Directional cues for growing axons forming the retinotectal projection

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Summary

In the formation of projections that map one area of the nervous system onto another, guidance of axonal growth cones by directional cues encoded in target tissues is well established by observations on misrouted fibres, though other mechanisms contribute to the precision of connectivity patterns. General physical principles indicate that, if there is directional guidance of normally as well as misrouted axons, leading them toward their appropriate target positions, the inference for a role of graded distributions of molecules is a strong one. As explained in this paper, curving, meandering, branching and shifting connections are fully consistent with a crucial role of directional cues encoded by graded molecular distri-

butions. A model is proposed according to which slight directional cues are strongly enhanced within the axonal growth cone; if the proximal part of the growth cone is activated indicating that the growth cone points in an entirely wrong direction, then a discontinuous directional change such as branching is elicited. Pathways are corrected in this way, and near the appropriate target position the terminal arbour is formed because from there all routes point towards less optimal positions, leading to multiple branching.

Key words: retina, tectum, projection, growth cone, axonal guidance, terminal arbour, pattern formation, neurogenesis.

Introduction

The formation of the retinotectal map in birds, amphibia and fish is a particularly suitable process for studying axonal pathfinding, leading to the topological order of a large number of neural fibres. Explanations proposed can be classified into mechanisms involving fibre–tectum (Sperry, 1963) and fibre–fibre interactions (Willshaw & von der Malsburg, 1979), order in time of arrival (Rager, 1976) and functional checks of neighbourhood relations by impulse activity (Willshaw & von der Malsburg, 1976). These mechanisms are not mutually exclusive and experimental evidence shows that several, if not all, of them play some role in map formation. For instance, impulse activity appears to be involved in the final sharpening of maps (Meyer, 1983; Schmidt, 1985). On the other hand, there is increasing evidence that, in the primary projection, directional cues on the tectum are crucially involved. Often, retinal fibres are capable of approaching their target position from different points of entry into the tectum, indicating that they sense the direction towards the target position across

a distance (for review, see Bonhoeffer & Gierer, 1984). On their way, they may cross other retinal fibres with other target areas. Mathematical models have been developed for directional guidance leading to topographic projections; they have been based on mechanisms of differential mechanical adhesion (Fraser, 1980), or on enhancement of slight directional cues within the growth cone (Gierer, 1981, 1983). In this paper, two aspects of map formation will be discussed which are relevant for the relation between experimental observations on one hand and the theoretical analysis of the underlying mechanisms on the other: first, the validity of the inference from directional cues to guidance by graded distributions of molecules; and second, the consistency of such a guidance mechanism with observations of curved pathways, and the meanderings and branchings of fibres in the course of map formation.

Directional cues specified by gradients

If axons are guided in a two-dimensional field towards their specific target positions and if this mechanism

operates for normally routed as well as for misrouted fibres (e.g. Fujisawa, Tani, Watanabe & Ibata, 1982; Thanos & Bonhoeffer, 1986), axons with the same target position will sense appropriate directions at different positions of the tectum (see Fig. 4A,B), and axons with different targets will sense different directions at the same position of the tectum. One may distinguish between two physically different possibilities for specifying directional cues. There exist either oriented molecular structures on the tectum which serve as tracks for fibre guidance, or spatially graded concentrations of guiding substances, directions of axonal growth being determined by the slopes of such gradients. In the first case, each part of the tectal area would have to be covered with tracks of different orientation to be selected by retinal fibres approaching different target positions. This is difficult to realize and not supported by any morphological evidence. The straightforward alternative is that axons respond to spatially graded substances. A two-dimensional field, guiding an axonal growth cone from any starting position to a specific target position, resembles a system in the environment of a stable steady state. The dynamics of such a system can be formally described as movement down the slope (the 'gradient') of a bowl-shaped 'potential', the distribution of a physical parameter in real or abstract space, until the point of minimal potential is reached. This notion of potential is a generalization of the concept of energy and is widely applicable to the physics of dynamic processes. It may also be applied to the mechanisms of axon navigation. If the growth cone approaches the target position independent of the point of entry, that is from any direction, the process can be described as growth along the slope of a guiding parameter until the optimal position is reached.

