optic tectum (Holt, 1984). Candidates for observation were reanaesthetized, the epidermis on the left side of the head was removed along with the left eye to expose the surface of the left side of the brain. At this point, the animal was beheaded just anterior to the heart and the head transferred to a petriperm hydrophil Petri dish (Heraeus) filled with Modified Barth’s Saline (MBS) (Gurdon, 1977) without anaesthetic. The head was held, left side down, slightly flattened by a nylon mesh and metal weights (Fig. 1). The specimen was then placed on an inverted microscope (Zeiss Axioskop) equipped with epifluorescence. Using brightfield optics, the tract and tectum were located. Then a 20 ms flash of green light (546 nm) was used to induce fluorescence. The fluorescent image (580 nm) was recorded on a SIT video camera with output to a Hamamatsu image-processing system which digitized the image and sent it to a DEC 11-23 computer where it was temporarily stored in memory. The image was then sent into a time-lapse tape recorder. The arrangement is shown in Fig. 1. Focusing and positioning of fluorescent axons and growth cones were done using these 20 ms flashes. Out of ten labelled embryos usually two or three were suitable for time-lapse recording. The others either showed no labelled axons or too many. This probably depended on the size and exact placement of the crystals of Dil. For the recording session, generally a new image from a 20 ms flash was taken every 1.3 or 2.7 min with the tape advancing one frame every 5-1 s. When viewed at normal speed (50 frames s⁻¹), these settings increased real time by a factor of about 250 and produced a slightly jerky (one new picture about every half second) time-lapse movie. We found that much more frequent flashes compromised the viability of the axon under observation. With the above protocol, we could

![Fig. 1. Basic set-up for making time-lapse videos of retinal axons in vivo (details in Materials and methods). Note how the sample is flattened against the bottom of the Petri dish using mesh and metal weights. This helps to keep the axons that are growing close to the pial surface in a limited plane of focus.](image-url)
observe living axons in an apparently healthy state for at least 12 h, although generally our time-lapse periods were shorter. We tried to concentrate our attention on fields of view where only one or a few axons were present. All experiments were performed at room temperature which varied between 20° and 24°C.

To observe axons growing free of their cell bodies, the entire right eye was simply removed from each anaesthetized animal breaking the optic nerve immediately postorbitally. The specimen was then mounted and observed as described above.

At the end of the time-lapse recording, the fluorescent shutter was opened for continuous real-time recording and, during this time, we focused 'through' the axons and arbors so that we could see what had happened out of the plane of focus. As a consequence of this continuous illumination period, the axon was assumed to be damaged and no further recordings were made, except in one case which is specially noted in the results.

Analysis of the video tape was done by tracing or photographing single frames from the video screen. The data in this paper come from 33 animals, 40 axons and a total recording time of 172 h.

### Results

#### Optic tract

A single small crystal of DiI in the retina usually labelled only a few ganglion cell axons, sometimes only one. Our best results were from specimens in which one to three axons were brightly labelled. Axons tipped with growth cones were found in the optic tract growing rapidly toward the tectum (Figs 3-6). For a given axon, the rate of growth was remarkably steady, with only slight accelerations and decelerations. There was substantial interfibre variability and it was not uncommon to witness a slower fibre being passed by a faster one. The average rate of all measured fibres was about 52 μm h⁻¹ (Fig. 2).

We saw no evidence of branching along the tract (Figs 3, 4, 6). Thus axons do not rely on throwing out short but randomly oriented branches which test for appropriate directionality. Rather the growth cone seems to lead the axon directly and unerringly to its target neuropil.

Some axons travel in rather straight lines toward the tectum while most seem to turn gently posteriorly as they reach the mid-diencephalon (Fig. 3). Others make abrupt and obtuse doglegs posteriorly and, in even these cases, there is no appreciable decrease in growth cone speed (Fig. 6).

