

#### **cones Reading of concentration gradients by axonal growth**

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# ETY<br> **Reading of concentration gradients**<br> **Reading of concentration gradients bu also concentration gradier**<br>by axonal growth cones

# Jürgen Löschinger<sup>\*</sup>, Franco Weth and Friedrich Bonhoeffer *Jürgen Löschinger*<sup>\*</sup>, Franco Weth and Friedrich Bonhoeffer<br>Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35, D72076 Tübingen, Germany

Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35, D72076 Tübingen, Germany<br>Wiring up the nervous system occurs as a self-organizing process during animal development. It has long<br>heen proposed that direction Wiring up the nervous system occurs as a self-organizing process during animal development. It has long<br>been proposed that directional growth of axons towards their targets is achieved by gradients of guiding<br>molecules and Wiring up the nervous system occurs as a self-organizing process during animal development. It has long<br>been proposed that directional growth of axons towards their targets is achieved by gradients of guiding<br>molecules and been proposed that directional growth of axons towards their targets is achieved by gradients of guiding<br>molecules and the conceptual framework of gradient guidance was introduced more than a decade ago.<br>Novel experimental molecules and the conceptual framework of gradient guidance was introduced more than a decade ago.<br>Novel experimental results now allow the formulation of models incorporating more mechanistic detail.<br>We first summarize so Novel experimental results now allow the formulation of models incorporating more mechanistic detail.<br>We first summarize some crucial *in vitro* and *in vivo* results concerning the development of the chick retino-<br>tectal We first summarize some crucial *in vitro* and *in vivo* results concerning the development of the chick retinotectal projection. We then review two recent theoretical models based on these findings (the models of Nakamoto tectal projection. We then review two recent theoretical models based on these findings (the models of Nakamoto and colleagues, and of Honda). Neither model considers the latest observation that putative guidance ligands, Nakamoto and colleagues, and of Honda). Neither model considers the latest observation that putative<br>guidance ligands, in addition to their tectal expression, are expressed in a similar pattern on the retina<br>and that a dis guidance ligands, in addition to their tectal expression, are expressed in a similar pattern on the retina<br>and that a disturbance of this expression affects topography. These findings suggest that retinal axons<br>might grow and that a disturbance of this expression affects topography. These findings suggest that retinal axons<br>might grow into the tectum until they have reached a ligand concentration matching that of their site of<br>origin. We ca might grow into the tectum until they have reached a ligand concentration matching that of their site of origin. We call this the imprint-matching concept of retinotectal guidance. As a framework for pinpointing logical di origin. We call this the imprint-matching concept of retinotectal guidance. As a framework for<br>pinpointing logical difficulties of the mechanistic description of the guidance process and to stimulate<br>further experiments we pinpointing logical difficulties of the mechanistic description of the guidance process and to stimulate further experiments we finally suggest two extended versions of Honda's model implementing imprint matching, which we further experiments we finally suggest two extendent<br>matching, which we call 'the variable set-point' and<br>and weaknesses of both mechanisms are discussed. and weaknesses of both mechanisms are discussed.<br>**Keywords:** topography; retinotectal projection; growth cone; gradient; axon guidance; ephrins

#### **1. INTRODUCTION**

**1. INTRODUCTION**<br>During development of the nervous system the growth<br>cones of projecting axons have to go to very specific sites COLOGETION<br>Cones of projecting axons have to go to very specific sites.<br>As shown in many instances they do so by directed During development of the nervous system the growth<br>cones of projecting axons have to go to very specific sites.<br>As shown in many instances they do so by directed<br>growth rather than by random walk and selection of the cones of projecting axons have to go to very specific sites.<br>As shown in many instances they do so by directed<br>growth rather than by random walk and selection of the<br>correct site. What molecular mechanisms bring them to As shown in many instances they do so by directed<br>growth rather than by random walk and selection of the<br>correct site. What molecular mechanisms bring them to<br>their appropriate positions? Since slopes of concentration growth rather than by random walk and selection of the correct site. What molecular mechanisms bring them to their appropriate positions? Since slopes of concentration gradients can define directions it was already suspect correct site. What molecular mechanisms bring them to to enlarge the concentration difference would be that the<br>their appropriate positions? Since slopes of concentration growth cones, like bacteria, would determine concen their appropriate positions? Since slopes of concentration<br>gradients can define directions, it was already suspected<br>many years ago (Sperry 1943, 1963) that axons might be<br>guided by graded distributions of some guiding mol gradients can define directions, it was already suspected<br>many years ago (Sperry 1943, 1963) that axons might be<br>guided by graded distributions of some guiding molecules.<br>At first sight, as long as one looks at the gradien many years ago (Sperry 1943, 1963) that axons might be<br>guided by graded distributions of some guiding molecules.<br>At first sight, as long as one looks at the gradients and the<br>growth cones (or migrating cells) at low magnif guided by graded distributions of some guiding molecules.<br>At first sight, as long as one looks at the gradients and the<br>growth cones (or migrating cells) at low magnification, At first sight, as long as one looks at the gradients and the<br>growth cones (or migrating cells) at low magnification,<br>this seems to be a very plausible explanation. It also<br>seems to be a rather economical way However looki growth cones (or migrating cells) at low magnification,<br>this seems to be a very plausible explanation. It also<br>seems to be a rather economical way. However, looking at<br>the growth cones at higher magnifications reveals the seems to be a rather economical way. However, looking at the growth cones at higher magnifications reveals the first seems to be a rather economical way. However, looking at<br>the growth cones at higher magnifications reveals the first<br>principal difficulty, which is illustrated in figure 1. The<br>steepness of the gradient at low magnificatio the growth cones at higher magnifications reveals the first<br>principal difficulty, which is illustrated in figure 1. The<br>steepness of the gradient at low magnification is easily<br>recognizable. However, the slope of the same principal difficulty, which is illustrated in figure 1. The<br>steepness of the gradient at low magnification is easily<br>recognizable. However, the slope of the same gradient<br>presented only at higher magnification is not easil steepness of the gradient at low magnification is easily recognizable. However, the slope of the same gradient presented only at higher magnification is not easily detected because the concentration differences between recognizable. However, the slope of the same gradient detail we studied the process *in vitro* and tried to interpret<br>presented only at higher magnification is not easily the experimental results on the basis of relatively presented only at higher magnification is not easily<br>detected because the concentration differences between<br>the various areas of the growth cone are extremely small.<br>Nevertheless if the gradient is the guiding que the grow detected because the concentration differences between<br>the various areas of the growth cone are extremely small.<br>Nevertheless, if the gradient is the guiding cue the growth<br>cone would have to be able to evaluate such small the various areas of the growth cone are extremely small.<br>Nevertheless, if the gradient is the guiding cue the growth<br>cone would have to be able to evaluate such small concen-<br>tration differences. Are growth, cones really Nevertheless, if the gradient is the guiding cue the growth<br>cone would have to be able to evaluate such small concen-<br>tration differences. Are growth cones really guided by<br>gradients and if so, what is the cellular mechani cone would have to be able to evaluate such small concentration differences. Are growth cones really guided by gradients and if so, what is the cellular mechanism of gradient guidance? How are the tiny concentration differ tration differences. Are growth cones really guided by<br>gradients and if so, what is the cellular mechanism of<br>gradient guidance? How are the tiny concentration differ-<br>ences between the various parts of the growth cone gradients and if so, what is the cellular mechanism of gradient guidance? How are the tiny concentration differences between the various parts of the growth cone

detected, amplified and converted to give a directional<br>signal to the cytoskeleton This might require an elaborate detected, amplified and converted to give a directional<br>signal to the cytoskeleton. This might require an elaborate<br>mechanism. Finding an answer to these questions is one detected, amplified and converted to give a directional<br>signal to the cytoskeleton. This might require an elaborate<br>mechanism. Finding an answer to these questions is one<br>of the goals of our research signal to the cytoskeleton. Thi<br>mechanism. Finding an answ<br>of the goals of our research.<br>In principle two different Echanism. Finding an answer to these questions is one<br>the goals of our research.<br>In principle two different strategies might solve the<br>positivity problem. One way to measure the gradient and

of the goals of our research.<br>In principle two different strategies might solve the<br>sensitivity problem. One way to measure the gradient and In principle two different strategies might solve the<br>sensitivity problem. One way to measure the gradient and<br>to enlarge the concentration difference would be that the<br>growth cones like bacteria would determine concentrasensitivity problem. One way to measure the gradient and<br>to enlarge the concentration difference would be that the<br>growth cones, like bacteria, would determine concentra-<br>tions only after having moved within the gradient f to enlarge the concentration difference would be that the<br>growth cones, like bacteria, would determine concentra-<br>tions only after having moved within the gradient for a<br>longer distance and thus having experienced a larger longer distance and thus having experienced a larger tions only after having moved within the gradient for a<br>longer distance and thus having experienced a larger<br>external concentration change. Alternatively, a small<br>external difference could be amplified by some internal longer distance and thus having experienced a larger<br>external concentration change. Alternatively, a small<br>external difference could be amplified by some internal<br>autocatalytic processes. Anatomical observations argue external concentration change. Alternatively, a small<br>external difference could be amplified by some internal<br>autocatalytic processes. Anatomical observations argue<br>against the former explanation (Fujisawa et al. 1981–1982 external difference could be amplified by some internal<br>autocatalytic processes. Anatomical observations argue<br>against the former explanation (Fujisawa *et al.* 1981, 1982;<br>Fujisawa 1987: Stuermer 1988*a b*). The involve autocatalytic processes. Anatomical observations argue<br>against the former explanation (Fujisawa *et al.* 1981, 1982;<br>Fujisawa 1987; Stuermer 1988*a*,*b*). The involvement of<br>autocatalytic processes in the growth cone orien against the former explanation (Fujisawa *et al.* 1981, 1982; Fujisawa 1987; Stuermer 1988*a*,*b*). The involvement of autocatalytic processes in the growth cone orientation has been suggested by Gierer (1981) Fujisawa 1987; Stuermer 1988 $a$ , $b$ ). The involvement of autocatalytic processes in the growth cone orientation has been suggested by Gierer (1981). to catalytic processes in the growth cone orientation has<br>en suggested by Gierer (1981).<br>To investigate the mechanism of axon guidance in more<br>tail we studied the process in vitra and tried to interpret

been suggested by Gierer (1981).<br>To investigate the mechanism of axon guidance in more<br>detail we studied the process *in vitro* and tried to interpret<br>the experimental results on the basis of relatively simple To investigate the mechanism of axon guidance in more<br>detail we studied the process *in vitro* and tried to interpret<br>the experimental results on the basis of relatively simple<br>models Pursuing this approach we experienced detail we studied the process *in vitro* and tried to interpret<br>the experimental results on the basis of relatively simple<br>models. Pursuing this approach we experienced a number<br>of unexpected difficulties, which will descr the experimental results on the basis of relatively simple models. Pursuing this approach we experienced a number of unexpected difficulties, which will describe and discuss in this article models. Pursuin<br>of unexpected c<br>in this article. **2.** *IN VIVO* **AND** *IN VITRO* **OBSERVATIONS**

#### **OF THE DEVELOPMENT OF THE RETINOTECTAL PROJECTION**

Our main experimental system is the retinotectal projection of chickens and fishes. During the formation of the retinotectal projection, retinal axons from a certain



Figure 1. A growth cone in a linear gradient. Drawing of an<br>axon growing in a graded field visualized at four different Figure 1. A growth cone in a linear gradient. Drawing of areas<br>axon growing in a graded field visualized at four different<br>magnifications. The steepness of the gradient in relation to to Figure 1. A growth cone in a linear gradient. Drawing of an<br>axon growing in a graded field visualized at four different<br>magnifications. The steepness of the gradient in relation to the<br>size of the growth cone is the same a axon growing in a graded field visualized at four different<br>magnifications. The steepness of the gradient in relation to the<br>size of the growth cone is the same at all four magnifications<br>and corresponds roughly to the in magnifications. The steepness of the gradient in relation to the<br>size of the growth cone is the same at all four magnifications<br>and corresponds roughly to the *in vivo* situation of a retinal<br>growth cone (10 um) migrating size of the growth cone is the same at all four magnifications<br>and corresponds roughly to the *in vivo* situation of a retinal<br>growth cone (10 µm) migrating on the tectal surface (5 mm).<br>Relative magnifications are  $(a) \times$ and corresponds roughly to the *in vivo* situation of a retinal<br>growth cone (10 µm) migrating on the tectal surface (5 mm).<br>Relative magnifications are (*a*)  $\times$ 1, (*b*)  $\times$ 3, (*c*)  $\times$ 10, and<br>(*d*)  $\times$ 100  $(d) \times 100.$ 

 $p$   $(1, 2)$   $\times$  100.<br>position within the retina have to find their very specific<br>target site in the target organ, the tectum opticum (figure 2) position within the retina have to find their very specific<br>target site in the target organ, the tectum opticum (figure 2).<br>Since it seemed very likely that the quiding cues are position within the retina have to find their very specific<br>target site in the target organ, the tectum opticum (figure 2).<br>Since it seemed very likely that the guiding cues are<br>membrane bound (a likely but by no means pro target site in the target organ, the tectum opticum (figure 2).<br>Since it seemed very likely that the guiding cues are<br>membrane bound (a likely, but by no means proven<br>assumption) we developed an *in nitro* assay in which Since it seemed very likely that the guiding cues are<br>membrane bound (a likely, but by no means proven<br>assumption) we developed an *in vitro* assay in which<br>growing retinal axons are offered a choice between two membrane bound (a likely, but by no means proven<br>assumption) we developed an *in vitro* assay in which<br>growing retinal axons are offered a choice between two<br>substrata to grow upon a membrane preparation derived assumption) we developed an *in vitro* assay in which is quite obvious that the repulsive activity at sharp<br>growing retinal axons are offered a choice between two boundaries of ephrins or posterior membranes has a<br>substra growing retinal axons are offered a choice between two<br>substrata to grow upon, a membrane preparation derived<br>from the anterior part of the tectum and another ana-<br>logous preparation derived from the posterior part. These substrata to grow upon, a membrane preparation derived<br>from the anterior part of the tectum and another ana-<br>logous preparation derived from the posterior part. These<br>two substrata were arranged in very narrow alternating from the anterior part of the tectum and another ana-<br>logous preparation derived from the posterior part. These<br>two substrata were arranged in very narrow alternating<br>strines so, that, axons, growing, on these strines are logous preparation derived from the posterior part. These two substrata were arranged in very narrow alternating stripes so that axons growing on these stripes are

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(anterior) (posterior)<br>Figure 2. The chick's visual system. Very schematic view<br>of the chick's visual system showing the projection from Figure 2. The chick's visual system. Very schematic view<br>of the chick's visual system showing the projection from<br>the retina to the tectum. The retinotectal projection is Figure 2. The chick's visual system. Very schematic view<br>of the chick's visual system showing the projection from<br>the retina to the tectum. The retinotectal projection is<br>tenographic i.e. peighbouring points in the retina of the chick's visual system showing the projection from<br>the retina to the tectum. The retinotectal projection is<br>topographic, i.e. neighbouring points in the retina are<br>connected to neighbouring sites in the target organ topographic, i.e. neighbouring points in the retina are<br>connected to neighbouring sites in the target organ.