A recent study of requirements for projections by gradient mechanisms (Gierer, 1981, 1983) demonstrates that countergraded effects are necessary in each of the two dimensions to generate a spatial distribution of a guiding parameter p with a minimum at a position *within* the tectal field. Two gradients with opposite slope, or two substances graded in the same direction, one activating and one inhibiting, or even a single gradient which is read by the axonal growth cone in two different manners, exerting an activating and an inhibitory effect, could create a minimum. (These tectal gradients involved in the generation of p could be distributions of relatively stable cell surface molecules which do not diffuse freely from cell to cell.) Further, it is necessary that the antagonistic effects arising from these graded distributions in the tectum depend quantitatively on molecular components of the axonal growth cone, for instance, on receptor concentrations, which are themselves

Fig. 1. Model for the retinal tectal projection based on counter gradients distributed exponentially in the two dimensions x, y of the tectal surface, and components (e.g. receptors) on the retinal growth cone with concentrations depending exponentially on the coordinates of origin u, v of the corresponding axon in the retina. In the model, the guiding parameter p resulting from the interaction of growth cone and target tissue is given by

$$p = e^{+\alpha x} \cdot e^{-\alpha u} + e^{-\alpha x} \cdot e^{+\alpha u} + e^{+\beta y} \cdot e^{-\beta v} + e^{-\beta y} \cdot e^{+\beta v}$$

Minimum of p is at $x = u, y = v$, corresponding to a simple projection. The plot shows, in the x dimension, the distributions ---- of gradient $e^{\alpha(x-u)}$ and counter gradient $e^{\alpha(u-x)}$, adding up to parameter $p(x)$ (—) for $u = 0.18$ (A) and $u = 0.68$ (B); minima of p are at $x = 0.18$ and $x = 0.68$, respectively.

graded with respect to the position of origin of the corresponding axon in the retina. Axons from different positions of the retina 'read' the gradients of the tectum differently, namely as bowl-shaped 'potentials' of the guiding parameter p with the minima at *different* positions within the tectum. Quite simple types of interactions of such axonal and tectal components were shown to be capable of generating reliable projections. An example for a model along these lines is given in Fig. 1. The same considerations hold for models guiding 'upwards' a gradient instead of 'downwards', with guiding distributions resembling a bowl turned upside down and target position at the maximum level. An example of this variant is shown in Fig. 2. Experimental evidence on the sign of guiding gradients is not yet available and the choice of sign does not strongly affect model properties. In this paper, we chose distributions as given in Fig. 2 for modelling; thus, the direction toward the target is 'up' the slope.

Which physical mechanisms would lead to axonal guidance determined by slopes of graded distributions of molecules? One possibility is differential adhesion. The axonal growth cone extends microprocesses that adhere, with different strengths, in different directions causing the growth cone to be pulled in

Fig. 2. A model for guidance towards the maximum of a guiding parameter p . Components of the tectal surface of distribution $e^{-\alpha x}$, $e^{-\beta y}$ interact with growth cone components of concentrations e^{au} , e^{bv} , thus generating components of concentration $q_x = e^{a(u-x)}$ and $q_y = e^{b(v-y)}$. These, in turn, affect the production of guiding parameter p by linear activation and 'overlinear' inhibition according to

$$p = q_x / (1 + q_x^2) + q_y / (1 + q_y^2)$$

The maximum of p is at $x = u$, $y = v$, corresponding to a projection. The distribution of p is shown for axons derived from different positions u , v of the retina, namely from $u = 0.21$, $v = 0.33$ in A and from $u = v = 0.62$ in B. The computer diagrams demonstrate that the coordinates x , y at which p assumes the maximal level depend on, and correspond to, the coordinates u , v of the origin of the axons of the retina; therefore, guidance of fibres towards their maxima of p leads to mapping of the retinal field (u, v) onto the tectal surface (x, y) . In the calculated example the field of p is anisotropic with $\alpha = 5/L$ and $\beta = 2/L$ (L is total field size).