#### Axons slow down when they reach the tectum

As growth cones approach the tectum, they slow down (Figs 2, 4). The transition to slower speeds (average for all fibres 16 μm h⁻¹, see Fig. 2) may happen smoothly or abruptly. It was impossible for us to tell if the point of deceleration coincides precisely with the proximal border of the tectal neuropil because there are no distinctive features that mark the tectal perimeters, especially in partially dissected live heads. We were able to make rough estimates of where the tectum was from the outline of the brain, as was done previously (Harris, 1986) but did not attempt a topographic analysis of the developing map. It is clear that the slowing usually occurs prior to the beginning of terminal branching (Fig. 2). Accompanying the deceleration, the growth cone often appears more complex (see below). As the axon slows in the target region, it often, but not always, also changes its direction (Figs 4, 9). What once seemed like a fast and highly directed growth cone now appears to be a lazy and meandering one.

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**Fig. 2.** Axon speed in the optic tract and tectum. The speed of 20 normal axons was measured in the optic tract with a total observation time of over 50 h. Their average speed and standard deviation, 53.7 ± 17.8 μm h⁻¹, are shown on the left side of the graph. Of these 20, a subset of 14 that reached tectum were observed for a total of 26 h. The beginning of the tectum was judged by anatomical criteria (see text) and was somewhat arbitrary. Nevertheless, it is clear from the data on the right (average speed = 15.7 ± 10.7 μm h⁻¹) that these 14 axons slowed down considerably as they reached their target area. The solid line illustrates data from a typical fibre. The two asterisks beside the line indicate when this fibre threw out its first and second branches. The asterisk with error bars above shows average data for first branch formation from five normal axons (a subset of the above mentioned 14) which were observed to branch in the tectum. The solid brackets at the extreme left and right of the graph give the average data and standard deviations for six somaless axons in the tract (left) (14 h of observation) and the subset of three of these that reached the target area (8 h of observation).
Eventually forward progress may become so slow it is inappreciable on our tapes.

**Observations of growth cones at high frequency and high magnification**

At the risk of compromising the viability of some growing axons, we made some short-term time-lapse recordings using more frequent exposures (up to one flash every 10 s). After about 3–10 h of such observation, the axon often appeared beaded and the growth cone rounded up and became immotile.

During the first hours of such high magnification, high frequency recordings showed extremely active and elaborate growth cones comparable to those seen with phase-contrast or Nomarski microscopy *in vitro* (Letourneau, 1982; Bray & Chapman, 1985). The

![Fig. 3](image_url)

**Fig. 3.** Still frames from an axon that grows to the optic tectum and begins to arborize. (A–E) In the dorsal tract, at half hour intervals; (F–J), near the target region, at one hour intervals. This illustrates how the axon advances more slowly near the target. Also evident are the worm-like branches which can be seen extending from behind the tip of the axon. (K) A higher magnification view of the terminal arbor taken as part of the 'focus-through' series. Bar in A applies to A–J, 100 µm; K, 50 µm.
Time lapse of CNS axons growing in vivo

Fig. 4. Another example of a single fibre growing in the tract and arborizing the tectum. Apparent again are (1) steady growth in the tract, (2) deceleration near the target, followed by (3) loss of normal growth cone morphology and (4) occurrence of back-branching. At the dotted line, the focus was switched onto a more superficial branch. At right is shown a 'focus-through' reconstruction of the terminal arbor.

growth cones were large, had numerous microspikes and lamelllodia, and changed their appearance completely every minute. By taking a new picture every 10–40 s, we could see some continuity in the changing morphology.

In the tract, the growth cones mostly had forward directed lamelllodia and newly extended microspikes (Fig. 5). The microspikes often seemed to swing rearwards (Fig. 5), while the advancing axon slipped forward into a front-facing lamellobodium. This is a common mode of axonal growth in vitro and has been described in detail by Bray & Chapman (1985). These growth cones looked like classical inverted cones at the end of the naked axons (Cajal, 1928). In the target region, the growth cones often radically changed their basic morphology, becoming first more complex and elongated with lamelllodia and microspikes extending at all angles often well behind the leading tip (Figs 3, 6, 9). We did not notice any obvious difference in the level of activity of the growth cone at these two regions.