repeatedly simultaneously exposed to a choice (figure 3)<br>repeatedly simultaneously exposed to a choice (figure 3)<br>of the two substrata originating from different positions of of the two substrata originating from different positions of<br>of the two substrata originating from different positions of<br>the target organ. In this assay, temporal retinal axons repeatedly simultaneously exposed to a choice (figure 3)<br>of the two substrata originating from different positions of<br>the target organ. In this assay, temporal retinal axons<br>show a strong preference for growing on membrane of the two substrata originating from different positions of<br>the target organ. In this assay, temporal retinal axons<br>show a strong preference for growing on membranes<br>derived from the anterior tectum which is their in nino show a strong preference for growing on membranes derived from the anterior tectum, which is their *in vivo* show a strong preference for growing on membranes<br>derived from the anterior tectum, which is their *in vivo*<br>target area. These axons actively avoid stripes of the<br>posterior membranes due to a repulsive activity of the derived from the anterior tectum, which is their *in vivo* target area. These axons actively avoid stripes of the posterior membranes due to a repulsive activity of the latter Surprisingly however pasal axons in this assay target area. These axons actively avoid stripes of the posterior membranes due to a repulsive activity of the latter. Surprisingly, however, nasal axons in this assay did not distinguish between the two substrata and the t posterior membranes due to a repulsive activity of the latter. Surprisingly, however, nasal axons in this assay did not distinguish between the two substrata and the transition between temporal, and nasal, behaviour in thi latter. Surprisingly, however, nasal axons in this assay did<br>not distinguish between the two substrata and the transi-<br>tion between temporal and nasal behaviour in this<br>respect was very abrupt (0.1 mm within the retina) not distinguish between the two substrata and the tion<br>tion between temporal and nasal behaviour in<br>respect was very abrupt  $(0.1 \text{ mm}$  within the retina).<br>The *in nitm* assay was used to identify the repo In between temporal and nasal behaviour in this<br>spect was very abrupt (0.1 mm within the retina).<br>The *in vitro* assay was used to identify the repulsive<br>embrane components. Some of these components have

respect was very abrupt (0.1 mm within the retina).<br>The *in vitro* assay was used to identify the repulsive<br>membrane components. Some of these components have<br>been cloned (Drescher *et al.* 1995) They turned out to be The *in vitro* assay was used to identify the repulsive<br>membrane components. Some of these components have<br>been cloned (Drescher *et al.* 1995). They turned out to be<br>ligands (nowadays called 'enhrins') of recentor typosin membrane components. Some of these components have<br>been cloned (Drescher *et al.* 1995). They turned out to be<br>ligands (nowadays called 'ephrins') of receptor tyrosine<br>kinases. Other, notential candidates, like the repulsi been cloned (Drescher *et al.* 1995). They turned out to be<br>ligands (nowadays called 'ephrins') of receptor tyrosine<br>kinases. Other potential candidates, like the repulsive<br>guidance molecule (Müller *et al.* 1996) are unfo ligands (nowadays called 'ephrins') of receptor tyrosine<br>kinases. Other potential candidates, like the repulsive<br>guidance molecule (Müller *et al.* 1996), are unfortunately<br>still resisting attempts to clone them. Both type kinases. Other potential candidates, like the repulsive guidance molecule (Müller *et al.* 1996), are unfortunately still resisting attempts to clone them. Both types of components act repulsively on temporal axons *in ni* guidance molecule (Müller *et al.* 1996), are unfortunately<br>still resisting attempts to clone them. Both types of<br>components act repulsively on temporal axons *in vitro* and<br>are capable of quiding axons in the *in vitro* s still resisting attempts to clone them. Both types of<br>components act repulsively on temporal axons *in vitro* and<br>are capable of guiding axons in the *in vitro* stripe assay<br>into lanes of anterior tectal membranes. They co components act repulsively on temporal axons *in vitro* and are capable of guiding axons in the *in vitro* stripe assay into lanes of anterior tectal membranes. They could be the molecules which are responsible for the formation of into lanes of anterior tectal membranes. They could be<br>the molecules which are responsible for the formation of<br>the topographic retinotectal projection along the anterior-<br>posterior axis because they occur concomitantly wi the topographic retinotectal projection along the anterior-<br>posterior axis because they occur concomitantly with the<br>development of this projection, they have a graded distri-<br>bution in the tectum, and at least some of the posterior axis because they occur concomitantly with the development of this projection, they have a graded distribution in the tectum, and at least some of these components have been conserved during evolution (reviewed b bution in the tectum, and at least some of these compo-Tessier-Lavigne & Goodman 1996). Nevertheless, the nents have been conserved during evolution (reviewed by<br>Tessier-Lavigne & Goodman 1996). Nevertheless, the<br>direct experimental proof that a smooth ephrin gradient<br>could guide axons in vitm is still lacking. This has also n Tessier-Lavigne & Goodman 1996). Nevertheless, the direct experimental proof that a smooth ephrin gradient could guide axons *in vitro* is still lacking. This has also not wet been shown for the posterior membranes. Howeve direct experimental proof that a smooth ephrin gradient<br>could guide axons *in vitro* is still lacking. This has also not<br>yet been shown for the posterior membranes. However, it<br>is quite obvious that the repulsive activity could guide axons *in vitro* is still lacking. This has also not<br>yet been shown for the posterior membranes. However, it<br>is quite obvious that the repulsive activity at sharp<br>boundaries of enhrins or posterior membranes ha yet been shown for the posterior membranes. However, it<br>is quite obvious that the repulsive activity at sharp<br>boundaries of ephrins or posterior membranes has a<br>guiding influence on temporal axons. At present we are is quite obvious that the repulsive activity at sharp<br>boundaries of ephrins or posterior membranes has a<br>guiding influence on temporal axons. At present we are<br>trying to design in vitro experiments with artificial gradiboundaries of ephrins or posterior membranes has a guiding influence on temporal axons. At present we are trying to design *in vitro* experiments with artificial gradients of ephrins with the aim of showing that the gradie guiding influence on temporal axons. At present we are<br>trying to design *in vitro* experiments with artificial gradients<br>of ephrins with the aim of showing that the gradients<br>influence the direction of axonal growth. Our f trying to design *in vitro* experiments with artificial gradients of ephrins with the aim of showing that the gradients influence the direction of axonal growth. Our first results indicate that in these gradient assays as ents of ephrins with the aim of showing that the gradients<br>influence the direction of axonal growth. Our first results<br>indicate that in these gradient assays, as in the stripe

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receptors in the retinotectal system. Experiments by<br>Drescher *et al.* (1995) and Cheng *et al.* (1995) had shown Figure 3. Stripe assay: guidance of retinal axons *in vitro*. The details of the experimental system are described in the materials<br>and methods section of Walter *et al.* (1987*a,b*). In essence a strip of embryonic chick and methods section of Walter *et al.* (1987*a,b*). In essence a strip of embryonic chick retina extending from nasal (N) to temporal (T) retina is explanted on a carpet of tectal membranes. The tectal cell membranes are and methods section of Walter *et al.* (1987*a*,*b*). In essence a strip of embryonic chick retina extending from nasal (N) to temporal (T) retina is explanted on a carpet of tectal membranes. The tectal cell membranes ar (T) retina is explanted on a carpet of tectal membranes. The tectal cell membranes are derived from the anterior (A) and the posterior (P) tectum and are arranged in alternating narrow (100  $\mu$ m) stripes. The retinal exp posterior (P) tectum and are arranged in alternating narrow (100  $\mu$ m) stripes. The retinal explant is incubated at 37 °C in 4%<br>CO<sub>2</sub> on the striped carpet for about two days. The retinal explant sends out temporal and n  $CO<sub>2</sub>$  on the striped carpet for about two days. The retinal explant sends out temporal and nasal axons. When temporal retinal axons reach the border between A and P lanes they show a clear preference for growing on A

assay, retinal axons do not show a graded but a binary<br>response with all temporal axons stopping at a certain assay, retinal axons do not show a graded but a binary<br>response with all temporal axons stopping at a certain<br>position and all nasal axons being non-responsive assay, retinal axons do not show a graded but a has<br>response with all temporal axons stopping at a c<br>position and all nasal axons being non-responsive.<br>It is not clear what makes the axons stop when response with all temporal axons stopping at a certain<br>position and all nasal axons being non-responsive.<br>It is not clear what makes the axons stop when they

position and all nasal axons being non-responsive.<br>It is not clear what makes the axons stop when they<br>have reached their *in vivo* target. There are at least two<br>conceivable mechanisms: first the axons might follow the It is not clear what makes the axons stop when they<br>have reached their *in vivo* target. There are at least two<br>conceivable mechanisms: first the axons might follow the<br>quiding gradient until they become exposed to an ant have reached their *in vivo* target. There are at least two conceivable mechanisms: first the axons might follow the guiding gradient until they become exposed to an antago-<br>nistic gradient which makes the axon stop. This conceivable mechanisms: first the axons might follow the<br>guiding gradient until they become exposed to an antago-<br>nistic gradient which makes the axon stop. This would<br>require two antagonistic gradients on the target organ guiding gradient until they become exposed to an antagonistic gradient which makes the axon stop. This would<br>require two antagonistic gradients on the target organ of<br>which for the retinotectal system only one has been nistic gradient which makes the axon stop. This would<br>require two antagonistic gradients on the target organ of<br>projects to the anterior pole of the tectum. Interestingly it<br>which for the retinotectal system only one has b require two antagonistic gradients on the target organ of<br>which for the retinotectal system only one has been<br>discovered so far. Alternatively, the gradient might serve<br>not only as a directional marker on the basis of its which for the retinotectal system only one has been<br>discovered so far. Alternatively, the gradient might serve<br>not only as a directional marker on the basis of its slope<br>but also as a positional marker depending on the abs discovered so far. Alternatively, the gradient might serve<br>not only as a directional marker on the basis of its slope<br>but also as a positional marker depending on the absolute<br>concentration values. Theoretical analysis (Gi not only as a directional marker on the basis of its slope<br>but also as a positional marker depending on the absolute<br>concentration values. Theoretical analysis (Gierer 1987)<br>shows that guidance to the target in each dimens but also as a positional marker depending on the absolute<br>concentration values. Theoretical analysis (Gierer 1987)<br>shows that guidance to the target in each dimension can<br>be achieved either by two antagonistic gradients or concentration values. Theoretical analysis (Gierer 1987)<br>shows that guidance to the target in each dimension can<br>be achieved either by two antagonistic gradients or by<br>one graded cue with two antagonistic evaluations. In t shows that guidance to the target in each dimension can<br>be achieved either by two antagonistic gradients or by<br>one graded cue with two antagonistic evaluations. In the<br>latter case, there must be internal processing in the be achieved either by two antagonistic gradients or by<br>one graded cue with two antagonistic evaluations. In the<br>latter case, there must be internal processing in the<br>growth cone for example attraction at low concentration one graded cue with two antagonistic evaluations. In the latter case, there must be internal processing in the growth cone, for example, attraction at low concentration levels of the gradient and inhibition at high levels latter case, there must be internal processing in the<br>growth cone, for example, attraction at low concentration<br>levels of the gradient and inhibition at high levels,<br>leading to an optimal position in between: the final pos growth cone, for example, attraction at low concentration<br>levels of the gradient and inhibition at high levels,<br>leading to an optimal position in between; the final posilevels of the gradient and inhibition at high levels,<br>leading to an optimal position in between; the final posi-<br>tion depends on interactive parameters of the searching<br>growth cone. In these versions of gradient models tar leading to an optimal position in between; the final position depends on interactive parameters of the searching<br>growth cone. In these versions of gradient models target<br>position is dependent on absolute concentrations of growth cone. In these versions of gradient models target<br>position is dependent on absolute concentrations of growth cone. In these versions of gradient models target<br>position is dependent on absolute concentrations of<br>guiding molecules, not on slope. Thus the gradient would<br>give two commands to the growth cone, for example position is dependent on absolute concentrations of<br>guiding molecules, not on slope. Thus the gradient would<br>give two commands to the growth cone, for example,<br>(i) grow upbill and (ii) stop at the concentration  $\zeta$ . The guiding molecules, not on slope. Thus the gradient would<br>give two commands to the growth cone, for example,<br>(i) grow uphill, and (ii) stop at the concentration  $c$ . The<br>latter view has recently been corroborated by experi give two commands to the growth cone, for example,<br>(i) grow uphill, and (ii) stop at the concentration  $\ell$ . The<br>latter view has recently been corroborated by experiments (i) grow uphill, and (ii) stop at the concentration  $c$ . The latter view has recently been corroborated by experiments of Rosentreter *et al.* (1998). They showed in *in vitro* gradient assays that temporal axons react to  $\mathbf S$ latter view has recently been corroborated by experiments<br>of Rosentreter *et al.* (1998). They showed in *in vitro*<br>gradient assays, that temporal axons react to the cue at a<br>defined concentration within the gradient irre of Rosentreter *et al.* (1998). They showed in *in vitro* gradient assays, that temporal axons react to the cue at a defined concentration within the gradient irrespective of gradient slope. However, if the absolute conce gradient assays, that temporal axons react to the cue at a defined concentration within the gradient irrespective of gradient slope. However, if the absolute concentration of the ligand is raised axons always climb un the defined concentration within the gradient irrespective of gradient slope. However, if the absolute concentration of the ligand is raised, axons always climb up the gradient for the same increment, as if they had adapted to gradient slope. However, if the absolute concentration of<br>the ligand is raised, axons always climb up the gradient<br>for the same increment, as if they had adapted to the<br>elevated basal level of the guidance cue ㅎ the ligand is raised, axons always climb up the gradient<br>for the same increment, as if they had adapted to the<br>elevated basal level of the guidance cue. the same increment, as if they had adapted to the<br>exated basal level of the guidance cue.<br>Before discussing conceivable guidance models we<br>suid like to summarize some of the most recent findings

elevated basal level of the guidance cue.<br>Before discussing conceivable guidance models we<br>would like to summarize some of the most recent findings<br>concerning the *in vine* distribution of the ephrins and their would like to summarize some of the most recent findings<br>concerning the *in vivo* distribution of the ephrins and their

some time ago that the ephrins A5 and A2 have a graded<br>distribution within the tectum with a maximum at the<br>posterior pole. The distributions of some of the corres-<br>ponding Eph receptors (Eph A4 and Eph A5) are not posterior pole. The distributions of some of the corres-<br>ponding Eph receptors (Eph A4 and Eph A5) are not posterior pole. The distributions of some of the corresponding Eph receptors (Eph A4 and Eph A5) are not graded. However, one of them (Eph A3) has a graded distribution with a maximum at the temporal side which ponding Eph receptors (Eph A4 and Eph A5) are not<br>graded. However, one of them (Eph A3) has a graded<br>distribution with a maximum at the temporal side which<br>projects to the anterior pole of the tectum Interestingly it graded. However, one of them (Eph A3) has a graded<br>distribution with a maximum at the temporal side which<br>projects to the anterior pole of the tectum. Interestingly it<br>was recently found that the ligands enhrin A2 and A5 a was recently found that the ligands ephrin A2 and A5 are projects to the anterior pole of the tectum. Interestingly it<br>was recently found that the ligands ephrin A2 and A5 are<br>not only expressed in a graded fashion on the target<br>organ but also on the projecting retinal axons (Ho was recently found that the ligands ephrin A2 and A5 are<br>not only expressed in a graded fashion on the target<br>organ but also on the projecting retinal axons (Horn-<br>herger et al. 1999) (figure 4) These authors have given not only expressed in a graded fashion on the target<br>organ but also on the projecting retinal axons (Horn-<br>berger *et al.* 1999) (figure 4). These authors have given<br>good evidence that the presence or absence of these organ but also on the projecting retinal axons (Horn-<br>berger *et al.* 1999) (figure 4). These authors have given<br>good evidence that the presence or absence of these<br>axonal ligands determines their temporal or nasal behaberger *et al.* 1999) (figure 4). These authors have given<br>good evidence that the presence or absence of these<br>axonal ligands determines their temporal or nasal beha-<br>viour *in vitro* and *in vivo* good evidence that the<br>axonal ligands determine<br>viour *in vitro* and *in vivo*. **Viour** *in vitro* and *in vivo*.<br>**3. MODEL SYSTEMS BASED S. MODEL SYSTEMS BASED<br>ON THE COMPLEMENTARY EXPRESSION** 3. MODEL SYSTEMS BASED<br>IE COMPLEMENTARY EXPRESSIO<br>OF RECEPTOR AND LIGAND

# **(a)** *The mass action model for topographic mapping:*<br> **(a)** *The mass action model for topographic mapping:*<br> **reading only positional information** *reading only positional information*<br>*reading only positional information***<br><b>***reading only positional information*

receptors in the retinotectal system. Experiments by<br>Drescher *et al.* (1995) and Cheng *et al.* (1995) had shown<br>some time ago that the enhrins A5 and A2 have a graded Drescher *et al.* (1995) and Cheng *et al.* (1995) had shown some time ago that the ephrins A5 and A2 have a graded Drescher *et al.* (1995) and Cheng *et al.* (1995) had shown<br>some time ago that the ephrins A5 and A2 have a graded<br>distribution within the tectum with a maximum at the<br>posterior pole. The distributions of some of the corr

**reading only positional information**<br>The conceptual features of axonal guidance by gradireading only positional information<br>The conceptual features of axonal guidance by gradi-<br>ents were discussed some years ago by Gierer (1987).<br>Rased on the new observations made by Nakamoto *et al* The conceptual features of axonal guidance by gradients were discussed some years ago by Gierer (1987).<br>Based on the new observations, made by Nakamoto *et al.*<br>(1996) that Eph receptors and the corresponding ligands ents were discussed some years ago by Gierer (1987).<br>Based on the new observations, made by Nakamoto *et al.*<br>(1996), that Eph receptors and the corresponding ligands<br>are expressed in complementary gradients in retina and Based on the new observations, made by Nakamoto *et al.* (1996), that Eph receptors and the corresponding ligands are expressed in complementary gradients in retina and (1996), that Eph receptors and the corresponding ligands<br>are expressed in complementary gradients in retina and<br>tectum these authors have suggested a mechanism for the<br>formation of the retinotonic projection. According to are expressed in complementary gradients in retina and<br>tectum these authors have suggested a mechanism for the<br>formation of the retinotopic projection. According to this<br>model a growth cone reads an input signal proportion tectum these authors have suggested a mechanism for the<br>formation of the retinotopic projection. According to this<br>model, a growth cone reads an input signal proportional<br>to the graded ligand concentration of the tectum formation of the retinotopic projection. According to this<br>model, a growth cone reads an input signal proportional<br>to the graded ligand concentration of the tectum,<br>compares this signal with an internal threshold value and model, a growth cone reads an input signal proportional<br>to the graded ligand concentration of the tectum,<br>compares this signal with an internal threshold value and<br>stops growing when the difference is zero. The strength of to the graded ligand concentration of the tectum,<br>compares this signal with an internal threshold value and<br>stops growing when the difference is zero. The strength of compares this signal with an internal threshold value and<br>stops growing when the difference is zero. The strength of<br>this input signal I depends on the concentration of the<br>receptor-ligand complexes. The threshold value is stops growing when the difference is zero. The strength of<br>this input signal  $I$  depends on the concentration of the<br>receptor–ligand complexes. The threshold value is called<br>the 'standard value'  $(S)$  and is the same for a this input signal  $I$  depends on the concentration of the receptor-ligand complexes. The threshold value is called the 'standard value'  $(S)$  and is the same for all growth cones. The concentration of receptors  $[R]$  on the receptor-ligand complexes. The threshold value is called<br>the 'standard value'  $(S)$  and is the same for all growth<br>cones. The concentration of receptors  $[R]$  on the growth