the direction of maximal adhesion on purely mechanical grounds. However, it is uncertain whether the (presumably slight) *differences* in adhesion in the small area of contact between growth cone and target tissue would suffice, by itself, for reliable directional guidance. It seems more likely that the growth cone has the capability of *enhancing* even very *slight* directional cues. Models for projections developed along these lines (Gierer, 1981) assume that a guiding substance of concentration p is produced by interaction of graded components of the tectum with components (e.g. receptors) of the axonal growth cone and that small differences of p across the growth cone are then strongly enhanced by intracellular pattern formation *within* the growth cone itself. In this way, a focus of activation is produced in the direction of the slope of p , which, in turn, causes axonal growth in this direction until the target position, the optimal level of p , is reached.

The mechanism would resemble the direction of chemotactic movement of cellular slime moulds (Bonner, 1947) by a gradient of cyclic AMP where a very small concentration difference (approx. 1%)

Fig. 3. Enhancement of directional cues by intracellular pattern formation within the growth cone. (A) Guiding parameter p , generated by interaction of the growth cone with graded components of target tissue (e.g. according to the equations given in the legend of Fig. 2) varies slightly across the area of the growth cone. The numerical values are arbitrarily chosen to illustrate the principle. Intracellular pattern formation by autocatalysis and lateral inhibition is assumed to enhance this directional cue, producing a focus of activation in the direction of the slope of p (arrows). In this way, the gradient of p exerts a steering effect on the navigating growth cone towards the target position (B,C).

between different parts of the cell suffice to orient the movement reliably. Physicochemical conditions leading to spatial concentration patterns have been discussed previously (Gierer & Meinhardt, 1972; Meinhardt & Gierer, 1974). Spatial patterns are formed if a short-range autocatalytic reaction is coupled to a 'lateral' inhibition or depletion effect extending into a wider range. Strongly polar distributions can be produced in this way, the orientation being reliably determined by very slight initial asymmetries. Models along these lines have been developed and discussed for pattern formation within multicellular tissues as well as within individual cells. They may be applicable to directional activation of the growth cone as well. A focus of activity may be produced within the cone or on its membrane, the direction of the focus being determined by the slope of a guiding gradient, however shallow; the focus would be capable of reliably determining the direction of growth towards the optimal (e.g. the maximal) level of p (Fig. 3). Possibly, the direction of maximal slope is assumed immediately; but a gradual steering effect in which the direction is gradually assumed in several steps would also suffice for guidance toward the target.

Fig. 4. Axonal pathways. Regenerating retinal axons invading newt tectum, according to Fujisawa *et al.* (1982). (— tectal margin, — axonal fibres). (A) Three retinal fibres that have reached about the same target position (as indicated by the terminal arbours) from different aspects of the tectum on different routes. There is some meandering *en route* but no selfcrossing. Note extensive terminal arborization. (B) Three fibres with a different target as in (A). (The pictures do not show branches *en route* that are transiently observed at earlier stages (Fujisawa *et al.* 1982; Gaze *et al.* 1974). In other systems, such as chick tectum, branching *en route* is rare or absent altogether (Crossland *et al.* 1974; Thanos *et al.* 1984; Thanos & Bonhoeffer, 1986).)

Such mechanisms do not exclude an involvement of molecular adhesion in guidance. Directional cues might be sensed, primarily, as slight differences of adhesion of microspikes extending in different directions of the growth cone; activation of a focus within the growth cone might elicit increased adhesivity in the direction of the focus. However, an essential role is likely to be played by *intracellular* enhancement of directional cues. The underlying autocatalytic mechanisms require further investigations. They might include, for instance, the activity of enzymes and receptors as well as selfassembly of cytoskeletal structures that contribute to the extension of microspikes and lamellipodia.