Terminal branching

One of the most exciting aspects of the time-lapse recordings was seeing the dynamics of terminal branching. From static views, it is obviously impossible to discern how the branches form, although the geometry of the tree may suggest possible sequences (Verwer & Van Pett, 1983). Do they, like trees, shoot out lateral buds some distance behind the central apex? Or does the growth cone bifurcate, becoming two growth cones each of which has the possibility of further bifurcations? The former, 'backbranching' scheme of arborization seems to be the major one. Most of the terminal branches that we saw form (36/42 or 85 %) clearly originated behind the leading growth cone (Figs 3, 4, 7). Another aspect of terminal arbor formation is the more meandering route that the leading growth cone begins to take in the target region.

The terminal branches did not, in general, grow in the same way as the tip of the primary axon. The primary axon always advanced by way of a distinct growth cone, whereas the secondary terminal branches rarely had growth cones (Figs 3, 4, 7). Some had small ones, but more often the branches appeared to worm their way out with no obvious terminal swelling, much less a growth cone to guide them. These branches often grew slowly and erratically. Some would appear and then later retract into

Fig. 5. Growth cone in optic tract at high magnification. These are stills from a videorecording on which a new picture was taken every 40 s so we could see continuity in the features of the growth cone. Arrows point to a microspike which in A points forward; but as the growth cone advances, it swings rearward. Time intervals from A are indicated. This case is similar to that shown in Fig. 8 of Bray & Chapman (1985).
Fig. 6. (A) Growth cone making a turn in the optic tract and arriving at the target. These tracings taken from a high magnification recording of a single axon are taken from two regions of its pathway. On the left, we see it growing quickly in the lower tract (see inset), turning suddenly without slowing. At right, 2 h later, the same axon is approaching the target. It slows down and becomes more complex. (B) Growth cone approaching the target and beginning to send out first branch. Here one can see a single fibre slowing down in the target region, its growth cone becoming at first more complex and then less obvious. The right-most tracing shows the emergence of the first branch about 30 μm behind the leading tip. It extends without a growth cone.

Fig. 7. Three examples of normal fibres branching in the tectum. In A and B it is clear that most of the branches originate by back-branching. In C three branches emerge from the tip.

The axon that grew past the tectum
One peculiar and interesting case, shown in Fig. 8, was that of an axon whose growth cone had been illuminated for about 20 s while we were focusing through the terminal branches of another axon that had already terminated. The growth cone we could see from its position was at the ventral edge of the tectum when it was illuminated. Instead of ending the session, we elected to observe this growth cone. The axon did not die as the result of the illumination, but it did act unusually. As it came close to the target zone, it moved slowly as is usually the case. In the target region, it put out a few back branches, again not unusual. But then the leading growth cone began to proceed more rapidly towards the posterior mid-brain, past the tectal neuropil and on towards the cerebellum at which point the recording was stopped. As it left the target region, not only did its speed increase but it stopped putting out branches in the vicinity of the growth cone so that when we focused through the axon at the end of the experiment the only branches visible were in the region of the tectal neuropil (Fig. 8).

Axons without cell bodies
In tissue culture, growth cones can continue to move and axons continue to elongate when severed from their parent cell bodies (Shaw & Bray, 1977). We suspected that this phenomenon might not exist in...
Fig. 8. This axon was exposed to the fluorescent light source for about 20s before it was videotaped (see text). It grew past the tectum, speeding up as it moved further away from the target region. Back-branches only formed near the tectum. No growth cone was seen in the final 'focus-through' because it grew too far away from the surface of the Petri dish.

Fig. 9. A growth cone detached from its cell body approaching the tectum. The growth cone turns abruptly as it nears the target and its morphology becomes more complex and less forward-directed as with normal axons.

Do isolated retinal axons continue to grow toward the tectum and are they still able to recognize their targets? The time-lapse recordings show that isolated axons continue to grow along the optic tract, without wandering off it, at a relatively fast and steady speed (Figs 2, 10), until they suddenly 'die' (Fig. 10A). If the axon was severed when the growth cone was near enough the target to reach it before dying, then we saw the normal speed transition from fast to slow on encountering the target area (Figs 2, 10B). If the severed axon was in or very near the tectum, the growth cone showed the normal characteristics of growth cones in the tectum: slow forward progress, turning and elaborate morphology (Figs 9, 10B).