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Figure 4. Expression of ephrin A ligands in the tectum and on retinal ganglion cell axons. Projection scheme of retinal Figure 4. Expression of ephrin A ligands in the tectum and<br>on retinal ganglion cell axons. Projection scheme of retinal<br>axons and a summary of the expression pattern of Eph A<br>family members in the retina and the tectum. Th on retinal ganglion cell axons. Projection scheme of retinal<br>axons and a summary of the expression pattern of Eph A<br>family members in the retina and the tectum. The Eph A<br>recentors Eph A4 and Eph A5 are uniformly expressed axons and a summary of the expression pattern of Eph A<br>family members in the retina and the tectum. The Eph A<br>receptors Eph A4 and Eph A5 are uniformly expressed in the<br>retina whereas Eph A3 is expressed in the temporal re family members in the retina and the tectum. The Eph A<br>receptors Eph A4 and Eph A5 are uniformly expressed in the<br>retina, whereas Eph A3 is expressed in the temporal retina receptors Eph A4 and Eph A5 are uniformly expressed in the<br>retina, whereas Eph A3 is expressed in the temporal retina<br>in a gradient, and there is little or no expression in the nasal<br>retina. Ephrin A2 and ephrin A5 are exp

retina, whereas Eph A3 is expressed in the temporal retina<br>in a gradient, and there is little or no expression in the nas:<br>retina. Ephrin A2 and ephrin A5 are expressed in retinal<br>ganglion cells including their axons in a retina. Ephrin A2 and ephrin A5 are expressed in retinal<br>ganglion cells including their axons in a high-nasal-to-lowretina. Ephrin A2 and ephrin A5 are expressed in retinal<br>ganglion cells including their axons in a high-nasal-to-low-<br>temporal gradient, whereby the expression domain of ephrin<br>A5 is restricted more to the nasal retina tha ganglion cells including their axons in a high-nasal-to-low-<br>temporal gradient, whereby the expression domain of ephrin<br>A5 is restricted more to the nasal retina than that of ephrin<br>A2. Both ligands are expressed in the te A5 is restricted more to the nasal retina than that of ephrin<br>A2. Both ligands are expressed in the tectum in a high-A5 is restricted more to the nasal retina than that of ephrin<br>A2. Both ligands are expressed in the tectum in a high-<br>posterior-to-low-anterior gradient. The expression domain of<br>ephrin A5 is restricted more to the posteri A2. Both ligands are expressed in the tectum in a high-<br>posterior-to-low-anterior gradient. The expression domain of<br>ephrin A5 is restricted more to the posterior half of the tectum<br>than that of ephrin A2. The projection ៑ posterior-to-low-anterior gradient. The expression domain of<br>ephrin A5 is restricted more to the posterior half of the tectum<br>than that of ephrin A2. The projection of temporal axons onto<br>the anterior tectum and the projec ephrin A5 is restricted more to the posterior half of the tectum<br>than that of ephrin A2. The projection of temporal axons onto<br>the anterior tectum and the projection of nasal axons onto the<br>posterior tectum are indicated the anterior tectum and the projection of nasal axons onto the posterior tectum are indicated.

cone depends on the position of its cell body in the retina.<br>The ligand concentration  $[I]$  within the tectum increases cone depends on the position of its cell body in the retina.<br>The ligand concentration [*L*] within the tectum increases<br>from the anterior to the posterior pole. On the basis of cone depends on the position of its cell body in the retina.<br>The ligand concentration  $[L]$  within the tectum increases<br>from the anterior to the posterior pole. On the basis of<br>this model the strength of the signal  $L$  is The ligand concentration [ $L$ ] within the tectum increases<br>from the anterior to the posterior pole. On the basis of<br>this model the strength of the signal  $I$  is proportional to<br>the concentration of the receptor  $[R]$  and t from the anterior to the posterior pole. On the basis of this model the strength of the signal  $I$  is proportional to the concentration of the receptor  $[R]$  and the ligand  $[L]$ this model the strength of the signal  $I$  is proportional to<br>the concentration of the receptor  $[R]$  and the ligand  $[L]$ <br>according to the law of mass action:  $I = [RL] = K[R][L]$ ,<br>assuming that the number of receptor-ligand compl the concentration of the receptor  $[R]$  and the ligand  $[L]$ <br>according to the law of mass action:  $I = [RL] = K[R][L]$ ,<br>assuming that the number of receptor-ligand complexes<br>is low in comparison to the number of free receptors and according to the law of mass action:  $I = [RL] = K[R][L]$ ,<br>assuming that the number of receptor-ligand complexes<br>is low in comparison to the number of free receptors and<br>ligands. Every growth, cone migrates until the signal assuming that the number of receptor-ligand complexes<br>is low in comparison to the number of free receptors and<br>ligands. Every growth cone migrates until the signal<br>strength  $I$  reaches the standard value  $S$ . Because all is low in comparison to the number of free receptors and<br>ligands. Every growth cone migrates until the signal<br>strength  $I$  reaches the standard value  $S$ . Because all<br>growth cones have the same  $S$  but different  $[R]$  they ligands. Every growth cone migrates until the signal strength  $I$  reaches the standard value  $S$ . Because all growth cones have the same  $S$  but different  $[R]$ , they will strength *I* reaches the standard value *S*. Because all growth cones have the same *S* but different [*R*], they will stop at different values of [*L*] in the tectum. For example, if the hypothetical value for  $S = 1200$  growth cones have the same *S* but different [*R*], they will<br>stop at different values of [*L*] in the tectum. For example,<br>if the hypothetical value for  $S = 1200$  and  $K = 1$ , then a<br>growth cone with  $[R] = 20$  will grow to stop at different values of [*L*] in the tectum. For example,<br>if the hypothetical value for  $S = 1200$  and  $K = 1$ , then a<br>growth cone with  $[R] = 20$  will grow to a position with<br> $[I1] = 60$  a growth cone with  $[R] = 30$  to  $[I1]$ if the hypothetical value for  $S = 1200$  and  $K = 1$ , then a<br>growth cone with  $[R] = 20$  will grow to a position with<br> $[L] = 60$ , a growth cone with  $[R] = 30$  to  $[L] = 40$  and so<br>on In other words to get the same strength I growt growth cone with  $[R] = 20$  will grow to a position with  $[L] = 60$ , a growth cone with  $[R] = 30$  to  $[L] = 40$  and so on. In other words, to get the same strength *I*, growth cones with low recentor concentrations (from the nas  $[L] = 60$ , a growth cone with  $[R] = 30$  to  $[L] = 40$  and so<br>on. In other words, to get the same strength *I*, growth<br>cones with low receptor concentrations (from the nasal<br>part of the retina) will grow until they reach a par on. In other words, to get the same strength  $I$ , growth cones with low receptor concentrations (from the nasal part of the retina) will grow until they reach a part of the cones with low receptor concentrations (from the nasal<br>part of the retina) will grow until they reach a part of the<br>tectum with high ligand concentration (posterior part)<br>whereas growth cones with a high receptor concentra part of the retina) will grow until they reach a part of the<br>tectum with high ligand concentration (posterior part)<br>whereas growth cones with a high receptor concentration<br>(from the temporal part of the retina) will alread tectum with high ligand concentration (posterior part)<br>whereas growth cones with a high receptor concentration<br>(from the temporal part of the retina) will already stop at<br>positions with low concentration of ligands (anteri whereas growth cones with a high receptor concentration (from the temporal part of the retina) will already stop at positions with low concentration of ligands (anterior part of the tectum). Thus the mass action model offe  $\mathsf{C}$ (from the temporal part of the retina) will already stop at vice versa. After having approached their final position, positions with low concentration of ligands (anterior part the site where the difference value  $D = 0$ , positions with low concentration of ligands (anterior part<br>of the tectum). Thus the mass action model offers an<br>explanation of how growth cones might recognize their<br>target position within the gradient. Importantly, the  $\mathbf{\Omega}$ of the tectum). Thus the mass action model offers an explanation of how growth cones might recognize their target position within the gradient. Importantly, the model does not explain how growth cones are guided to explanation of how growth cones might recognize their<br>target position within the gradient. Importantly, the<br>model does not explain how growth cones are guided to<br>this target position target position with<br>model does not expla<br>this target position.  $\overline{C}$ 

# **(b)** *The servomechanism model: reading both directional and positional information*

(b) The servomechanism model: reading both<br>directional and positional information<br>In principle, gradients carry two different kinds of<br>formation: a positional information based on the local *directional and positional information*<br>In principle, gradients carry two different kinds of<br>information: a positional information based on the local<br>concentration of ligands, and a directional information In principle, gradients carry two different kinds of it would not migrate at all. However, in fact growth information: a positional information based on the local cones do also migrate on homogeneous substrates. In the con

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based on the slope of the concentration gradient. Honda<br>(1998) developed an extended mass action model which based on the slope of the concentration gradient. Honda<br>(1998) developed an extended mass action model which<br>describes how growth cones make use of both the direc-(1998) developed an extended mass action model which describes how growth cones make use of both the direc-(1998) developed an extended mass action model which<br>describes how growth cones make use of both the direc-<br>tional and the positional information of a gradient to find<br>their targets describes how<br>tional and the<br>their targets.<br>The key co mal and the positional information of a gradient to find<br>eir targets.<br>The key concept in Honda's model is a servome-<br>anism (Honda 1998). As in the mass action model the

their targets.<br>The key concept in Honda's model is a servome-<br>chanism (Honda 1998). As in the mass action model the<br>target position of a growth cone is defined by the require-The key concept in Honda's model is a servome-<br>chanism (Honda 1998). As in the mass action model the<br>target position of a growth cone is defined by the require-<br>ment that the input strength  $I$  has to reach the intrinsic chanism (Honda 1998). As in the mass action model the target position of a growth cone is defined by the requirement that the input strength  $I$  has to reach the intrinsic standard value  $S$ . In addition, however, the mod target position of a growth cone is defined by the requirement that the input strength *I* has to reach the intrinsic standard value *S*. In addition, however, the model makes use of the directional information provided by ment that the input strength  $I$  has to reach the intrinsic<br>standard value  $S$ . In addition, however, the model makes<br>use of the directional information provided by the<br>gradient by introducing a novel parameter namely the use of the directional information provided by the gradient by introducing a novel parameter, namely the use of the directional information provided by the gradient by introducing a novel parameter, namely the difference of the local input strength *I* and the standard value *S*. The smaller this difference the smaller is the gradient by introducing a novel parameter, namely the difference of the local input strength *I* and the standard value *S*. The smaller this difference, the smaller is the distance of the growth cone to its final position difference of the local input strength  $I$  and the standard value  $S$ . The smaller this difference, the smaller is the distance of the growth cone to its final position. To navi-<br>gate in two spatial dimensions a growth co value  $S$ . The smaller this difference, the smaller is the distance of the growth cone to its final position. To navigate in two spatial dimensions, a growth cone needs two distance of the growth cone to its final position. To navigate in two spatial dimensions, a growth cone needs two independent sets of complementary gradients and two independent S-values, one set for each dimension. The gate in two spatial dimensions, a growth cone needs two<br>independent sets of complementary gradients and two<br>independent *S*-values, one set for each dimension. The<br>following description is only for one dimension (x): an independent sets of complementary gradients and two<br>independent *S*-values, one set for each dimension. The<br>following description is only for one dimension  $\langle x \rangle$ ; an<br>analogous formalism is used for the other dimension independent *S*-values, one set for each dimension. The following description is only for one dimension  $(x)$ ; an analogous formalism is used for the other dimension  $(y)$ . Let  $[R_A]$  be the receptor concentration on a growt following description is only for one dimension  $(x)$ ; an analogous formalism is used for the other dimension  $(y)$ .<br>Let  $[R_A]$  be the receptor concentration on a growth cone with its cell body at the position A in the retin analogous formalism is used for the other dimension  $(y)$ .<br>Let  $[R_A]$  be the receptor concentration on a growth cone<br>with its cell body at the position A in the retina and<br> $[I]$  the ligand concentration at the position x in with its cell body at the position A in the retina and  $[L_x]$  the ligand concentration at the position *x* in the with its cell body at the position A in the retina and  $[L_x]$  the ligand concentration at the position x in the tectum with S being the constant standard value. Then  $I = K[R, 1[L]$  represents the signal strepath measured [*L*<sub>*x*</sub>] the ligand concentration at the position *x* in the tectum with *S* being the constant standard value. Then  $I_x = K[R_A][L_x]$  represents the signal strength measured by a growth cone at position *x* in the tectum an tectum with *S* being the constant standard value. Then  $I_x = K[R_A][L_x]$  represents the signal strength measured<br>by a growth cone at position *x* in the tectum and<br> $D = S - I$  is the difference between the standard value *S*  $D_x = S - I_x$  is the difference between the standard vand the local input strength  $I_x$ . In order to det the direction of growth the growth cone has to  $[L_x]$  represents the signal strength measured<br>vth cone at position  $x$  in the tectum and<br>is the difference between the standard value  $S$ <br>cal input strength  $I$  In order to determine by a growth cone at position *x* in the tectum and  $D_x = S - I_x$  is the difference between the standard value *S* and the local input strength  $I_x$ . In order to determine the direction of growth the growth cone has to (i) calculate the local difference signal  $D_x = S - I_x$ ;<br>(i) calculate the local difference signal  $D_x = S - I_x$ ;<br>(ii) sense the ligand concentration at a new position

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- (i) calculate the local difference signal  $D_x = S I_x$ ;<br>(ii) sense the ligand concentration at a new position in a<br>+ or direction: + or - direction; (ii) sense the ligand concentration at a new position in a<br>+ or - direction;<br>(iii) calculate the difference signal at the new position<br> $D_{\text{max}} = S - I_{\text{max}}$ .
- $D_{\text{probed}} = S I_{\text{probed}};$ (iii) calculate the difference signal at the new position<br>  $D_{\text{probed}} = S - I_{\text{probed}};$ <br>
(iv) compare the difference signals  $D_x$  and  $D_{\text{probed}}$  at the<br>
local and the probed position:
- $D_{\text{probed}} = S I_{\text{probed}};$ <br>compare the difference signals<br>local and the probed position;<br>calculate an output correspondi (iv) compare the difference signals  $D_x$  and  $D_{\text{probed}}$  at the local and the probed position;<br>(v) calculate an output corresponding to the probability<br>for accepting the new versus staving at the current
- $f(x)$  calculate an output corresponding to the probability<br>for accepting the new versus staying at the current position.

The probability considerations are introduced in order position.<br>The probability considerations are introduced in order<br>to account for fluctuations in the input strength *I*. The<br>probability *P* of the growth cone to remain at the current The probability considerations are introduced in order<br>to account for fluctuations in the input strength *I*. The<br>probability *P* of the growth cone to remain at the current<br>position is  $P - h(D)/(h(D) + h(D - \lambda))$  The probabilities probability *P* of the growth cone to remain at the current position is  $P = p(D_x)/(p(D_x) + p(D_{\text{probed}}))$ . The probabilities probability *P* of the growth cone to remain at the current<br>position is  $P = p(D_x)/(p(D_x) + p(D_{\text{probe}}))$ . The probabilities<br> $p(D_x)$  and  $p(D_{\text{probe}})$  depend on the distance of the local<br>position from the final position and have a maxi position is  $P = p(D_x)/(p(D_x) + p(D_{\text{probed}}))$ . The probabilities  $p(D_x)$  and  $p(D_{\text{probed}})$  depend on the distance of the local position from the final position and have a maximum where  $D = 0$ . If the difference simple at the probed p  $p(D_x)$  and  $p(D_{\text{probed}})$  depend on the distance of the local position from the final position and have a maximum where  $D = 0$ . If the difference signal at the probed position  $D_{\text{per}}$ , is smaller than that at the current position from the final position and have a maximum<br>where  $D = 0$ . If the difference signal at the probed posi-<br>tion  $D_{\text{probed}}$  is smaller than that at the current position<br> $D$ , the probability of staying at the current po  $D_x$ , the probability of staying at the current position is lower than that of moving to the probed position, and vice versa. After having approached their final position, the site where the difference value  $D = 0$  forwar here  $D = 0$ . If the difference signal at the probed position  $D_{\text{probed}}$  is smaller than that at the current position is the probability of staying at the current position is wer than that of moving to the probed position tion  $D_{\text{probed}}$  is smaller than that at the current position  $D_x$ , the probability of staying at the current position is lower than that of moving to the probed position, and vice versa. After having approached their fina lower than that of moving to the probed position, and vice versa. After having approached their final position,<br>the site where the difference value  $D = 0$ , forward and<br>backward migration of the growth cone will have the<br>same probability. Thus net growth will be zero. This the site where the difference value  $D = 0$ , forward and backward migration of the growth cone will have the same probability. Thus net growth will be zero. This could be interpreted to mean that growth cones do not backward migration of the growth cone will have the same probability. Thus net growth will be zero. This could be interpreted to mean that growth cones do not ston moving but are getting tranned at their final region same probability. Thus net growth will be zero. This<br>could be interpreted to mean that growth cones do not<br>stop moving but are getting trapped at their final region.<br>The difference signal D of local and probed positions uld be interpreted to mean that growth cones do not<br>p moving but are getting trapped at their final region.<br>The difference signal *D* of local and probed positions<br>a homogeneous substrate do not denend on the direc-

stop moving but are getting trapped at their final region.<br>The difference signal  $D$  of local and probed positions<br>on a homogeneous substrate do not depend on the direc-<br>tion of growth and therefore the probability of a g The difference signal  $D$  of local and probed positions<br>on a homogeneous substrate do not depend on the direc-<br>tion of growth and therefore the probability of a growth on a homogeneous substrate do not depend on the direction of growth and therefore the probability of a growth cone to accept the new position is 50% everywhere. Thus it would not migrate at all However in fact growth tion of growth and therefore the probability of a growth<br>cone to accept the new position is 50% everywhere. Thus<br>it would not migrate at all. However, in fact growth<br>cones do also migrate on homogeneous substrates. In the cone to accept the new position is 50% everywhere. Thus<br>it would not migrate at all. However, in fact growth<br>cones do also migrate on homogeneous substrates. In the<br>servomechanism model this problem has been solved by servomechanism model this problem has been solved by

assuming that the tendency of probing the forward direc-<br>tion  $(+)$  is higher than the tendency of probing the backassuming that the tendency of probing the forward direc-<br>tion  $(+)$  is higher than the tendency of probing the back-<br>ward direction  $(-)$ . This, results, in a net, forward tion  $(+)$  is higher than the tendency of probing the back-<br>ward direction  $(-)$ . This results in a net forward and is thus a strong indication that guidance by gradients tion  $(+)$  is higher than the tendency of probing the backward direction  $(-)$ . This results in a net forward movement, i.e. in growth of the axon. According to diverse experimental observations  $(e \sigma$  Bray 1979) the ward direction  $(-)$ . This results in a net forward<br>movement, i.e. in growth of the axon. According to<br>diverse experimental observations (e.g. Bray 1979) the<br>forward tendency is a plausible assumption diverse experimental observations (e.g. Bray 1979) the forward tendency is a plausible assumption. verse experimental observations (e.g. Bray 1979) the<br>ward tendency is a plausible assumption.<br>The described algorithm is independent of whether the<br>obed position is lying within the range of a filopodium

forward tendency is a plausible assumption. The described algorithm is independent of whether the<br>probed position is lying within the range of a filopodium<br>or far away for example at the axon shaft. Therefore probed position is lying within the range of a filopodium<br>or far away, for example, at the axon shaft. Therefore probed position is lying within the range of a filopodium get trapped, staying active and mobile in their specific<br>or far away, for example, at the axon shaft. Therefore termination area.<br>there is no *a priori* need to amp or far away, for example, at the axon shaft. Therefore<br>there is no *a priori* need to amplify small concentration<br>differences measured within various parts of a growth<br>cone. In simulations the model appears to be rather there is no *a priori* need to amplify small concentration<br>differences measured within various parts of a growth<br>cone. In simulations the model appears to be rather<br>tolerant of noisy input signals and the probability for a differences measured within various parts of a growth<br>cone. In simulations the model appears to be rather<br>tolerant of noisy input signals and the probability for a<br>growth cone to get tranned in local minima is low cone. In simulations the model appears to be 1<br>tolerant of noisy input signals and the probability<br>growth cone to get trapped in local minima is low.