Curving, meandering and branching pathways

In its simplest form, guidance by gradients would lead to smooth, straight or slightly curved pathways from the point of entry into the target tissue to the final target position. In some cases, fibre pathways nearly correspond to this scheme. On the other hand, there are also cases in which a considerable degree of curving, meandering and branching is observed (Fujisawa *et al.* 1982), as in the example of Fig. 4A,B. In the course of termination, extensive arborization may occur (Fujisawa *et al.* 1982; Fujisawa, 1987; Stürmer, 1984). Such features are sometimes interpreted intuitively as disproving directional guidance, and of demonstrating, instead, a large-scale random search until the correct target is reached. As will be explained below, this interpretation is inconclusive and is unlikely to be correct; curving, meandering and

branching fit very well into concepts based primarily on directional guidance by gradients.

Curving

On some simple assumptions, the target position should be approached on nearly straight pathways. Curved pathways are expected, however, if the steepness of gradients involved in guidance differs in the two dimensions: in this case, the fibres tend to first follow the dimension with the steeper gradient and then approach the target position in the dimension of the shallower gradient (Fig. 5A). Curved pathways are also expected if the coordinate system specified by gradients is not Cartesian, or if the guiding gradients are of complex (e.g. sigmoid) form.

Meandering

In some cases where individual fibres invading the tectum have been traced, considerable meandering was observed with respect to the pathway. This applies especially to misrouted fibres. Meandering indicates that fluctuations of directions are superimposed on systematic directional cues; it does not imply that the fibre undertakes a predominantly or purely random search until the correct target area is hit. A truly random search, with arbitrary changes of direction at short intervals, would give rise to a diffusion-type pathway with many self-crossings; the actual pathway across the tectum would be, on average, *much* longer than the shortest distance to the target. For n random directional changes, the actual pathlength would be of the order of $\sqrt{n}$ times the shortest route (a computer stimulation for $n = 300$ is shown in Fig. 5C). Experimentally detected axons take perhaps twice the shortest distance (see Fig 4),

Fig. 5. Curving and meandering pathways.

(A) Curved pathways. Nearly straight or moderately curved pathways (—) between points of entry (*) and (**) and target position (•) may result if, in a Cartesian coordinate system, guiding gradients are of similar steepness ($\alpha = \beta = 2.5/L$, see legend of Fig. 2). Strong differences in steepness parameters ($\alpha = 1/L$; $\beta = 2.5/L$) give rise to more strongly curved pathways (—). The target is approached from different aspects (*) and (**) of the field. Growth is assumed to be terminated where the growth cone senses its direction as going 'down' instead of 'up' the guiding gradient.

(B) Meandering. The model is extended to include a random directional fluctuation. At each step of progression, proceeding 1.5 % of the field size, there is a change of direction of + or -17° , the probability being affected by the slope of the gradient of the guiding parameter p which exerts a steering effect in the dimension perpendicular to the forward direction (see Fig. 3). The computer simulation shows meandering pathways of similar tortuousness as actually observed pathways (see Fig. 4). (In most cases, the target area is reached; in some of the cases, however, there is premature termination of growth because random fluctuations lead to directions going down the gradient on the way. This may be avoided by including branching into the model (see Fig. 7)).

(C) Random pathways. For comparison, an example for a completely random pathway is given, without directional guidance, assuming random changes of directions between 0° and $\pm 90^\circ$ at each step of progression. The computer simulation shows a pathway of 300 steps. Steplength is 3 % of total field size. Even such long pathways showing many selfcrossings have a low chance of coming close to the target position; such pathways are in no way characteristic of actually observed axonal pathways (see Fig. 4).

and they cross their own pathway very rarely, if at all: therefore, although some random directional fluctuations occur, we are led to conclude that they are *superimposed* on directed growth, the latter dominating the path – just as a real meandering river is *systematically* driven, despite all detours, towards the ocean by the *gradient* in altitude. Fig. 5B shows a computer simulation of pathways directed by gradients, with superimposed random fluctuation of directions leading to meandering comparable to the examples shown in Fig. 4.