Axons without somata were also capable of initiating branches in the target. One axon that had already sent out its first branch was separated from its cell body and approximately 1½ h later a new second branch began to form (Fig. 10C). These observations show that somaless growth cones are capable of navigating to the target and of recognizing and of responding to it.

Discussion

In these studies, we followed axons after they had crossed the chiasm and were proceeding in the optic tract. Knowledge of the dynamics of axonal growth in the retina, optic nerve and chiasm must await advances in the method, for in our studies the orientation of the specimen together with the halo of fluorescence caused by the crystal of dye in the retina prevented observation of these regions. From our restricted view, we can nevertheless sketch a scenario of axonal growth and terminal arbor formation in the embryonic CNS.

Axons grow directly to their targets led by active growth cones which resemble those in vitro in their morphology and dynamics. The growth cones lead the axons at fairly constant rates toward the tectal neuropil. This is in contrast to the 'stop and start'
growth of insect pioneers (Raper et al. 1983) and the suggested saltatory progress of growth cones in the mouse retinofugal pathway (Bovolenta & Mason, 1987), in the amphibian tail (Speidel, 1933), and in culture (see below). As the growth cone approaches the target, three things happen: (1) its rate of extension slows, (2) it becomes more elaborate and extended and (3) it may lead the axon on a meandering path with sharp turns. After the arrival at the target region, the axon may begin to branch, usually budding well behind the tip. Secondary branches usually elongate in a blind and worm-like fashion without the aid of guiding growth cones. Whether these features will be found to be common to growing axons in other parts of the CNS awaits future studies.

Could it be that labelled growth cones suffer from exposure to the light flashes and this photo-induced damage is what is causing the growth cone to slow down and branch? This is unlikely for a number of reasons. First, Honig & Hume (1985) showed that Dil was nontoxic in long-term multiply-exposed cultures. Second, in our study, the axons slowed down as they neared the target and not after a particular amount of observation time. Those that were already near the target decelerated soon after the recording session began. Those that were in the ventral part of the optic tract did not slow down for many hours, until they reached the tectum. Third, the axon that grew past the tectum was exposed for 20 s continuously, longer than the total exposure time of the axon shown in Figs 3 and 4, yet it continued to advance and even accelerated. Whether this axon’s behaviour was the result of the illumination or a case of a developmental mistake that we fortuitously recorded is not clear.

When the entire retinal projection is labelled with HRP at these early stages it is usual to see a few fibres dorsal to the basal optic root and ventral to the tectum (Harris, 1986; Fig. 1G,H). These may be selectively eliminated as they are not seen in older larvae.

Many laboratories have begun to study the molecular and cell biology of neurite outgrowth in tissue culture (see Letourneau, 1982, for a review). Good substrates for outgrowth are generally considered to be those that support fast rates of neurite extension. They are adhesive enough for good growth cone attachment, but not so sticky that they impede outgrowth. They also have factors, such as components of the extracellular matrix, that promote neurite extension. In line with this idea, the average rate of outgrowth we have seen in the optic tract in vivo in *Xenopus* is faster than anything we have yet observed for these axons in culture (Harris, Holt, Smith & Gallenson, 1985; Sakaguchi, Moeller, Gallenson & Harris, 1987). The most natural substrate we were able to reproduce in culture, a monolayer of glial cells or their cell surface components, supports the fastest in vitro rate of outgrowth, which is $32 \pm 9 \mu m h^{-1}$ (S.E.M.) (Sakaguchi et al. 1987). In culture, it has been our experience that axons spend some time in bouts of retraction. Although this is less common on glial-cell-conditioned collagen, it happens even there. In vivo, along the optic tract we saw no evidence of retraction. If we limit our in vitro analysis solely to bouts of forward progression then the average rate of outgrowth on glial cells is $51 \pm 7 \mu m h^{-1}$ (S.E.M.), nearly as fast as that seen here in vivo. Thus the main difference between growth in vitro and in vivo may be in what causes or eliminates these bouts of retraction.