#### Frowth cone to get trapped in local minima is low.<br>
(c) In the servomechanism model one and the same<br> *andance* molecule can be either an attractive or a *guidance molecule can be either an attractive or a repulsive cue for the growth cone*

**repulsive cue for the growth cone**<br>Depending on the relationship between local position<br>and the intrinsic standard value *S* of the growth cone the<br>effect of a ligand gradient can be either attractive or Depending on the relationship between local position<br>and the intrinsic standard value S of the growth cone the<br>effect of a ligand gradient can be either attractive or<br>repulsive (figure 6) This is in contrast to the mass a effect of a ligand gradient can be either attractive or originating from areas between these two poles should repulsive (figure 6). This is in contrast to the mass action show a graded transition between these two extremes model. Here the authors of that model postulate that the repulsive (figure 6). This is in contrast to the mass action<br>model. Here the authors of that model postulate that the<br>ligand has to have a repellent effect on the growth cone<br>(Nakamoto *et al.* 1996). However, the idea th model. Here the authors of that model postulate that the ligand has to have a repellent effect on the growth cone (Nakamoto *et al.* 1996). However, the idea that the same molecule could act repulsively or attractively fit ligand has to have a repellent effect on the growth cone (Nakamoto *et al.* 1996). However, the idea that the same molecule could act repulsively or attractively fits well with results of Poo and co-workers (Song *et al.* (Nakamoto *et al.* 1996). However, the idea that the same<br>molecule could act repulsively or attractively fits well<br>with results of Poo and co-workers (Song *et al.* 1997; Ming<br>et al. 1997) in an analogous system. They demo molecule could act repulsively or attractively fits well<br>with results of Poo and co-workers (Song *et al.* 1997; Ming<br>*et al.* 1997) in an analogous system. They demonstrated<br>that the effect of certain guidance molecules o with results of Poo and co-workers (Song *et al.* 1997; Ming *et al.* 1997) in an analogous system. They demonstrated that the effect of certain guidance molecules on growth cones depends on the cAMP-cGMP concentration wit *et al.* 1997) in an analogous system. They demonstrated<br>that the effect of certain guidance molecules on growth<br>cones depends on the cAMP-cGMP concentration within<br>the neuron itself. A decrease of one of these second that the effect of certain guidance molecules on growth<br>cones depends on the cAMP–cGMP concentration within<br>the neuron itself. A decrease of one of these second<br>messengers converts attraction by the source of the molecones depends on the cAMP–cGMP concentration within<br>the neuron itself. A decrease of one of these second<br>messengers converts attraction by the source of the mole-<br>cules into a repulsion and vice versa, i.e. the effect of t messengers converts attraction by the source of the molemessengers converts attraction by the source of the mole-<br>cules into a repulsion and vice versa, i.e. the effect of the<br>guidance molecules depends on the internal state of the<br>growth cone cules into a rep<br>guidance mole<br>growth cone.

## **4. COMPARISON OF THE SERVOMECHANISM MODEL**<br>4. COMPARISON OF THE SERVOMECHANISM MODEL **WITH** *IN VIVO* **AND** *IN VITRO* **RESULTS**

#### WITH *IN VIVO AND IN VITRO RESULTS*<br>(a) *The model fits well with certain aspects of the* **is well with certain a**<br>in vivo situation<br>amonstrated for the Sperry (1943) demonstrated for the first time that

**in vivo situation**<br>Sperry (1943) demonstrated for the first time that<br>axons read positional information from their target area.<br>Regeneration studies of the ontic nerve in newts (Fujisawa Sperry (1943) demonstrated for the first time that<br>axons read positional information from their target area.<br>Regeneration studies of the optic nerve in newts (Fujisawa<br> $et$ al 1982) and in fishes (Stuermer 1988a b) have pro axons read positional information from their target area.<br> *Regeneration studies of the optic nerve in newts (Fujisawa et al.* 1982) and in fishes (Stuermer 1988*a*,*b*) have provided clear evidence that both normally and Regeneration studies of the optic nerve in newts (Fujisawa *et al.* 1982) and in fishes (Stuermer 1988*a*,*b*) have provided clear evidence that both normally and ectopically incrowing retinal axons show directed (non-ran *et al.* 1982) and in fishes (Stuermer 1988*a,b*) have provided clear evidence that both normally and ectopically ingrowing retinal axons show directed (non-random) growth towards the target zone Similarly in the chick clear evidence that both normally and ectopically<br>ingrowing retinal axons show directed (non-random)<br>growth towards the target zone. Similarly, in the chick, growth towards the target zone. Similarly, in the chick,<br>an initially loose projection is sharpened by the interstitial<br>branch formation followed by elimination of specific<br>axonal backbranches and to a lesser extent, by ab an initially loose projection is sharpened by the interstitial an initially loose projection is sharpened by the interstitial<br>branch formation followed by elimination of specific<br>axonal backbranches and, to a lesser extent, by abrupt<br>course corrections of the growth cone towards the a branch formation followed by elimination of specific<br>axonal backbranches and, to a lesser extent, by abrupt<br>course corrections of the growth cone towards the appro-<br>priate target zone (Thanos & Bonboeffer 1987: Nakamura  $\bigcup$ axonal backbranches and, to a lesser extent, by abrupt<br>course corrections of the growth cone towards the appro-<br>priate target zone (Thanos & Bonhoeffer 1987; Nakamura<br>& Q'I eary 1989). Betinal axons of some zebrafish path-% course corrections of the growth cone towards the appropriate target zone (Thanos & Bonhoeffer 1987; Nakamura<br>& O'Leary 1989). Retinal axons of some zebrafish path-<br>finding mutants enter the tectum from an ectonic positi priate target zone (Thanos & Bonhoeffer 1987; Nakamura those with high receptor concentration there are three<br>
& O'Leary 1989). Retinal axons of some zebrafish path-<br>
finding mutants enter the tectum from an ectopic positi & O'Leary 1989). Retinal axons of some zebrafish path-<br>finding mutants enter the tectum from an ectopic position<br>but grow directly to their correct termination zones<br>(Trowe et al. 1996). In the mouse, the strategy seems t finding mutants enter the tectum from an ectopic position<br>but grow directly to their correct termination zones<br>(Trowe *et al.* 1996). In the mouse, the strategy seems to be<br>different There the majority of the growth cones but grow directly to their correct termination zones<br>(Trowe *et al.* 1996). In the mouse, the strategy seems to be<br>different. There the majority of the growth cones over-<br>shoots the retinotonic projection is established by (Trowe *et al.* 1996). In the mouse, the strategy seems to be different. There the majority of the growth cones overshoots, the retinotopic projection is established by the formation of interstitial branches and eliminati different. There the majority of the growth cones over-<br>shoots, the retinotopic projection is established by the<br>formation of interstitial branches and elimination of the<br>major growth cones (Roskies & O'Leary 1994) However shoots, the retinotopic projection is established by the formation of interstitial branches and elimination of the major growth cones (Roskies & O'Leary 1994). However, despite of all interspecies differences the in viva formation of interstitial branches and elimination of the<br>major growth cones (Roskies & O'Leary 1994). However,<br>despite of all interspecies differences, the *in vivo* situations<br>demonstrate that growth cones find their tar major growth cones (Roskies & O'Leary 1994). However, despite of all interspecies differences, the *in vivo* situations demonstrate that growth cones find their target position demonstrate that growth cones find their target position<br>*Phil. Trans. R. Soc. Lond.* B (2000)

independently of their entry point into the tectum. This is<br>a general feature of gradient models of axonal targeting independently of their entry point into the tectum. This is<br>a general feature of gradient models of axonal targeting<br>and is thus a strong indication that guidance by gradients independently of their entry point into the tectum. This is<br>a general feature of gradient models of axonal targeting<br>and is thus a strong indication that guidance by gradients<br>is involved somehow. Indeed simulations with t a general feature of gradient models of axonal targeting<br>and is thus a strong indication that guidance by gradients<br>is involved somehow. Indeed, simulations with the servo-<br>mechanism model showed that all virtual growth co is involved somehow. Indeed, simulations with the servomechanism model showed that all virtual growth cones migrate towards their target zone regardless of the posimigrate towards their target zone regardless of the position of the starting point in the graded field. Then they get trapped, staying active and mobile in their specific termination area tion of the starting<br>get trapped, stayin<br>termination area.

## **(b)** *Some originally unexpected results of the stripe assay become easy to explain* (a) Some originally unexpected results of the stripe<br>assay become easy to explain<br>As mentioned in  $\S$ 2, the stripe assay was developed to<br>derstand the mechanisms of the retinotonic projection

Depending on the relationship between local position growth decision for the posterior stripes and the extreme **assay become easy to explain**<br>As mentioned in  $\S$ 2, the stripe assay was developed to<br>understand the mechanisms of the retinotopic projection<br>and to identify guidance molecules. Some results of this As mentioned in  $\S$ 2, the stripe assay was developed to understand the mechanisms of the retinotopic projection and to identify guidance molecules. Some results of this assay were rather unexpected. The initial expectatio understand the mechanisms of the retinotopic projection<br>and to identify guidance molecules. Some results of this<br>assay were rather unexpected. The initial expectation was<br>a graded response in the behaviour of the axonal po and to identify guidance molecules. Some results of this<br>assay were rather unexpected. The initial expectation was<br>a graded response in the behaviour of the axonal popula-<br>tion. The extreme nasal growth cones should show a assay were rather unexpected. The initial expectation was<br>a graded response in the behaviour of the axonal popula-<br>tion. The extreme nasal growth cones should show a clear<br>growth decision for the posterior stripes and the tion. The extreme nasal growth cones should show a clear tion. The extreme nasal growth cones should show a clear<br>growth decision for the posterior stripes. Growth cones<br>temporal ones for the anterior stripes. Growth cones<br>originating from areas between these two poles should growth decision for the posterior stripes and the extreme<br>temporal ones for the anterior stripes. Growth cones<br>originating from areas between these two poles should<br>show a graded transition between these two extremes originating from areas between these two poles should However, in the stripe assay experiments only two show a graded transition between these two extremes.<br>However, in the stripe assay experiments only two<br>different behaviours were observed: all temporal axons<br>irrespective of their temporal position grow on stripes However, in the stripe assay experiments only two<br>different behaviours were observed: all temporal axons<br>irrespective of their temporal position grow on stripes<br>made un of anterior membranes whereas all nasal axons different behaviours were observed: all temporal axons<br>irrespective of their temporal position grow on stripes<br>made up of anterior membranes, whereas all nasal axons<br>show no preference and grow equally well on stripes of irrespective of their temporal position grow on stripes<br>made up of anterior membranes, whereas all nasal axons<br>show no preference and grow equally well on stripes of<br>both anterior and posterior membranes. Also unexpected made up of anterior membranes, whereas all nasal axons<br>show no preference and grow equally well on stripes of<br>both, anterior and posterior membranes. Also unexpected<br>was a sharp transition between the behaviour of these show no preference and grow equally well on stripes of<br>both, anterior and posterior membranes. Also unexpected<br>was a sharp transition between the behaviour of these<br>two populations (Walter *et al.* 1987*a*) both, anterior and posterior membranes. Also unexpected<br>was a sharp transition between the behaviour of these<br>two populations (Walter *et al.* 1987*a*).<br>One possible explanation for this outcome of the stripe was a sharp transition between the behaviour of these

two populations (Walter *et al.* 1987*a*).<br>One possible explanation for this outcome of the stripe<br>assay experiments could be that the *in vitro* system is<br>somehow incomplete i.e., a quidance factor for nasal One possible explanation for this outcome of the stripe assay experiments could be that the *in vitro* system is somehow incomplete, i.e. a guidance factor for nasal axons is lost or diluted during the membrane nurificati assay experiments could be that the *in vitro* system is somehow incomplete, i.e. a guidance factor for nasal axons is lost or diluted during the membrane purification procedure. A nother explanation also not satisfying s somehow incomplete, i.e. a guidance factor for nasal axons is lost or diluted during the membrane purification procedure. Another explanation, also not satisfying, specaxons is lost or diluted during the membrane purification<br>procedure. Another explanation, also not satisfying, spec-<br>ulates that the function of a graded distribution of<br>guidance molecules would only be to define the poste procedure. Another explanation, also not satisfying, speculates that the function of a graded distribution of guidance molecules would only be to define the posterior part of the tectum in a step-like function. However ulates that the function of a graded distribution of guidance molecules would only be to define the posterior part of the tectum in a step-like function. However, experiments with the strine assay showed that temporal guidance molecules would only be to define the posterior<br>part of the tectum in a step-like function. However,<br>experiments with the stripe assay showed that temporal<br>axons can distinguish between stripes made of part of the tectum in a step-like function. However,<br>experiments with the stripe assay showed that temporal<br>axons can distinguish between stripes made of<br>membranes from neighbouring tectal areas independent experiments with the stripe assay showed that temporal<br>axons can distinguish between stripes made of<br>membranes from neighbouring tectal areas, independent axons can distinguish between stripes made of<br>membranes from neighbouring tectal areas, independent<br>of the absolute positions in the tectum, indicating a<br>graded distribution of the cue (Bonhoeffer & Huf 1982) membranes from neighbouring tectal areas, independent<br>of the absolute positions in the tectum, indicating a<br>graded distribution of the cue (Bonhoeffer & Huf 1982).<br>The servomechanism model offers an interesting alterthe absolute positions in the tectum, indicating a<br>aded distribution of the cue (Bonhoeffer & Huf 1982).<br>The servomechanism model offers an interesting alter-<br>tive explanation. Figure 5*b* shows the result of a simula-

graded distribution of the cue (Bonhoeffer & Huf 1982).<br>The servomechanism model offers an interesting alternative explanation. Figure 5*b* shows the result of a simula-The servomechanism model offers an interesting alternative explanation. Figure  $5b$  shows the result of a simulation of the stripe assay on a carpet of alternating lanes of high and low ligand concentrations. On the left mative explanation. Figure  $5b$  shows the result of a simulation of the stripe assay on a carpet of alternating lanes of high and low ligand concentrations. On the left side of the figure are the starting points of axons. tion of the stripe assay on a carpet of alternating lanes of<br>high and low ligand concentrations. On the left side of<br>the figure are the starting points of axons. Their guidance<br>receptor concentration increases from the low high and low ligand concentrations. On the left side of<br>the figure are the starting points of axons. Their guidance<br>receptor concentration increases from the lower to the<br>unner lanes. This is equivalent to an explant strip the figure are the starting points of axons. Their guidance<br>receptor concentration increases from the lower to the<br>upper lanes. This is equivalent to an explant strip<br>reaching from the nasal to the temporal part of the receptor concentration increases from the lower to the upper lanes. This is equivalent to an explant strip reaching from the nasal to the temporal part of the retina. From axons with low receptor concentration to upper lanes. This is equivalent to an explant strip those with high receptor concentration there are three retina. From axons with low receptor concentration to<br>those with high receptor concentration there are three<br>distinguishable axonal behaviours: (i) decision for the<br>strines with a bigh ligand concentration (lower part of those with high receptor concentration there are three<br>distinguishable axonal behaviours: (i) decision for the<br>stripes with a high ligand concentration (lower part of<br>figure 5*a b*): (ii) no decision at all (middle part distinguishable axonal behaviours: (i) decision for the stripes with a high ligand concentration (lower part of figure  $5a,b$ ); (ii) no decision at all (middle part of figure  $5a,b$ ); and (iii) decision for stripes with a l stripes with a high ligand concentration (lower part of figure 5*a*,*b*); (ii) no decision at all (middle part of figure 5*a*,*b*); and (iii) decision for stripes with a low ligand concentration (upper part of figure 5*a* figure 5*a*,*b*); (ii) no decision at all (middle part of figure 5*a*,*b*); and (iii) decision for stripes with a low ligand concentration (upper part of figure 5*a*,*b*). The transition between these behaviours is sharp.  $5a,b$ ; and (iii) decision for stripes with a low ligand concentration (upper part of figure  $5a,b$ ). The transition concentration (upper part of figure  $5a,b$ ). The transition<br>between these behaviours is sharp. The position of these<br>transitions depends on the ligand concentration of the<br>strines relative to the intrinsic standard value S between these behaviours is sharp. The position of these transitions depends on the ligand concentration of the stripes relative to the intrinsic standard value *S* of the axons and could be changed in such a way that only transitions depends on the ligand concentration of the stripes relative to the intrinsic standard value  $S$  of the axons and could be changed in such a way that only two axonal behaviours remain. Under these assumptions the