Kinks of pathways and initiation of pathfinding

In some systems, axonal fibres invade the tectum by following fascicles of other fibres; then they change directions and deviate from the fascicle in order to approach their appropriate target position (Easter & Stürmer, 1984; Stürmer & Easter, 1984; Thanos, Bonhoeffer & Rutishauser, 1984). Generally, the position of *initiation* of directional guidance towards the target within the two-dimensional field need not be identical with the position at which the fibre

reaches the target area first. The signal for a striking directional change (be it deviation from a bundle of other fibres, or just from an initial forward direction of invasion) may itself result from the response of the growth cone to the tectal gradients. Simple rules may govern cases in which the directional change occurs at nearly right angles, as for misrouted retinal fibres invading chick tectum (Fig. 6A) (Thanos *et al.* 1984). As long as the slope of the guiding parameter is positive, i.e. the axons is still going '*upward*' upon following the direction of a fascicle, the *distal* part of the growth cone is activated. We assume that, in this case, the fibre continues to grow *forward*, because it is energetically favourable to grow along its fascicle. However, as soon as the guiding parameter goes *downward*, the slope being negative, the *proximal* part of the growth cone is activated. This, in turn, may initiate branching off at nearly right angles as is indicated in Fig. 6B–D. From there onwards, the target position is approached along the slope of the guiding gradient as explained above (see Fig. 3).

Fig. 6. Model for deviation from the track of invasion. In the chick, treatment with an antigen of cell adhesion molecules leads to a misrouting of retinal fibres invading the tectum; there, often, misrouted fibres first follow other fibres in the anterior–posterior (A–P) direction, but turn by right angle and then approach the appropriate target in the dorsoventral (DV) dimension within the tectum field (A). The pathway is redrawn according to Thanos *et al.* (1984). Our model for such behaviour is to postulate that first the axon is guided along a track which would not eventually lead to the appropriate target position, in the present example on a fascicle of other fibres; it continues to grow along this track, as long as the guiding parameter p goes ‘up’ and therefore the distal part of the growth cone is activated (B). As soon as the guiding parameter goes ‘down’ leading to proximal activation of the growth cone (C), this is read as a signal for deviation (e.g. by right angle) from the track; from there on, the target position is approached, directed by the slope of the guiding parameter p (D).

Branching

Extensive branching is, as a rule, found in terminal arbours (e.g. Stürmer, 1984). Branching is also observed, in some but not all systems, along the pathway across the tectum. It may be misleading to consider this branching as a random search for the ‘true’ target itself as identified by position-specific chemical markers which are qualitatively different from each other. Such a search, to be efficient, would require that a major part of the tectal field would have to be covered by fibre pathways before branches stop growing (or are retracted); this is in contrast to observations reported in the literature, both for primary development (Fujisawa, 1987) and regeneration (Fujisawa *et al.* 1982). They indicate that only one (or few) branches dominate, whereas most branches remain short and stop growing, or are even retracted, while the search for the target still continues. It appears that, in these cases, branching *en route* may contribute to sensing, and correcting for, the *relatively* more appropriate directions toward the target position even if the latter is still far from the tips of the growing axon.

Generally, one expects that there is a variety of mechanisms capable of causing axonal branching. With respect to branching of retinal axons on the

tectum, a hypothesis will now be proposed which connects this phenomenon directly with guidance by gradients. Its basic postulate is simple: when the axonal growth cone, upon sensing the slope of the guiding gradient, finds that the optimal direction is no longer forward, the proximal, ‘backward’ part of the growth cone becomes activated. Such proximal activation, we propose, elicits with high probability a second direction of growth, thus leading to branching (Fig. 7A,B).

In mathematical terms, proximal activation is assumed to occur if there is a decrease (or, in a minor generalization of the model, no significant increase) of the guiding parameter p in the forward direction of the growth cone. In addition, two further probability values are introduced into the model, one for continuation without branching, another for cessation of growth without branching in situations of proximal activation of the growth cone. Widely different branching patterns can be modelled by suitable quantitative choices of the probability values mentioned. Our concept for branching of a growth cone navigating freely on the tectum is very similar to that postulated in the preceding section for deviation from a fascicle, namely that activation of the proximal part of the growth cone produces a distinct directional

Fig. 7. Branching models.