On very adhesive substrates, in vitro and in vivo, axons travel slowly and leave microspikes behind as minibranches on the axon trunk (Letourneau, 1982; Bray & Chapman, 1985; Caudy & Bentley, 1986). Could it be then that the tectal neuropil, by being more adhesive, causes the axon both to decelerate...
and to begin branching by leaving microspikes behind the growing tip? Since branches do not appear to originate from the growth cone, but rather from the clear stretch of axon well behind the growth cone, another mechanism seems to be called for. The only means of arbor formation yet observed in two-dimensional culture systems seems to be by bifurcation of the growth cone (Bray, 1973; Shaw & Bray, 1977; Wessells & Nuttall, 1978). Since in culture neurites tend to be attached to the substrate at their growth cones, which in turn exert tension on the axon, neurones with branched neurites tend to be delicate balances of vectorial tension (Bray, 1973) and a growth cone of such a neurone cannot easily turn 90° without the balance of a bifurcating partner heading in the opposite direction. In fact, the release of proximal tension, by cutting the axon stem, often causes the growth cone to bifurcate in opposite directions in order to reestablish a balance in tension (Shaw & Bray, 1977). From our observations in vivo, this does not appear to be a motive force behind branch formation since branches (a) originate well behind the leading growth cone and (b) are not apparently equipped with growth cones of their own.

The question then remains, what causes the branches to form? Since branch formation occurs where forward progression is slow and meandering, it may be that the two processes are causally linked, such that the retardation or cessation of forward movement is itself a signal for branching. Possibly, upon retardation, materials shipped up from the cell body have nowhere else to go. This possibility may be tested in tissue culture by causing axons to grow from a fast to a very adhesive and therefore slow substrate. Alternatively, positional cues in the target which inform the axon terminal that it has reached an optimal position may cause forward movement to stop and induce backward branching (Gierer, 1987). The fact that we see branches forming behind the growth cone is in agreement with the segmental growth model of tree formation based on studies of the geometry of mature arbors. In this model, 'protrusion of new branches occurs with equal probability from any existing segment' (Verwer & Van Pelt, 1983).

Our studies strongly support a number of recent static observations on growth cones as they arrive in the target and begin to branch. Two studies in different systems (chick limb: Tosney & Landmesser, 1985; mouse visual system: Bovolenta & Mason, 1987) have shown that growth cones in the target region are more complex on average than those in pathway. Stretevan & Shatz (1987) and Bovolenta & Mason (1987) studied early terminals of visual fibres in the mammalian thalamus and it is particularly clear from these studies that the tips of newly arrived retinogeniculate terminals are not like enlarged growth cones, but are 'hairy' for quite a distance behind their tips indicating that secondary branch structures must form along the length of the terminal segment not just at its very tip. Sakaguchi & Murphey (1985) in *Xenopus* tectum and Thanos & Bonhoeffer (1987) in chick tectum studied early events in the growth of topographic terminal arbors. At first there are only a few branches that define the perimeters of a certain target area. More secondary and tertiary branches are then added to fill in the area more completely (Thanos & Bonhoeffer, 1987). Our examples of individual arbors in the process of forming support this mode of initial development.

Our results show that the growth cones themselves have the machinery to recognize and respond to pathway and target cues. This rules out the alternative hypothesis that receptors on the growth cone mediate retrograde signals to the nucleus which then ships back messages to the growth cone telling it such things as whether or not it is on course or whether to slow down because it is approaching the target. Since HRP can be shipped from the cell body to growth cones in or near the tectum in less than 15 min in the embryonic *Xenopus* (Harris et al. 1985) and Lucifer Yellow travels the same distance in a couple of minutes (Holt, unpublished observation), such a hypothesis could not have been ruled out a priori as being too slow.

Other experimental manipulations with growth cone dynamics, such as barrier avoidance and pharmacological interference, may also prove to be informative in this in vivo system which promises to be useful in testing new ideas about the normal development of axons in the CNS.

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