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Figure 5. Von Boxberg's guidance experiments and Honda's simulation. (a) A temporal to nasal retinal explant was placed<br>on a membrane carpet, which consists of alternating lanes of anterior and posterior tectal membranes. on a membrane carpet, which consists of alternating lanes of anterior and posterior tectal membranes. In contrast to the<br>standard stripe assay the tectal membranes for this assay have been prepared by a different mild puri on a membrane carpet, which consists of alternating lanes of anterior and posterior tectal membranes. In contrast to the<br>standard stripe assay the tectal membranes for this assay have been prepared by a different mild puri standard stripe assay the tectal membranes for this assay have been prepared by a different mild purification procedure, involving<br>iso-electric focusing (Von Boxberg *et al.* 1993). The position of the anterior lanes is in iso-electric focusing (Von Boxberg *et al.* 1993). The position of the anterior lanes is indicated by bars. Temporal axons grow<br>membranes derived from the anterior tectal pole, nasal axons show a preference for growing on membranes derived from the anterior tectal pole, nasal axons show a preference for growing on membranes from the posterior<br>pole indicated by triangles. Given the choice, nasal and temporal axons prefer to grow on membranes pole indicated by triangles. Given the choice, nasal and temporal axons prefer to grow on membranes of their own target<br>position. (b) Corresponding simulation of axonal growth and guidance on the basis of the Honda model ( figure shows the trajectories of virtual growth cones starting on the left side of the figure growing into a field consisting of<br>alternating stripes with two different ligand concentrations. The lanes with low ligand conce figure shows the trajectories of virtual growth cones starting on the left side of the figure growing into a field consisting of<br>alternating stripes with two different ligand concentrations. The lanes with low ligand conce alternating stripes with two different ligand concentrations. The lanes with low ligand concentrations are marked with vertical<br>bars (anterior membranes  $L_a$ ), the lanes with high ligand concentrations with triangles (pos bars (anterior membranes  $L_a$ ), the lanes with high ligand concentrations with triangles (posterior membranes  $L_p$ ). The receptor concentration of the growth cones increases linearly from the lower (nasal axons) to the up concentration of the growth cones increases linearly from the lower (nasal axons) to the upper (temporal axons) stripes. There<br>are three different behaviours distinguishable: decision for stripes with anterior membranes (v are three different behaviours distinguishable: decision for stripes with anterior membranes (vertical bars) in the upper part; no<br>decision in the middle and lower part; and a preference for stripes with posterior membrane decision in the middle and lower part; and a preference for stripes with posterior membranes (triangles) in a region within the<br>lower third. The behaviour of the growth cones (preference or no preference) depend on the li lower third. The behaviour of the growth cones (preference or no preference) depend on the ligand concentrations, the standary value *S* and the receptor concentration of the growth cone. Depending on these factors a simu value *S* and the receptor concentration of the growth cone. Depending only one of the described decisions. The following parameters have b  $L_{\rm p}$  = 50, *S* = 2500, the receptor concentration ranges from 0 to 110.

 $L_p = 50$ ,  $S = 2500$ , the receptor concentration ranges from 0 to<br>outcome of the simulation looks like the usual experi-<br>mental result: no decision for the nasal axons (low outcome of the simulation looks like the usual experimental result: no decision for the nasal axons (low tireceptor concentration) and decision against one type of the outcome of the simulation looks like the usual experi-<br>mental result: no decision for the nasal axons (low<br>receptor concentration) and decision against one type of<br>the strines for the temporal axons (high receptor concenmental result: no decision for the nasal axons (low receptor concentration) and decision against one type of the stripes for the temporal axons (high receptor concenreceptor concentration) and decision against one type of<br>the stripes for the temporal axons (high receptor concen-<br>tration). According to this model, it should be possible to<br>see one, two or all three different behaviours the stripes for the temporal axons (high receptor concentration). According to this model, it should be possible to<br>see one, two or all three different behaviours in one assay<br>depending on the relative ligand concentration tration). According to this model, it should be possible to<br>see one, two or all three different behaviours in one assay<br>depending on the relative ligand concentration of the two<br>sorts, of strines. Modifying, the standard p see one, two or all three different behaviours in one assay further experiments, for example, by changing the depending on the relative ligand concentration of the two relative ligand concentration in the stripes by specif depending on the relative ligand concentration of the two<br>sorts of stripes. Modifying the standard purification<br>procedure, Von Boxberg *et al.* (1993) were indeed able to<br>show a stripe assay where temporal axons grow on an sorts of stripes. Modifying the standard purification procedure, Von Boxberg *et al.* (1993) were indeed able to show a stripe assay where temporal axons grow on ante-<br>rior stripes and nasal axons on posterior stripes (fi procedure, Von Boxberg *et al.* (1993) were indeed able to show a stripe assay where temporal axons grow on ante-<br>rior stripes and nasal axons on posterior stripes (figure  $5a$ ). The experiments contain as internal contro show a stripe assay where temporal axons grow on ante-<br>rior stripes and nasal axons on posterior stripes (figure<br>5*a*). The experiments contain as internal controls the<br>normal decision of temporal axons. This makes them rior stripes and nasal axons on posterior stripes (figure  $ta$  5*a*). The experiments contain as internal controls the normal decision of temporal axons. This makes them<br>trustworthy and convincing despite their not being v 5*a*). The experiments contain as internal controls the<br>normal decision of temporal axons. This makes them<br>trustworthy and convincing despite their not being very<br>reproducible. One possible explanation for the lack of normal decision of temporal axons. This makes them<br>trustworthy and convincing despite their not being very<br>reproducible. One possible explanation for the lack of<br>reproducibility was originally that the activity of a factor trustworthy and convincing despite their not being very<br>reproducible. One possible explanation for the lack of<br>reproducibility was originally that the activity of a factor<br>which influences nasal axons is labile and is thus reproducible. One possible explanation for the lack of reproducibility was originally that the activity of a factor which influences nasal axons is labile and is thus only sometimes preserved reproducibility was originally that the activity of a factor which influences nasal axons is labile and is thus only sometimes preserved. ich influences nasal axons is labile and is thus only<br>metimes preserved.<br>The servomechanism model offers a better explanation<br>the preference of nasal axons for the posterior

sometimes preserved.<br>The servomechanism model offers a better explanation<br>for the preference of nasal axons for the posterior<br>strines: the novel purification scheme might change the The servomechanism model offers a better explanation<br>for the preference of nasal axons for the posterior<br>stripes: the novel purification scheme might change the *stripes:* the novel purification scheme might change the *Phil. Trans. R. Soc. Lond.* B (2000)

concentration of active ligands in the membrane preparaconcentration of active ligands in the membrane prepara-<br>tions derived from anterior and posterior tectal tissue and<br>the preference of pasal axons for posterior membranes concentration of active ligands in the membrane prepara-<br>tions derived from anterior and posterior tectal tissue and<br>the preference of nasal axons for posterior membranes<br>could critically dependent on their concentration d tions derived from anterior and posterior tectal tissue and<br>the preference of nasal axons for posterior membranes<br>could critically dependent on their concentration differthe preference of nasal axons for posterior membranes<br>could critically dependent on their concentration differ-<br>ence in the stripes. This notion can possibly be verified by<br>further experiments for example by changing the further concentration difference in the stripes. This notion can possibly be verified by further experiments, for example, by changing the relative ligand concentration in the stripes by specific ence in the stripes. This notion can possibly be verified by<br>further experiments, for example, by changing the<br>relative ligand concentration in the stripes by specific<br>antibodies antibodies.

#### **(c)** *A graded response* **in vivo** *is not in contradiction to a step-like transition between nasal and temporal behavionse* in vivo *is not in contr*<br>*ie transition between nasal and t*<br>*behaviour in the stripe assay*<br>*w* the results of the simulations she

**behaviour in the stripe assay**<br>Surprisingly the results of the simulations show clearly that a stepwise transition from nasal to temporal beha-Surprisingly the results of the simulations show clearly<br>that a stepwise transition from nasal to temporal beha-<br>viour of the axons in the stripe assay can be produced<br>even if the very same axons display a graded response that a stepwise transition from nasal to temporal behaviour of the axons in the stripe assay can be produced<br>even if the very same axons display a graded response in<br>a gradient field. They show further that the very same viour of the axons in the stripe assay can be produced<br>even if the very same axons display a graded response in<br>a gradient field. They show further that the very same<br>factor can bave a repellent or an attractive effect on even if the very same axons display a graded response in<br>a gradient field. They show further that the very same<br>factor can have a repellent or an attractive effect on an a gradient field. They show further that the very same<br>factor can have a repellent or an attractive effect on an<br>axon population (figure 6). The character of the effect<br>denends exclusively on the relationship between the s factor can have a repellent or an attractive effect on an<br>axon population (figure 6). The character of the effect<br>depends exclusively on the relationship between the signal<br>strength Lat the current position of the growth c axon population (figure 6). The character of the effect depends exclusively on the relationship between the signal strength  $I$  at the current position of the growth cone and

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Figure 6. Repulsive and attractive concentration ranges. The graph shows a linear ligand gradient and the trajectories Figure 6. Repulsive and attractive concentration ranges.<br>The graph shows a linear ligand gradient and the trajectories<br>of two migrating growth cones within this gradient (dashed<br>lines). They stop at two different ligand co The graph shows a linear ligand gradient and the trajector of two migrating growth cones within this gradient (dash<br>lines). They stop at two different ligand concentrations.<br>The effect of the ligands on the growth cones (a of two migrating growth cones within this gradient (dashed<br>lines). They stop at two different ligand concentrations.<br>The effect of the ligands on the growth cones (attractive or<br>repulsive) is dependent on their target posi lines). They stop at two different ligand concentrations.<br>The effect of the ligands on the growth cones (attractive or<br>repulsive) is dependent on their target positions. The whole<br>concentration range (white area) up to the The effect of the ligands on the growth cones (attractive or repulsive) is dependent on their target positions. The whole concentration range (white area) up to the first stop-point is attractive  $(A|A)$  for both growth co repulsive) is dependent on their target positions. The whole<br>concentration range (white area) up to the first stop-point is<br>attractive  $(A/A)$  for both growth cones. The concentration<br>range (light grey area) between the two attractive  $(A/A)$  for both growth cones. The concentration<br>range (light grey area) between the two stop-points has a<br>repellent  $(R)$  effect on one growth cone and an attractive range (light grey area) between the two stop-points has a  $(A)$  effect on the other growth cone. Any concentration (dark grey area) above the second stop-point has a repellent (A) effect on the other growth cone. Any concentration<br>(dark grey area) above the second stop-point has a repellent<br>( $R/R$ ) effect for both growth cones. Thus, the molecule itself<br>has neither an attractive nor a repulsive (dark grey area) above the second stop-point has  $(R/R)$  effect for both growth cones. Thus, the m<br>has neither an attractive nor a repulsive effect.

the internal standard value *S*. The explanation for the<br>uniform behaviour of a group of different axons within a the internal standard value  $S$ . The explanation for the uniform behaviour of a group of different axons within a certain concentration range is simple: if the probability the internal standard value S. The explanation for the<br>uniform behaviour of a group of different axons within a<br>certain concentration range is simple: if the probability<br>for preference for one sort of stripe is high enough uniform behaviour of a group of different axons within a certain concentration range is simple: if the probability for preference for one sort of stripe is high enough, then a large majority of axons grow onto that stripe. certain concentration range is simple: if the probability<br>for preference for one sort of stripe is high enough, then a<br>large majority of axons grow onto that stripe. This has the<br>appearance of a decision. An even higher pr for preference for one sort of stripe is high enough, then a<br>large majority of axons grow onto that stripe. This has the<br>appearance of a decision. An even higher probability does<br>not change the apparent behaviour of the ax appearance of a decision. An even higher probability does not change the apparent behaviour of the axons because most of them have already made their decision.

# **(d)** *A step -like behaviour in the gradient assay remains difficult in the gradient*<br>*remains difficult to explain*<br> $\alpha$  investigate axons growing a

substrates, we have developed an *in vitro* assay (Rosentreter In order to investigate axons growing on graded<br>substrates, we have developed an *in vitro* assay (Rosentreter<br>*et al.* 1998) in which the substrate is offered as a linear<br>concentration gradient of guidance molecules. This substrates, we have developed an *in vitro* assay (Rosentreter *et al.* 1998) in which the substrate is offered as a linear concentration gradient of guidance molecules. This has been done in both stringd and non-stringd g *et al.* 1998) in which the substrate is offered as a linear concentration gradient of guidance molecules. This has been done in both striped and non-striped gradients.<br>These assays allow growing retinal axons to grow into concentration gradient of guidance molecules. This has<br>been done in both striped and non-striped gradients.<br>These assays allow growing retinal axons to grow into line-<br>arly increasing concentrations of posterior membranes been done in both striped and non-striped gradients.<br>These assays allow growing retinal axons to grow into linearly increasing concentrations of posterior membranes or<br>membranes derived from cells transfected with guidance These assays allow growing retinal axons to grow into line-<br>arly increasing concentrations of posterior membranes or<br>membranes derived from cells transfected with guidance<br>molecules (ephrin A5, A9) Temporal axons enter the arly increasing concentrations of posterior membranes or<br>membranes derived from cells transfected with guidance<br>molecules (ephrin A5, A2). Temporal axons enter the membranes derived from cells transfected with guidance<br>molecules (ephrin A5, A2). Temporal axons enter the<br>gradient and grow unaffectedly until they reach a certain<br>threshold concentration. In the unstrined version of the molecules (ephrin A5, A2). Temporal axons enter the gradient and grow unaffectedly until they reach a certain threshold concentration. In the unstriped version of the assay temporal axons stop growing but the growth cones gradient and grow unaffectedly until they reach a certain<br>threshold concentration. In the unstriped version of the<br>assay, temporal axons stop growing, but the growth cones<br>stay, mobile and active, as the model predicts. In threshold concentration. In the unstriped version of the<br>assay, temporal axons stop growing, but the growth cones<br>stay mobile and active, as the model predicts. In the<br>strined version of the assay temporal axons try to avo assay, temporal axons stop growing, but the growth cones<br>stay mobile and active, as the model predicts. In the<br>striped version of the assay, temporal axons try to avoid<br>higher concentrations within the gradient and escape stay mobile and active, as the model predicts. In the striped version of the assay, temporal axons try to avoid higher concentrations within the gradient and escape to lower concentrations at the borders of the (non-ideal) striped version of the assay, temporal axons try to avoid<br>higher concentrations within the gradient and escape to<br>lower concentrations at the borders of the (non-ideal) higher concentrations within the gradient and escape to<br>lower concentrations at the borders of the (non-ideal)<br>stripes. The nasal axons enter the gradient and keep<br>growing in both versions of the assay lower concentrations at the borders<br>stripes. The nasal axons enter the<br>growing in both versions of the assay.<br>Like the stripe assay the grad ipes. The nasal axons enter the gradient and keep<br>owing in both versions of the assay.<br>Like the stripe assay, the gradient assay reveals<br>moral and nasal behaviours but no intermediate be-

growing in both versions of the assay.<br>Like the stripe assay, the gradient assay reveals<br>temporal and nasal behaviours but no intermediate be-Like the stripe assay, the gradient assay reveals<br>temporal and nasal behaviours but no intermediate be-<br>haviour (Rosentreter *et al.* 1998). All temporal axons are<br>shorter than the nasal axons. The expectation was that temporal and nasal behaviours but no intermediate behaviour (Rosentreter *et al.* 1998). All temporal axons are shorter than the nasal axons. The expectation was that axons growing into increasing concentrations of guidanc haviour (Rosentreter *et al.* 1998). All temporal axons are shorter than the nasal axons. The expectation was that axons growing into increasing concentrations of guidance molecules would react in a graded manner i.e. that shorter than the nasal axons. The expectation was that axons growing into increasing concentrations of guidance molecules would react in a graded manner, i.e. that

individual axons depending on their receptor composition<br>would stop at different positions in the gradient and individual axons depending on their receptor composition<br>would stop at different positions in the gradient and<br>would therefore have different lengths. Honda (1998) would stop at different positions in the gradient and<br>would therefore have different lengths. Honda (1998) would stop at different positions in the gradient and<br>would therefore have different lengths. Honda (1998)<br>showed in computer simulations of the gradient assay<br>that the appearance of the result depends very much on would therefore have different lengths. Honda (1998)<br>showed in computer simulations of the gradient assay<br>that the appearance of the result depends very much on<br>the shape of the concentration distribution of the ligand showed in computer simulations of the gradient assay<br>that the appearance of the result depends very much on<br>the shape of the concentration distribution of the ligand.<br>Simulations with linear gradients show the expected that the appearance of the result depends very much on<br>the shape of the concentration distribution of the ligand.<br>Simulations with linear gradients show the expected<br>graded distribution of axonal stop positions. Simulation the shape of the concentration distribution of the ligand.<br>Simulations with linear gradients show the expected<br>graded distribution of axonal stop positions. Simulations<br>with sigmoidal ligand gradients show a more or less s Simulations with linear gradients show the expected<br>graded distribution of axonal stop positions. Simulations<br>with sigmoidal ligand gradients show a more or less step-<br>like distribution of axonal lengths (Honda 1998) It wo graded distribution of axonal stop positions. Simulations<br>with sigmoidal ligand gradients show a more or less step-<br>like distribution of axonal lengths (Honda 1998). It would<br>be interesting to find out if simulations of th with sigmoidal ligand gradients show a more or less step-<br>like distribution of axonal lengths (Honda 1998). It would<br>be interesting to find out if simulations of the gradient<br>assay with a nonlinear receptor distribution an be interesting to find out if simulations of the gradient assay with a nonlinear receptor distribution and linear be interesting to find out if simulations of the gradient<br>assay with a nonlinear receptor distribution and linear<br>ligand gradients result in a similar step-like nasal-<br>temporal switch assay with a nonl<br>ligand gradients<br>temporal switch.<br>Although there and gradients result in a similar step-like nasal-<br>nporal switch.<br>Although there is admittedly a discrepancy between<br>e simulations and the real experiments, the results

temporal switch.<br>Although there is admittedly a discrepancy between<br>the simulations and the real experiments, the results Although there is admittedly a discrepancy between<br>the simulations and the real experiments, the results<br>certainly show that, depending on the exact parameters<br>of the gradient the graded response of the axons might the simulations and the real experiments, the results<br>certainly show that, depending on the exact parameters<br>of the gradient, the graded response of the axons might<br>sometimes be hard to see of the gradient, the graded response of the axons might sometimes be hard to see.