(A–C) Forward branching. If an axonal growth cone encounters, in its forward direction, a ‘downward’ slope of the guiding gradient, the proximal part of the growth cone becomes activated; this is proposed to lead, with high probability, to a second focus of activation (A) and this, in turn, causes branching (B). The model assumes that proximal activation occurs if the slope of p in the forward direction is below a threshold. In addition, finite probabilities are introduced for termination of growth as well as for continuation of growth without branching in cases of proximal activation. Branching *may* occur along the pathway if meandering leads to entirely wrong directions and *must* occur in the area of termination where all slopes go ‘down’. A computer simulation based on the distribution of guidance parameters explicated in Fig. 2 with $\alpha = 2.5/L$, $\beta = 1/L$ is given in C; * and ** starting points, ● target position. Random directional fluctuations superimposed on directional guidance give rise to meandering pathways as in Fig. 5B. Growth is assumed to be terminated if a certain amount of branching has occurred. Widely different branching patterns can be simulated by suitable parameter choices. In the simulation in C, parameters were adapted such that terminal arbours are of similar appearance as those shown in Fig. 4.

(D–F) Backward branching. In this version of the branching model, it is assumed that proximal activation of the growth cone (D) stops further growth and causes a new branch to occur at some position not far behind the original growth cone (E). After a certain amount of branching has occurred, growth stops altogether. (F) Computer simulation of a pathway resulting from such back-branching, in conjunction with random meandering.

(G–I) Random branching. Random branching is assumed to occur along the pathway (G), followed by a lateral inhibition between the two growth cones, causing selective activation of the cone with the relatively higher level of guiding parameter p ; the branch with the lower level stops growing (H). The model thus allows for guidance by gradients by sensing differences *between* nearby growth cones instead of *within* an individual growth cone. (I) Computer simulation of the model. Most pathways lead to the target position, but they do not terminate there unless combined with another mechanism, such as gradient-directed branching (see Fig. 8). * starting point of search for the target, ● target position.

change. According to the model, branching *en route* depends on the degree of meandering and may correct for wrong directions; it is not a necessary feature of directional guidance and need not, therefore, occur in all systems. In the target area where the guiding parameter has its maximum, the signal ‘down’ is encountered in any direction of fibre growth;

according to the model, this leads to high probabilities for consecutive branching. The mechanism thus accounts automatically for terminal arborization once the target area is reached. After a certain amount of branching has occurred, the search is assumed to be terminated. A computer simulation of pathways with terminal branching is given in Fig. 7C,

Fig. 8. Combination of forward branching with random branching *en route* (see Fig. 7A–C and 7G–I). Computer simulation of fibre tracks approaching the same target position ● from different starting positions *.

with general characteristics of curving, tortuousness and branching patterns similar to those of empirically found pathways as exemplified in Fig. 4.

Another conceivable mechanism for the formation of branches is by 'back-branching' (Fig. 7D,E). If the growth cone senses that the direction of the guiding parameter goes 'down' and its proximal part becomes activated, growth may stop altogether, and a new growth cone may be formed at some distance behind the original one; there, a new branch grows out, whereas the old one may persist for a while. In this way, pathways are correctable and extensive terminal arborization may occur. A computer simulation is given in Fig. 7F. Recently, 'back-branching' has been experimentally detected and analysed by time-lapse studies on the innervation of tectal tissue *in vivo* (Harris, Holt & Bonhoeffer, 1987).