#### **5. MODELS BASED ON THE IMPRINT-MATCHING CONCEPT OF RETINOTECTAL GUIDANCE**

#### **(a)** *Novel experimental results suggest an* (a) *Novel experimental results suggest an*<br>*imprint-matching concept of retinotectal guidance*<br>As presented up to now. Honda's model relies on the (a) *Novel experimental results suggest an*<br> *mprint-matching concept of retinotectal guidance*<br>
As presented up to now, Honda's model relies on the<br>
mplementary expression of guidance receptors and

(d) *A step-like behaviour in the gradient assay* on the nasal side. When Hornberger *et al.* (1999) enzym-<br> **remains difficult to explain** atically removed the ligand from the retinal ganglion<br>
In order to investigate ax As presented up to now, Honda's model relies on the complementary expression of guidance receptors and As presented up to now, Honda's model relies on the complementary expression of guidance receptors and ligands on the projecting area and the target organ as well as on a set-point value which is the same for all complementary expression of guidance receptors and<br>ligands on the projecting area and the target organ as<br>well as on a set-point value, which is the same for all<br>retinal ganglion cells. Novel experimental results by well as on a set-point value, which is the same for all retinal ganglion cells. Novel experimental results by well as on a set-point value, which is the same for all<br>retinal ganglion cells. Novel experimental results by<br>Hornberger *et al.* (1999) and Dütting *et al.* (1999),<br>however indicate an additional function of the guidance retinal ganglion cells. Novel experimental results by<br>Hornberger *et al.* (1999) and Dütting *et al.* (1999),<br>however, indicate an additional function of the guidance<br>ligands These showed that the ligands are expressed no Hornberger *et al.* (1999) and Dütting *et al.* (1999), however, indicate an additional function of the guidance ligands. These showed that the ligands are expressed not only on the tectum but also on retinal ganglion cel however, indicate an additional function of the guidance<br>ligands. These showed that the ligands are expressed not<br>only on the tectum, but also on retinal ganglion cells. The<br>expression pattern on the retinal resembles that ligands. These showed that the ligands are expressed not<br>only on the tectum, but also on retinal ganglion cells. The<br>expression pattern on the retina resembles that on the<br>target organ. It corresponds to a gradient with lo only on the tectum, but also on retinal ganglion cells. The<br>expression pattern on the retina resembles that on the<br>target organ. It corresponds to a gradient with low<br>concentrations on the temporal and high concentrations expression pattern on the retina resembles that on the target organ. It corresponds to a gradient with low concentrations on the temporal and high concentrations on the nasal side When Hornberger *et al.* (1999) enzymtarget organ. It corresponds to a gradient with low<br>concentrations on the temporal and high concentrations<br>on the nasal side. When Hornberger *et al.* (1999) enzym-<br>atically removed the ligand from the retinal ganglion concentrations on the temporal and high concentrations<br>on the nasal side. When Hornberger *et al.* (1999) enzym-<br>atically removed the ligand from the retinal ganglion<br>cells nasal axons became responsive to the guidance cu on the nasal side. When Hornberger *et al.* (1999) enzymatically removed the ligand from the retinal ganglion cells, nasal axons became responsive to the guidance cue, i.e. they all behaved like temporal axons in the *in* atically removed the ligand from the retinal ganglion<br>cells, nasal axons became responsive to the guidance cue,<br>i.e. they all behaved like temporal axons in the *in vitro*<br>strine assay Eurthermore, when they overexpressed cells, nasal axons became responsive to the guidance cue,<br>i.e. they all behaved like temporal axons in the *in vitro*<br>stripe assay. Furthermore, when they overexpressed the<br>ligand in retina, the overexpressing axons were n i.e. they all behaved like temporal axons in the *in vitro* stripe assay. Furthermore, when they overexpressed the ligand in retina, the overexpressing axons were non-<br>responsive to the quidance cue i.e. they behaved like stripe assay. Furthermore, when they overexpressed the ligand in retina, the overexpressing axons were non-<br>responsive to the guidance cue, i.e. they behaved like<br>nasal axons in the stripe assay. These results indicate tha ligand in retina, the overexpressing axons were non-<br>responsive to the guidance cue, i.e. they behaved like<br>nasal axons in the stripe assay. These results indicate that<br>the retinal ligand is crucially involved in the deter responsive to the guidance cue, i.e. they behaved like<br>nasal axons in the stripe assay. These results indicate that<br>the retinal ligand is crucially involved in the determina-<br>tion of target destination. The resemblance of nasal axons in the stripe assay. These results indicate that<br>the retinal ligand is crucially involved in the determina-<br>tion of target destination. The resemblance of the ligand<br>expression patterns on retina and tectum ind the retinal ligand is crucially involved in the determination of target destination. The resemblance of the ligand expression patterns on retina and tectum indicates that tion of target destination. The resemblance of the ligand<br>expression patterns on retina and tectum indicates that<br>retinal axons might grow into the tectum until they reach<br>a ligand concentration that corresponds to that of expression patterns on retina and tectum indicates that<br>retinal axons might grow into the tectum until they reach<br>a ligand concentration that corresponds to that of their<br>retinal site of origin. We would like to call this retinal axons might grow into the tectum until they reach<br>a ligand concentration that corresponds to that of their<br>retinal site of origin. We would like to call this the<br>imprint-matching concent of retinatestal guidance a ligand concentration that corresponds to that of their retinal site of origin. We would like to call this the imprint-matching concept of retinotectal guidance.

## **(b)** *Implementation of the imprint-matching concept by a set-point variation mechanism* If **Implementation of the imprint-matching concept**<br>by **a set-point variation mechanism**<br>If the above-mentioned results  $(\S5(a))$  are to be in-<br>proceted into a theoretical model of the guidance

by a set-point variation mechanism<br>If the above-mentioned results  $(\S5(a))$  are to be in-<br>corporated into a theoretical model of the guidance<br>mechanism the first nuzzling issue to be solved is the If the above-mentioned results  $(\$5(a))$  are to be incorporated into a theoretical model of the guidance mechanism the first puzzling issue to be solved is the problem why the densely interminated axons expression corporated into a theoretical model of the guidance<br>mechanism the first puzzling issue to be solved is the<br>problem why the densely intermingled axons expressing mechanism the first puzzling issue to be solved is the problem why the densely intermingled axons expressing both, ligand and receptor, do not disturb each other during the pathfinding and guidance process. It is thereproblem why the densely intermingled axons expressing<br>both, ligand and receptor, do not disturb each other<br>during the pathfinding and guidance process. It is there-<br>fore important to postulate that ligands on one cell bind both, ligand and receptor, do not disturb each other<br>during the pathfinding and guidance process. It is there-<br>fore important to postulate that ligands on one cell bind<br>exclusively to receptors of the same cell *(cis-inter* during the pathfinding and guidance process. It is there-<br>fore important to postulate that ligands on one cell bind<br>exclusively to receptors of the same cell *(cis*-interaction)



Figure 7. The set-point variation mechanism. A possible mechanistic implementation of the set-point variation mechanism.<br>Two retinal ganglion cells are shown, whose somata (depicted as circles) reside at different position Figure 7. The set-point variation mechanism. A possible mechanistic implementation of the set-point variation mechanism.<br>Two retinal ganglion cells are shown, whose somata (depicted as circles) reside at different position Figure 7. The set-point variation mechanism. A possible mechanistic implementation of the set-point variation mechanism.<br>Two retinal ganglion cells are shown, whose somata (depicted as circles) reside at different position Two retinal ganglion cells are shown, whose somata (depicted as circles) reside at different positions on the retina, one more<br>temporal (T) and one more nasal (N). Their axons are guided to correspondingly different posit temporal (T) and one more nasal (N). Their axons are guided to correspondingly different positions of the tectum (A, anterior;<br>P, posterior) by growth cones symbolized as trapezoids. In the most-parsimonious mechanistic i lion cells express roughly the same concentration of a guidance receptor (e.g. an Eph receptor tyrosine kinase). The first salient<br>feature of the suggested mechanism is that matching concentration gradients of the same lig lion cells express roughly the same concentration of a guidance receptor (e.g. an Eph receptor tyrosine kinase). The first salient<br>feature of the suggested mechanism is that matching concentration gradients of the same li feature of the suggested mechanism is that matching concentration gradients of the same ligand (e.g. an ephrin ligand), which<br>by itself is neither attractive nor repulsive, are used both to determine target destination of their retinal position and to provide the guiding cue to the growth cone on the tectal target. The ligand is therefore expressed<br>by retinal ganglion cells (temporal low, nasal high) as well as by cells of the tectal target their retinal position and to provide the guiding cue to the growth cone on the tectal target. The ligand is therefore expressed<br>by retinal ganglion cells (temporal low, nasal high) as well as by cells of the tectal target by retinal ganglion cells (temporal low, nasal high) as well as by cells of the tectal target (anterior low, posterior high;<br>represented by the density of ligand symbols and grey gradient shading). The second salient featu two independent modes of receptor-ligand interaction resulting in two different pathways of signal transduction. When<br>challenged with a ligand presented on the same cell *(cis*-interaction, green colour) the receptor gener challenged with a ligand presented on the same cell *(cis*-interaction, green colour) the receptor generates one signal, the set-point challenged with a ligand presented on the same cell *(cis-*interaction, green colour) the receptor generates one signal, the set-point<br>signal, when challenged with a ligand in *trans* (red colour) an antagonistic signal is because retinal growth a ligand in *trans* (red colour) an antagonistic signal is produced. The set-point signal, which<br>provides the position-specific imprint, is encoded by the concentration of ligand–receptor *cis*-compl provides the position-specific imprint, is encoded by the concentration of ligand–receptor *cis*-complexes. Topography is achieved<br>because retinal growth cones search for that ligand concentration on the tectum that matche because retinal growth cones search for that ligand concentration on the tectum that matches their own imprinted ligand<br>concentration. The guidance problem therefore amounts to a match-to-sample task. The growth cone stops between both signals (symbolized within the growth cone by different shades of green and red in proportion to signal strength) becomes zero (white). As on each cell the same receptor governs both the set-point and the anta becomes zero (white). As on each cell the same receptor governs both the set-point and the antagonistic signal the model is basibecomes zero (white). As on each cell the same receptor governs both the set-point and the antagonistic signal the model is basi<br>cally independent of receptor concentration as long as saturation is not reached. The graded cally independent of receptor concentration as long as saturation is not reached. The graded distribution observed for some<br>candidate receptors (e.g. Eph A3) might actually be used for an optimization of signal strength ( candidate receptors (e.g. Eph A3) might actually be used for an optimization of signal strength (see  $\S 5(b)$ ). The actual signal<br>integration might be compartmentalized to the growth cone as indicated in this figure. Alter integration might be compartmentalized to the growth cone as indicated in this figure. Alternatively, the whole neurite might<br>involved depending on whether the axon participates in gradient sensing or not. The spatial sepa involved depending on whether the axon participates in gradient sensing or not. The spatial separation of the signals within the growth cone is only for the sake of clarity of the graphical presentation. The subpopulation growth cone is only for the sake of clarity of the graphical presentation. The subpopulation of receptors already bound to a<br>ligand in *cis* might or might not bind an additional ligand in *trans*. If it does, the produced ligand in *cis* might or might not bind an additional ligand in *trans*. If it does, the produced signals have to be strictly additive, i.e. the ternary complex has to produce both signals at once or the additional *trans* the ternary comple<br>respective receptor<br>steric inhibition.  $\overline{\mathbf{S}}$ 

and not to those of neighbouring cells, which are in turn<br>and not to those of neighbouring cells, which are in turn<br>engaged in interactions with their respective *c*is-receptors and not to those of neighbouring cells, which are in turn<br>engaged in interactions with their respective *cis*-receptors.<br>The exclusivity of these interactions might simply be due and not to those of neighbouring cells, which are in turn<br>engaged in interactions with their respective *cis*-receptors.<br>The exclusivity of these interactions might simply be due<br>to steric hindrance. Clearly, separate from engaged in interactions with their respective *cis*-receptors.<br>The exclusivity of these interactions might simply be due<br>to steric hindrance. Clearly separate from the *cis*-The exclusivity of these interactions might simply be due<br>to steric hindrance. Clearly separate from the *cis*-<br>interaction is the interaction of receptors with ligands on<br>the target organ *(trans-interaction*) to steric hindrance. Clearly separate<br>interaction is the interaction of rece-<br>the target organ (*trans*-interaction).<br>The immuint-matching concent can Exercacion is the interaction of receptors with ligands on<br>target organ *(trans-*interaction).<br>The imprint-matching concept can now be incorporated<br>to Honda's model by assuming the novel *dis-interaction* to

the target organ *(trans*-interaction).<br>The imprint-matching concept can now be incorporated<br>into Honda's model by assuming the novel *cis*-interaction to

adjust the set-point *<sup>S</sup>*. We call this the set-point variation mechanism. just the set-point *S*. We call this the set-point variation<br>echanism.<br>As described above, the servomechanism uses the<br>fference parameter *D* between the input signal *L* and

As described above, the servomechanism uses the difference parameter  $D$  between the input signal  $I$  and the standard value *<sup>S</sup>* to calculate a directional and a difference parameter *D* between the input signal *I* and<br>the standard value *S* to calculate a directional and a<br>positional information:  $D = S - I = S - K[R][L_T]$ ,  $L_T$ <br>being the ligand concentration in the tectum. *S* need not b positional information:  $D = S - I = S - K[R][L_T]$ ,  $L_T$ <br>being the ligand concentration in the tectum. *S* need not be<br>a constant, it might be dependent on the concentration of

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Figure 8. The gradient-sensitive adaptation mechanism. A growth cone is shown endowed with the necessary elements for gradient-sensitive adaptation. As for the set-point variation mechanism receptor^ligand *cis*- and *trans*-interactions are modelled Figure 8. The gradient-sensitive adaptation mechanism. A growth cone is shown endowed with the necessary elements for<br>gradient-sensitive adaptation. As for the set-point variation mechanism receptor–ligand *cis*- and *tran* gradient-sensitive adaptation. As for the set-point variation mechanism receptor–ligand *cis*- and *trans*-interactions are m<br>to be strictly exclusive; now, however, they are assumed to produce the same input signal.  $\delta I$ to be strictly exclusive; now, however, they are assumed to produce the same input signal.  $\delta I$  is meant to symbolize a differential sensor for spatial inhomogeneity of the input signal. The autocatalytic reaction sugges sensor for spatial inhomogeneity of the input signal. The autocatalytic reaction suggested to amplify the small external<br>concentration differences across a growth cone into a large internal signal is well suited for this p concentration differences across a growth cone into a large internal signal is well suited for this purpose. The output of the<br>should be inhibitory for a default receptor desensitization–resensitization machinery consistin should be inhibitory for a default receptor desensitization–resensitization machinery consisting of a desensitizing (D) and a<br>resensitizing enzyme (R). As in standard receptor adaptation biochemistry the substrates of thes resensitizing enzyme (R). As in standard receptor adaptation biochemistry the substrates of these enzymes are the ligand-<br>activated receptor for the desensitizing enzyme and the desensitized but ligand-depleted receptor fo Desensitized receptors are shown in grey. If such a growth cone is migrating within a concentration gradient of a guidance cue,<br>which is represented by the density of ligand symbols and grey gradient shading, the spatial d Desensitized receptors are shown in grey. If such a growth cone is migrating within a concentration gradient of a guidance cue,<br>which is represented by the density of ligand symbols and grey gradient shading, the spatial d which is represented by the density of ligand symbols and grey gradient shading, the spatial differential sensor will switch off the desensitization apparatus. This allows the receptors which were in a sensitized state at proportional sensors for the input signal, thereby providing the machinery for concentration increment reading. The growth<br>cone will stop when the input signal encoding the concentration increment reaches the constant setsymbols are confined to the leading edge of the growth cone only for the sake of clarity.