We may also envisage that there is some random branching on the way to the target area. Soon after branching has occurred, lateral inhibition between nearby growth cones may lead to selective activation of the one growth cone with the *relatively* more optimal level of the guiding parameter for further growth, whereas the 'weaker' branch stops growing (Fig. 7G,H) due to inhibition extending from the activated branch. This mechanism is capable of leading an axon toward the target position by selecting appropriate directions; however, it does not, by itself, cause the cessation of fibre growth once the target position is reached (Fig. 7I). Termination occurs if such random branching *en route* is combined with the mechanism of predominantly terminal branching as described in Fig. 7A–C. Computer simulations of this combination are given in Fig. 8A–C.

All variants of the branching scheme proposed are capable of guiding axonal growth toward the target position from different starting positions and of

correcting misrouted pathways. Terminal arborization appears as the immediate *consequence* of reaching the optimal position (Fig. 2) of the guiding parameter. From here, all pathways lead to 'worse' positions. This may result in extensive branching; the amount of branching may serve, by itself, as the signal to terminate further search. No additional regional-specific chemical identification of the target is required.

Explicitly, our model is dealing only with fibre tracks in the dimensions parallel to the tectal surface. However, it is conceivable that growth in the third dimension, i.e. a shift towards deeper layers of the tectum as it occurs *en route* in goldfish (Easter & Stürmer, 1984) and near the terminal site in the chick (Crossland *et al.* 1974), could also be elicited by proximal activation of the growth cone in response to tectal gradients. In the chick, this shift occurs when the target position in the dimension of the tectal surface is reached. There, the fibre dives into the deeper tectal layers where the terminal arbour is formed and synapses are made. Such a shift from one layer to another implies that the growth cone encounters a qualitatively different environment which may, in turn, contribute signals for branching. In goldfish tectum, which grows continuously, invading retinal fibres depart from fascicles *en route*, shift to deeper layers of the tectum, continue growth in that layer (mainly in the rostrocaudal dimension), approach their target position and form terminal arbours there (Easter & Stürmer, 1984). Some of the main elements of such behaviour have been dealt with in this paper, though the rules for the departure from the fascicle *en route* require further studies.

Terminal arbours within a tectum differ considerably in size (Stürmer, 1984). It seems likely that the size of the smallest class of terminal arbours (of the order of 0.1 mm) may be a reasonable measure of

the accuracy of target specification by gradients (big arbours may be big for functional reasons, not because of limitations of accuracy of axonal guidance). In the environment of the target position, the guiding gradients are very shallow, the slope reducing to zero at the target position itself (see Figs 1, 2). An accuracy of target identification of, say, 5% of tectal diameter would require that small differences in guiding parameter (roughly of the order of 5% of 5%, i.e. $\frac{1}{4}\%$) still suffice for directional guidance. According to our model, intracellular pattern formation within the growth cone would indeed be capable of enhancing such small differences, giving rise to strong directional activation. Limits of accuracy would be mainly due to random fluctuations of concentrations sensed in the region of contact between the growth cone and the target tissue. At later stages, projections may be refined by functional checks (involving synaptic activity) of correct neighbourhood relations (Meyer, 1983; Schmidt, 1985).

Discussion

The example of the retinotectal map suggests that directional cues encoded in spatially graded distributions of molecules are crucially involved in the generation of topological order in the nervous system though other effects such as fibre-fibre interactions and functional checks of neighbourhood relations play a role, too, especially at later stages of map formation.

Guidance by gradients allows fibres to correct their pathways and to approach their appropriate target position, even after misrouting. Further regulatory features such as expansion and contraction of maps upon surgical ablations of retinal, or tectal, tissue, as well as the adaptation of the retinotectal map to the growing tecta (Gaze, Keating & Chung, 1974), can easily be incorporated into gradient models; this is possible because the mechanism proposed does not depend on irreversibly fixed markers but allows the gradients to be developmentally regulated as discussed in detail elsewhere (Gierer, 1983). Gradient models do not generally predict whether and to what extent transient synapses are formed while the axon approaches its target position, and so sliding connections (Gaze *et al.* 1974; Stürmer & Easter, 1984) are consistent with, but not required by, such models.