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> symbols are conined to the leading edge of the growth cone only if<br>the ligand and the receptor on the growth cone, e.g. on the<br>product of [R][L\_] where L\_ is the ligand concentration of the ligand and the receptor on the growth cone, e.g. on the product of  $[R][L_{\rm R}]$ , where  $L_{\rm R}$  is the ligand concentration of the retinal growth cone. The retinal signal  $[R][L_{\rm R}]$  would the ligand and the receptor on the growth cone, e.g. on the<br>product of  $[R][L_R]$ , where  $L_R$  is the ligand concentration of<br>the retinal growth cone. The retinal signal  $[R][L_R]$  would<br>therefore be due to receptor-ligand *c*is product of  $[R][L_R]$ , where  $L_R$  is the ligand concentration of<br>the retinal growth cone. The retinal signal  $[R][L_R]$  would<br>therefore be due to receptor-ligand *cis*-interaction and the<br>tectal signal  $[RIL_R]$  to receptor-ligan the retinal growth cone. The retinal signal  $[R][L_R]$  would<br>therefore be due to receptor-ligand *tis*-interaction and the<br>tectal signal  $[R][L_T]$  to receptor-ligand *trans*-interaction.<br>It should be noted that in the set-poin therefore be due to receptor-ligand *cis*-interaction and the tectal signal  $[R][L_{\Gamma}]$  to receptor-ligand *trans*-interaction.<br>It should be noted that in the set-point variation mechanism *cis*- and *trans*-interactions le tectal signal  $[R][L_{\Gamma}]$  to receptor-ligand *trans*-interaction.<br>It should be noted that in the set-point variation<br>mechanism *cis*- and *trans*-interactions lead to two distin-<br>mushable signals (set-point and reading sign It should be noted that in the set-point variation<br>mechanism *cis*- and *trans*-interactions lead to two distinguishable signals (set-point and reading signals). The<br>difference signal D would therefore be  $D - K[R]$ D mechanism *cis*- and *trans*-interactions lead to two distinguishable signals (set-point and reading signals). The difference signal *D* would therefore be  $D = K_1[R][L_R]$  $-K_2[R][L_T]$ . In the simplest case, that  $K_1 = K_2 = 1$ , the

difference *D* is  $D = [R]([L_R] - [L_T])$ .<br>Growth cones have reached their final position when the

difference *D* is  $D = [R] ([L_R] - [L_T])$ .<br>Growth cones have reached their final position when the difference signal  $D = 0$ , i.e. when the input signal derived from the tectal ligand concentration matches the signal Growth cones have reached their final position when the difference signal  $D = 0$ , i.e. when the input signal derived from the tectal ligand concentration matches the signal derived from the retinal ligand concentration (f difference signal  $D = 0$ , i.e. when the input signal derived<br>from the tectal ligand concentration matches the signal<br>derived from the retinal ligand concentration (figure 7).<br>The final position would only be dependent on from the tectal ligand concentration matches the signal<br>derived from the retinal ligand concentration (figure 7).<br>The final position would only be dependent on the ligand<br>concentrations  $I_2$  and  $I_3$ . Thus in order to s derived from the retinal ligand concentration (figure 7).<br>The final position would only be dependent on the ligand<br>concentrations  $L_R$  and  $L_T$ . Thus in order to stop at different<br>termination zones growth cones must have The final position would only be dependent on the ligand<br>concentrations  $L_R$  and  $L_T$ . Thus in order to stop at different<br>termination zones growth cones must have their own<br>specific ligand concentration  $L$ , determining t concentrations  $L_R$  and  $L_T$ . Thus in order to stop at different termination zones growth cones must have their own specific ligand concentration  $L_R$  determining the set-point.

The final position  $(D = 0)$  would not be changed as long as<br>the difference between the ligand concentration in the The final position  $(D = 0)$  would not be changed as long as<br>the difference between the ligand concentration in the<br>growth cone  $(L_2)$  and in the tectum  $(L_2)$  stays constant The final position  $(D = 0)$  would not be changed as long as<br>the difference between the ligand concentration in the<br>growth cone  $(L_R)$  and in the tectum  $(L_T)$  stays constant.<br>The function of the recentor in this model is not the difference between the ligand concentration in the growth cone  $(L_R)$  and in the tectum  $(L_T)$  stays constant.<br>The function of the receptor in this model is not to define a growth cone  $(L_R)$  and in the tectum  $(L_T)$  stays constant.<br>The function of the receptor in this model is not to define a<br>target position as in the servomechanism model but just to<br>detect the signal. The complementary expre The function of the receptor in this model is not to define a<br>target position as in the servomechanism model but just to<br>detect the signal. The complementary expression of the<br>tectal ligands and of the retinal receptors wo target position as in the servomechanism model but just to<br>detect the signal. The complementary expression of the<br>tectal ligands and of the retinal receptors would have the<br>function to adjust the signal strength of the inp detect the signal. The complementary expression of the tectal ligands and of the retinal receptors would have the function to adjust the signal strength of the input signal, i.e. low ligand concentrations are amplified by tectal ligands and of the retinal receptors would have the function to adjust the signal strength of the input signal, i.e. low ligand concentrations are amplified by high receptor concentrations and vice versa. function to adjust the signal strength of the input signal,

A strength of this model is the relative independence of the path¢nding process of absolute receptor and the A strength of this model is the relative independence of<br>the pathfinding process of absolute receptor and the<br>ligand concentrations. This corresponds to a gain of<br>robustness over the servomechanism model in which the the pathfinding process of absolute receptor and the ligand concentrations. This corresponds to a gain of robustness over the servomechanism model in which the absolute value  $R$   $I$  and  $S$  have a strong influence on the ligand concentrations. This corresponds to a gain of robustness over the servomechanism model in which the absolute value *R, L* and *S* have a strong influence on the target position robustness over the servomechanism model in which the absolute value  $R$ ,  $L$  and  $S$  have a strong influence on the target position.<br>The second messenger for the *cis*-activation of the absolute value  $R$ ,  $L$  and  $S$  have a strong influence on the

target position.<br>The second messenger for the *cis*-activation of the<br>receptor might for example be cAMP or cGMP. An<br>increase in the intracellular cAMP level would shift the The second messenger for the *cis*-activation of the receptor might for example be cAMP or cGMP. An increase in the intracellular cAMP level would shift the

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set-point signal (*cis*-signal) and therefore the final position<br>of a growth cone towards a region with higher ligand set-point signal (*cis*-signal) and therefore the final position<br>of a growth cone towards a region with higher ligand<br>concentration in the tectum i.e. the attractive region in set-point signal (*cis*-signal) and therefore the final position<br>of a growth cone towards a region with higher ligand<br>concentration in the tectum, i.e. the attractive region in<br>figure 6 would be enlarged. Decreasing the cA % of a growth cone towards a region with higher ligand<br>concentration in the tectum, i.e. the attractive region in<br>figure 6 would be enlarged. Decreasing the cAMP level<br>would shift the final position towards lower concentra concentration in the tectum, i.e. the attractive region in<br>figure 6 would be enlarged. Decreasing the cAMP level<br>would shift the final position towards lower concentration<br>within the tectum i.e. the repulsive region in fig figure 6 would be enlarged. Decreasing the cAMP level<br>would shift the final position towards lower concentration<br>within the tectum, i.e. the repulsive region in figure 6<br>would be enlarged This fits well with results of Poo would shift the final position towards lower concentration<br>within the tectum, i.e. the repulsive region in figure 6<br>would be enlarged. This fits well with results of Poo and<br>co-workers (Song et al. 1997: Ming et al. 1997) within the tectum, i.e. the repulsive region in figure 6 would be enlarged. This fits well with results of Poo and co-workers (Song *et al.* 1997; Ming *et al.* 1997) who demon-strated that an increase in cAMP or cGMP lead would be enlarged. This fits well with results of Poo and<br>co-workers (Song *et al.* 1997; Ming *et al.* 1997) who demon-<br>strated that an increase in cAMP or cGMP leads to an<br>attractive response and a decrease to a repulsiv co-workers (Song *et al.* 1997; Ming *et al.* 1997) who demonstrated that an increase in cAMP or cGMP leads to an attractive response and a decrease to a repulsive response of growth cones to guidance molecules strated that an increase in cAMP or cC<br>attractive response and a decrease to a re<br>of growth cones to guidance molecules.<br>A weakness of the set-point variati A weakness of the set-point variation model is its

of growth cones to guidance molecules.<br>A weakness of the set-point variation model is its<br>inability to deal with adaptation phenomena observed in<br>some other experimental situations A weakness of the set-point variability to deal with adaptation phe<br>some other experimental situations.

## **(c)** In vitro *experimental situations.*<br> **(c)** In vitro *experiments indicate the existence*<br>
of adaptation processes in the growth cone *In vitro experiments indicate the existence of adaptation processes in the growth cone***<br>***s* **known from the strine assay that temporal axe** (c) In vitro experiments indicate the existence<br>of adaptation processes in the growth cone<br>It is known from the stripe assay that temporal axons<br>ow well even on high concentrations of posterior

of *adaptation processes in the growth cone*<br>It is known from the stripe assay that temporal axons<br>grow well even on high concentrations of posterior<br>membranes if they do not have a choice between different It is known from the stripe assay that temporal axons<br>grow well even on high concentrations of posterior<br>membranes if they do not have a choice between different<br>substrates. Albeit seeming like adaptation this behaviour grow well even on high concentrations of posterior<br>membranes if they do not have a choice between different<br>substrates. Albeit seeming like adaptation this behaviour<br>could still be explained without the assumption of recep membranes if they do not have a choice between different<br>substrates. Albeit seeming like adaptation this behaviour<br>could still be explained without the assumption of receptor<br>desensitization. As described above, the servom substrates. Albeit seeming like adaptation this behaviour ligand-activated receptor and that of the resensitizing<br>could still be explained without the assumption of receptor enzyme would be the desensitized but ligand-depl could still be explained without the assumption of receptor<br>desensitization. As described above, the servomechanism<br>explains growth on homogeneous substrates if there is an<br>inherent tendency of growth cones to grow forward desensitization. As described above, the servomecha<br>explains growth on homogeneous substrates if there<br>inherent tendency of growth cones to grow forward.<br>However, there is some evidence for recentor ad plains growth on homogeneous substrates if there is an herent tendency of growth cones to grow forward.<br>However, there is some evidence for receptor adapta-<br>in the gradient assay (Rosentreter *et al* 1998)

inherent tendency of growth cones to grow forward.<br>However, there is some evidence for receptor adaptation in the gradient assay (Rosentreter *et al.* 1998). However, there is some evidence for receptor adaptation in the gradient assay (Rosentreter *et al.* 1998). Temporal axons stop at a certain ligand concentration regardless of the steenness of the gradient. If the explant tion in the gradient assay (Rosentreter *et al.* 1998).<br>Temporal axons stop at a certain ligand concentration<br>regardless of the steepness of the gradient. If the explant<br>is placed on a constant plateau of the guidance cue Temporal axons stop at a certain ligand concentration<br>regardless of the steepness of the gradient. If the explant<br>is placed on a constant plateau of the guidance cue, which<br>is continuous with the gradient i.e. if the whole regardless of the steepness of the gradient. If the explant<br>is placed on a constant plateau of the guidance cue, which<br>is continuous with the gradient, i.e. if the whole experi-<br>ment is carried out at an increased absolute is placed on a constant plateau of the guidance cue, which<br>is continuous with the gradient, i.e. if the whole experi-<br>ment is carried out at an increased absolute concentration<br>of the guidance cue, then the axons stop at a of the guidance cue, then the axons stop at a correspondment is carried out at an increased absolute concentration<br>of the guidance cue, then the axons stop at a correspond-<br>ingly higher absolute, but at the same relative position<br>within the gradient. It therefore seems as if th of the guidance cue, then the axons stop at a correspondingly higher absolute, but at the same relative position within the gradient. It therefore seems as if they would try to reach a certain concentration increment. This ingly higher absolute, but at the same relative position<br>within the gradient. It therefore seems as if they would<br>try to reach a certain concentration increment. This<br>observation suggests that the guidance machinery has within the gradient. It therefore seems as if they would<br>try to reach a certain concentration increment. This<br>observation suggests that the guidance machinery has<br>adapted to the basal concentration. If only the explant is try to reach a certain concentration increment. This<br>observation suggests that the guidance machinery has<br>adapted to the basal concentration. If only the explant is<br>put onto a pedestal of the guidance cue but the gradient observation suggests that the guidance machinery has<br>adapted to the basal concentration. If only the explant is<br>put onto a pedestal of the guidance cue but the gradient is<br>left unchanged the axons start their ingrowth into adapted to the basal concentration. If only the explant is<br>put onto a pedestal of the guidance cue but the gradient is<br>left unchanged, the axons start their ingrowth into the<br>gradient from a zero basal level of the guidanc put onto a pedestal of the guidance cue but the gradient is also able to provide an explanation of the *in vitro* gradient left unchanged, the axons start their ingrowth into the experiments by Rosentreter *et al.* (1998) left unchanged, the axons start their ingrowth into the gradient from a zero basal level of the guidance cue. In this case there is no difference to the standard situation, in which the explant also resides on the zero lev gradient from a zero basal level of the guidance cue. In<br>this case there is no difference to the standard situation,<br>in which the explant also resides on the zero level. This<br>result indicates that the adaptation process se this case there is no difference to the standard situation,<br>in which the explant also resides on the zero level. This<br>result indicates that the adaptation process seen in the<br>first experiment does not take place at the lev in which the explant also resides on the zero level. This result indicates that the adaptation process seen in the first experiment does not take place at the level of the cell result indicates that the adaptation process seen in the<br>first experiment does not take place at the level of the cell<br>soma but most likely in the growth cone itself. Adaptation<br>within the growth cone is not included in th first experiment does not take place at the level of the cell<br>soma but most likely in the growth cone itself. Adaptation<br>within the growth cone is not included in the set-point<br>variation mechanism as described above soma but most likely in the growth cone its<br>within the growth cone is not included it<br>variation mechanism as described above.

## **(d)** *Incorporating the imprint-matching concept via gradient-sensitive adaptation* A is a proporating the imprint-matching concept via<br>
a gradient-sensitive adaptation<br>
Adaptation of the receptors has the inherent dis-<br>
vantage of turning the receptors from proportional

**gradient-sensitive adaptation**<br>Adaptation of the receptors has the inherent dis-<br>advantage of turning the receptors from proportional<br>sensors into differential sensors. Within a gradient the Adaptation of the receptors has the inherent dis-<br>advantage of turning the receptors from proportional<br>sensors into differential sensors. Within a gradient the<br>growth cone would thus lose its ability to detect a concenadvantage of turning the receptors from proportional<br>sensors into differential sensors. Within a gradient the<br>growth cone would thus lose its ability to detect a concen-<br>tration increment, which is needed to locate the fin sensors into differential sensors. Within a gradient the growth cone would thus lose its ability to detect a concentration increment, which is needed to locate the final target. The key feature of our assumption is therefo growth cone would thus lose its ability to detect a concentration increment, which is needed to locate the final target. The key feature of our assumption is therefore that the adaptation mechanism has to be switched off tration increment, which is needed to locate the final<br>target. The key feature of our assumption is therefore<br>that the adaptation mechanism has to be switched off<br>within the gradient Therefore two elements are needed target. The key feature of our assumption is therefore and they would stay so, as their adaptation mechanism is<br>that the adaptation mechanism has to be switched off silenced within a gradient. They would therefore be<br>withi that the adaptation mechanism has to be switched off<br>within the gradient. Therefore two elements are needed<br>to implement gradient-sensitive adaptation (figure 8): a<br>downstream differential sensor for spatial inhomogeneity within the gradient. Therefore two elements are needed<br>to implement gradient-sensitive adaptation (figure 8): a<br>downstream differential sensor for spatial inhomogeneity<br>of the intracellular guidance signal and an adaptatio to implement gradient-sensitive adaptation (figure 8): a<br>downstream differential sensor for spatial inhomogeneity<br>of the intracellular guidance signal and an adaptation<br>machinery acting on the guidance receptors which is downstream differential sensor for spatial inhomogeneity<br>of the intracellular guidance signal and an adaptation<br>machinery acting on the guidance receptors which is

inhibited by the output of that sensor. A differential sensor for spatial inhomogeneity is already a vital part of the servomechanism model. It is the apparatus detersensor for spatial inhomogeneity is already a vital part of<br>the servomechanism model. It is the apparatus deter-<br>mining the difference between the input strength at the<br>current and a probed position for biasing the probabi the servomechanism model. It is the apparatus deter-<br>mining the difference between the input strength at the<br>current and a probed position for biasing the probability<br>of an orientation change. Needless to say that when inc mining the difference between the input strength at the current and a probed position for biasing the probability of an orientation change. Needless to say that when incor-<br>porating adaptation into that model the time cons current and a probed position for biasing the probability<br>of an orientation change. Needless to say that when incor-<br>porating adaptation into that model the time constants<br>have to be adjusted in such a way that no adaptati of an orientation change. Needless to say that when incor-<br>porating adaptation into that model the time constants<br>have to be adjusted in such a way that no adaptation can<br>take place in the interval between the measurements porating adaptation into that model the time constants<br>have to be adjusted in such a way that no adaptation can<br>take place in the interval between the measurements at<br>the current and the probed position. Otherwise the have to be adjusted in such a way that no adaptation can<br>take place in the interval between the measurements at<br>the current and the probed position. Otherwise the<br>spatial differential sensor would not work any more take place in the interval between the measurements at<br>the current and the probed position. Otherwise the<br>spatial differential sensor would not work any more.<br>Another option for the required differential sensor for spatial differential sensor would not work any more.<br>Another option for the required differential sensor for spatial differential sensor would not work any more.<br>Another option for the required differential sensor for<br>spatial inhomogeneity would be the above-mentioned<br>autocatalytic reaction (8.1) that might amplify the small Another option for the required differential sensor for<br>spatial inhomogeneity would be the above-mentioned<br>autocatalytic reaction  $(\S 1)$  that might amplify the small<br>external concentration differences of the gradient in spatial inhomogeneity would be the above-mentioned<br>autocatalytic reaction  $(\S 1)$  that might amplify the small<br>external concentration differences of the gradient in the<br>vicinity of a growth cone into a large internal sign autocatalytic reaction  $(\S 1)$  that might amplify the small<br>external concentration differences of the gradient in the<br>vicinity of a growth cone into a large internal signal. The<br>adaptation mechanism might consist of a pai external concentration differences of the gradient in the<br>vicinity of a growth cone into a large internal signal. The<br>adaptation mechanism might consist of a pair of a desen-<br>sitizing and a resensitizing enzyme comparable vicinity of a growth cone into a large internal signal. The<br>adaptation mechanism might consist of a pair of a desen-<br>sitizing and a resensitizing enzyme, comparable to CheR<br>and CheB in the bacterial chemotaxis, apparatus. adaptation mechanism might consist of a pair of a desensitizing and a resensitizing enzyme, comparable to CheR<br>and CheB in the bacterial chemotaxis apparatus. The<br>cognate substrate of the desensitizing enzyme is the sitizing and a resensitizing enzyme, comparable to CheR<br>and CheB in the bacterial chemotaxis apparatus. The<br>cognate substrate of the desensitizing enzyme is the and CheB in the bacterial chemotaxis apparatus. The<br>cognate substrate of the desensitizing enzyme is the<br>ligand-activated receptor and that of the resensitizing<br>enzyme would be the desensitized but ligand-depleted cognate substrate of the desensitizing enzyme is the ligand-activated receptor and that of the resensitizing enzyme would be the desensitized but ligand-depleted receptor receptor. zyme would be the desensitized but ligand-depleted<br>ceptor.<br>To prevent ligand-expressing axons from seeing each<br>her again a *ci*s- and a *trans*-receptor-ligand interaction

receptor.<br>To prevent ligand-expressing axons from seeing each<br>other, again a *cis*- and a *trans*-receptor-ligand interaction<br>have to be distinguished. In contrast to the set-point To prevent ligand-expressing axons from seeing each other, again a *cis*- and a *trans*-receptor-ligand interaction have to be distinguished. In contrast to the set-point variation model however in this case both are sugg other, again a *cis*- and a *trans*-receptor-ligand interaction<br>have to be distinguished. In contrast to the set-point<br>variation model, however, in this case both are suggested to lead to the same intracellular signal.