We have shown that guidance by gradients is capable of directing navigating axons in the presence of considerable random fluctuations, but there are limits to this. For unnatural situations, such as surgical interference and regeneration, pathways may show relatively low degrees of order. In such cases, axons could undergo considerable detours before eventually approaching the target position, but, even

then, we expect that directional guidance is crucially involved if fibres are to reach their target at all. If there is too much stochastic interference, or if directional cues are inaccessible, weak or absent, the target position is likely to be missed (Thanos *et al.* 1984) and the corresponding fibre may eventually be eliminated by cell death.

Guidance by gradients allows a limited set of substances, and thus a limited set of genes, to control spatial order of a large number of neural fibres. Experimentally, graded distributions of molecules have been detected in both retina (Trisler, Schneider & Nirenberg, 1981) and tectum (Bonhoeffer & Huff, 1982), though their chemical nature and their participation in generating projections require further investigations. Guidance of fibres *towards* the appropriate target tissue (e.g. the tectum) probably involves recognition of *qualitatively* different chemical markers in fibre-tissue and fibre-fibre interaction. In addition, directional cues were demonstrated in the brain which are capable of guiding misrouted retinal fibres across considerable distances towards the tectum (Harris, 1986), suggesting a guiding role of gradients extending over considerable parts of the brain.

Gradient models presume that graded distributions of molecules are formed in neural tissue at early stages of development. The primary process of establishing such gradients is not the subject of this paper, but it is remarkable that general physical principles underlying *intercellular* pattern formation (such as the formation of graded distributions within cell sheets) may be the same as those for *intracellular* pattern formation (such as the activation of part of a growth cone as discussed in this paper). Generally, autocatalysis in conjunction with long-range 'lateral' inhibition is capable of generating spatial concentration patterns. The molecular mechanisms of activation and inhibition are not yet known and are expected to be different for intracellular and intercellular pattern formation.

In this paper, it is shown that curving, meandering, kink-like changes of direction, branching *en route* and terminal arborization are fully consistent with axonal guidance by gradients. A crucial feature of our type of model is that if directional cues lead to activation of the *proximal* part of the growth cone (indicating that the growth cone points in an entirely wrong direction) this may elicit a discrete response; a strong kink-like change of direction, or termination of growth (possibly in conjunction with 'back-branching'), or forward-branching. These responses and their probabilities depend on the biochemical parameters and may thus vary from system to system. All responses are consistent with the theory of pattern formation by autocatalysis and lateral inhibition mentioned above

(Gierer & Meinhardt, 1972), applied to intracellular activation within the growth cone. For instance, forward-branching (Fig. 7A–C) can be explained as the formation of two well-spaced focuses of activation within the growth cone instead of one, leading to two different branches for further growth. This requires only moderate changes of quantitative parameters; it would occur, for instance, if activation of the proximal part of the growth cone would produce somewhat lower levels of inhibition as compared to more distal parts. The straightforward, discrete transition from monopolar to bipolar activation is a basic feature of pattern formation by autocatalysis and lateral inhibition, easy to simulate on computer. (If guided by differential adhesion only, axons would be expected to approach the target position and stop there; additional mechanisms would be required for the production of terminal arbours.)

Size and topological features of terminal arbours (such as the degree of branching *en route* and close to the terminal position, the amount of symmetric and asymmetric branching and the length of the branches) strongly depend on the parameters involved in the specification of branching rules. Moreover, one and the same regime can produce, by mere statistical fluctuations, pathways and arbours of widely different appearance. The experience of computer simulations casts doubt on simple classifications of arbours by appearance only, and even more on the singling out of particularly tortuous and complicated pathways in the search for specific explanations; such fibres may be nothing but extreme stochastic variants. On the other hand, the models discussed in this paper provide a basis for more detailed mathematical analysis of experimentally determined pathways, directional fluctuations and arborization patterns in space and time; this, in turn, might lead to detailed insights into actual gradient shapes and branching rules. A particularly promising approach is the analysis of the time sequence of branching in time-lapse video studies on growing single fibres *in vivo* (Harris *et al.* 1987).

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