With these assumptions the gradient-sensitive adaptation mechanism, like the set-point variation model, is With these assumptions the gradient-sensitive adapta-<br>tion mechanism, like the set-point variation model, is<br>suited to explain the results of the above-mentioned<br> $(\delta 5(a))$  retinal ligand-overgy pression and removal experition mechanism, like the set-point variation model, is<br>suited to explain the results of the above-mentioned<br>(§5(a)) retinal ligand-overexpression and -removal experi-<br>ments by Hornberger *et al.* (1999) by desensitization suited to explain the results of the above-mentioned  $(\S 5(a))$  retinal ligand-overexpression and-removal experiments by Hornberger *et al.* (1999) by desensitization-resensitization of the receptors interacting with the *c*  $(\S 5(a))$  retinal ligand-overexpression and -removal experiments by Hornberger *et al.* (1999) by desensitization-resensitization of the receptors interacting with the *cis*ments by Hornberger *et al.* (1999) by desensitization–<br>resensitization of the receptors interacting with the *cis*-<br>ligand. Imprint matching in this model is due to the fact<br>that the *cis*-ligands induce a desensitizatio resensitization of the receptors interacting with the *cis*-ligand. Imprint matching in this model is due to the fact that the *cis*-ligands induce a desensitization of their bound receptors thereby determining the concent that the  $cis$ -ligands induce a desensitization of their bound receptors, thereby determining the concentration that the *cis*-ligands induce a desensitization of their<br>bound receptors, thereby determining the concentration<br>of remaining sensitive receptors. In addition the model is<br>also able to provide an explanation of the *in vit* bound receptors, thereby determining the concentration<br>of remaining sensitive receptors. In addition the model is<br>also able to provide an explanation of the *in vitro* gradient<br>experiments by Rosentreter *et al.* (1998) de of remaining sensitive receptors. In addition the model is<br>also able to provide an explanation of the *in vitro* gradient<br>experiments by Rosentreter *et al.* (1998) described in<br> $8.5(c)$  As long as the growth cone migrates also able to provide an explanation of the  $\dot{m}$  vitro gradient neous substrate the differential sensor for the spatial inho- $\S$  5(c). As long as the growth cone migrates on a homogeneous substrate the differential sensor for the spatial inhomogeneity of the guidance signal would be silent, thereby permitting desensitization of the signalling r neous substrate the differential sensor for the spatial inhomogeneity of the guidance signal would be silent, thereby<br>permitting desensitization of the signalling receptors. As<br>soon as the growth cone reaches the gradient mogeneity of the guidance signal would be silent, thereby<br>permitting desensitization of the signalling receptors. As<br>soon as the growth cone reaches the gradient the sensor<br>would become active thereby preventing any furthe permitting desensitization of the signalling receptors. As<br>soon as the growth cone reaches the gradient the sensor<br>would become active, thereby preventing any further<br>adaptation. Finally, the gradient-sensitive adaptation soon as the growth cone reaches the gradient the sensor<br>would become active, thereby preventing any further<br>adaptation. Finally the gradient-sensitive adaptation<br>mechanism also provides an explanation for the preliwould become active, thereby preventing any further<br>adaptation. Finally the gradient-sensitive adaptation<br>mechanism also provides an explanation for the preli-<br>minary result that axons growing down the gradient in adaptation. Finally the gradient-sensitive adaptation<br>mechanism also provides an explanation for the preli-<br>minary result that axons growing down the gradient *in*<br>*nitro* seemingly do not stop at all. As the differential mechanism also provides an explanation for the preli-<br>minary result that axons growing down the gradient *in*<br>*vitro* seemingly do not stop at all. As the differential<br>sensor for the spatial inhomogeneity of the input sign minary result that axons growing down the gradient *in vitro* seemingly do not stop at all. As the differential sensor for the spatial inhomogeneity of the input signal is suggested to reside in the growth cone and not *vitro* seemingly do not stop at all. As the differential sensor for the spatial inhomogeneity of the input signal is suggested to reside in the growth cone and not in the cell body, cells of an explant placed onto the gra sensor for the spatial inhomogeneity of the input signal is suggested to reside in the growth cone and not in the cell<br>body, cells of an explant placed onto the gradient itself<br>would not be prevented from adapting to the local<br>concentration of the guidance cue. Growth cones emerbody, cells of an explant placed onto the gradient itself<br>would not be prevented from adapting to the local<br>concentration of the guidance cue. Growth cones emer-<br>ging from the explant would therefore be pre-adapted would not be prevented from adapting to the local<br>concentration of the guidance cue. Growth cones emer-<br>ging from the explant would therefore be pre-adapted<br>and they would stay so as their adaptation mechanism is concentration of the guidance cue. Growth cones emerging from the explant would therefore be pre-adapted<br>and they would stay so, as their adaptation mechanism is<br>silenced within a gradient. They would therefore be ging from the explant would therefore be pre-adapted<br>and they would stay so, as their adaptation mechanism is<br>silenced within a gradient. They would therefore be<br>insensitive for decreasing concentrations of the guidance and they would stay so, as their adaptation mechanism is cue, which are met during downhill growth.

It should be mentioned that gradient-sensitive adaptation instead of being incorporated into the servomechanism model might also be added to the set-point

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variation model. In this case the adaptation machinery has<br>to be strictly specific for the *trans*-signal and has to leave the to be strictly specific for the *trans*-signal and has to leave the *cis*-signal unaltered. Although there is no logical need to variation model. In this case the adaptation machinery has<br>to be strictly specific for the *trans*-signal and has to leave the<br>*cis*-signal unaltered. Although there is no logical need to<br>fuse both models, the advantage wo to be strictly specific for the *trans*-signal and has to leave the *cis*-signal unaltered. Although there is no logical need to fuse both models, the advantage would be a gain of robust-ness introduced by the set-point v fuse both models, the advantage would be a gain of robustness introduced by the set-point variation mechanism. Exercise both models, the advantage would be a gain of robust-<br>ss introduced by the set-point variation mechanism.<br>There is one important weakness of the gradient-<br>nitive adaptation mechanism. In contrast to the set-

ness introduced by the set-point variation mechanism.<br>There is one important weakness of the gradient-<br>sensitive adaptation mechanism. In contrast to the set-<br>point variation model it fails to provide an explanation There is one important weakness of the gradient-<br>sensitive adaptation mechanism. In contrast to the set-<br>point variation model it fails to provide an explanation<br>for the experiments by Von Boxberg *et al.* (1993) Adaptasensitive adaptation mechanism. In contrast to the set-<br>point variation model it fails to provide an explanation<br>for the experiments by Von Boxberg *et al.* (1993). Adapta-<br>tion would interfere with the preference of nasal point variation model it fails to provide an explanation<br>for the experiments by Von Boxberg *et al.* (1993). Adapta-<br>tion would interfere with the preference of nasal axons for<br>posterior membranes observed in the special s for the experiments by Von Boxberg *et al.* (1993). Adaptation would interfere with the preference of nasal axons for posterior membranes observed in the special stripe assay described in  $\delta 4$ (b). The alternating lanes posterior membranes observed in the special stripe assay described in  $\S 4(b)$ . The alternating lanes in the stripe posterior membranes observed in the special stripe assay<br>described in  $\S 4(b)$ . The alternating lanes in the stripe<br>assay consist of homogeneously distributed membrane<br>vesicles with low (anterior stripes) and high (posteri described in  $\S 4(b)$ . The alternating lanes in the stripe<br>assay consist of homogeneously distributed membrane<br>vesicles with low (anterior stripes) and high (posterior<br>stripes) ligand concentrations. According to the model assay consist of homogeneously distributed membrane<br>vesicles with low (anterior stripes) and high (posterior<br>stripes) ligand concentrations. According to the model,<br>growth on homogeneous substrates permits the adaptavesicles with low (anterior stripes) and high (posterior<br>stripes) ligand concentrations. According to the model,<br>growth on homogeneous substrates permits the adapta-<br>tion mechanism to act. Because nasal growth cones which stripes) ligand concentrations. According to the model,<br>growth on homogeneous substrates permits the adapta-<br>tion mechanism to act. Because nasal growth cones which<br>grow on posterior stripes adapt to high ligand concentragrowth on homogeneous substrates permits the adaptation mechanism to act. Because nasal growth cones which<br>grow on posterior stripes adapt to high ligand concentra-<br>tions they cannot recognize the lower concentration of grow on posterior stripes adapt to high ligand concentrations they cannot recognize the lower concentration of grow on posterior stripes adapt to high ligand concentrations they cannot recognize the lower concentration of<br>the ligands in anterior stripes. This makes the borderline<br>invisible for nasal axons and therefore they would n

tions they cannot recognize the lower concentration of<br>the ligands in anterior stripes. This makes the borderline<br>invisible for nasal axons and therefore they would not be<br>tranned in posterior lanes, as they are in Von Box the ligands in anterior stripes. This makes the borderline<br>invisible for nasal axons and therefore they would not be<br>trapped in posterior lanes, as they are in Von Boxberg's<br>experiments. This argumentation would not be val invisible for nasal axons and therefore they would not be trapped in posterior lanes, as they are in Von Boxberg's experiments. This argumentation would not be valid, if trapped in posterior lanes, as they are in Von Boxberg's<br>experiments. This argumentation would not be valid, if<br>the inhibition of adaptation following a signal from the<br>spatial differential sensor is prolonged or if the on experiments. This argumentation would not be valid, if<br>the inhibition of adaptation following a signal from the<br>spatial differential sensor is prolonged or if the onset of<br>adaptation is delayed. For that purpose the time c the inhibition of adaptation following a signal from the spatial differential sensor is prolonged or if the onset of adaptation is delayed. For that purpose the time constants of these processes must be within the range of spatial differential sensor is prolonged or if the onset of adaptation is delayed. For that purpose the time constants of these processes must be within the range of the time adaptation is delayed. For that purpose the time constants growth. The model not only implements the directionality<br>of these processes must be within the range of the time of axon guidance. By virtue of its comparative nat of these processes must be within the range of the time<br>between two border contacts of the growth cone. Both<br>assumptions lead to no or only partial adaptation of the<br>recentors which might prevent growth cones from between two border contacts of the growth cone. Both<br>assumptions lead to no or only partial adaptation of the<br>receptors which might prevent growth cones from guarantee borders of the lanes assumptions lead to no or only pa<br>receptors which might prevent<br>crossing the borders of the lanes. **(e)** *The presented models can be distinguished*

## *experimentally*

The major feature of the set-point variation model is **Experimentally**<br>
The major feature of the set-point variation model is<br>
its independence of the receptor concentration. The<br>
model therefore predicts that receptor overexpression The major feature of the set-point variation model is<br>its independence of the receptor concentration. The<br>model therefore predicts that receptor overexpression<br>should show no effect as long as the receptor can read its independence of the receptor concentration. The<br>model therefore predicts that receptor overexpression<br>should show no effect as long as the receptor can read<br>hoth the *trans*- and the *cis*-signal A conclusion of the model therefore predicts that receptor overexpression<br>should show no effect as long as the receptor can read<br>both the *trans*- and the *cis*-signal. A conclusion of the<br>gradient-sensitive adaptation mechanism is that a pla should show no effect as long as the receptor can read<br>both the *trans*- and the *cis*-signal. A conclusion of the<br>gradient-sensitive adaptation mechanism is that a plateau<br>of sufficient length within a gradient would lead both the *trans*- and the *cis*-signal. A conclusion of the gradient-sensitive adaptation mechanism is that a plateau of sufficient length within a gradient would lead to adapgradient-sensitive adaptation mechanism is that a plateau<br>of sufficient length within a gradient would lead to adap-<br>tation and would therefore allow the axons to continue<br>growth beyond their proper target. Both of these e of sufficient length within a gradient would lead to adaptation and would therefore allow the axons to continue<br>growth beyond their proper target. Both of these experiments seem to be feasible. Both models fall short of tation and would therefore allow the axons to continue<br>growth beyond their proper target. Both of these experi-<br>ments seem to be feasible. Both models fall short of<br>explaining the complexity of the experimental evidence growth beyond their proper target. Both of these experiments seem to be feasible. Both models fall short of explaining the complexity of the experimental evidence and therefore both will be wrong in detail but the experime ments seem to be feasible. Both models fall short of<br>explaining the complexity of the experimental evidence<br>and therefore both will be wrong in detail, but the experi-<br>mental tests might provide hints as to which of them explaining the complexity of the experimental evidence<br>and therefore both will be wrong in detail, but the experimental tests might provide hints as to which of them<br>deserves further elaboration and therefore both will be wrong in detail, but the experimental tests might provide hints as to which of them deserves further elaboration.

#### **6. CONCLUSION**

In this paper we first reviewed the results of some **conceptually important** *in vivo* and *in vitro* experiments addressing the establishment of topographic projections in In this paper we first reviewed the results of some conceptually important *in vivo* and *in vitro* experiments addressing the establishment of topographic projections in the developing nervous system. Although similar co conceptually important *in vivo* and *in vitro* experiments<br>addressing the establishment of topographic projections in<br>the developing nervous system. Although similar con-<br>clusions can be drawn from other systems we focuse addressing the establishment of topographic projections in<br>the developing nervous system. Although similar con-<br>clusions can be drawn from other systems we focused on<br>the chick retinotectal projection as an experimental the developing nervous system. Although similar conclusions can be drawn from other systems we focused on the chick retinotectal projection as an experimental paradigm Whereas the concentional framework of axon clusions can be drawn from other systems we focused on<br>trem the tectal gradient. Complete balancing is achieved<br>the chick retinotectal projection as an experimental when the ligand concentration at the position of the<br>para guidance by gradients of guidance molecules has long paradigm. Whereas the conceptional framework of axon<br>guidance by gradients of guidance molecules has long<br>been established (Gierer 1987), novel experimental results<br>allow the formulation of models adopting more guidance by gradients of guidance molecules has long<br>been established (Gierer 1987), novel experimental results<br>allow the formulation of models adopting more<br>mechanistic detail been established (Gi<br>allow the formula<br>mechanistic detail. *Phil. Trans. R. Soc. Lond.* B (2000)

As a first of these evidence-based models we discussed the mass action model by Nakamoto *et al.* (1996). This model implements the observation that certain putative the mass action model by Nakamoto *et al.* (1996). This model implements the observation that certain putative guidance molecules and their receptors are expressed as complementary gradients in the respective projecting an model implements the observation that certain putative<br>guidance molecules and their receptors are expressed as<br>complementary gradients in the respective projecting and<br>target areas. In Nakamoto's description an input signa guidance molecules and their receptors are expressed as<br>complementary gradients in the respective projecting and<br>target areas. In Nakamoto's description an input signal is<br>generated in the randomly searching growth cone by complementary gradients in the respective projecting and<br>target areas. In Nakamoto's description an input signal is<br>generated in the randomly searching growth cone by the<br>interaction of receptor and ligand according to mas target areas. In Nakamoto's description an input signal is<br>generated in the randomly searching growth cone by the<br>interaction of receptor and ligand according to mass<br>action kinetics. The projection target is reached when generated in the randomly searching growth cone by the<br>interaction of receptor and ligand according to mass<br>action kinetics. The projection target is reached when the<br>input signal reaches a set-point value, which is indepe interaction of receptor and ligand according to mass<br>action kinetics. The projection target is reached when the<br>input signal reaches a set-point value, which is indepen-<br>dent of retinal position. As the receptor concentrat action kinetics. The projection target is reached when the<br>input signal reaches a set-point value, which is indepen-<br>dent of retinal position. As the receptor concentration<br>varies with retinal position axons from different dent of retinal position. As the receptor concentration varies with retinal position axons from different retinal dent of retinal position. As the receptor concentration<br>varies with retinal position axons from different retinal<br>sites of origin terminate at different tectal positions. The<br>model makes use of the positional information o varies with retinal position axons from different retinal<br>sites of origin terminate at different tectal positions. The<br>model makes use of the positional information of a<br>gradient but it fails to describe the observed direc sites of origin terminate at different tectal positions. The<br>model makes use of the positional information of a<br>gradient, but it fails to describe the observed directional<br>growth of axons This feature is taken into account model makes use of the positional information of a<br>gradient, but it fails to describe the observed directional<br>growth of axons. This feature is taken into account by a<br>more sophisticated elaboration of the mass action mode gradient, but it fails to describe the observed directional<br>growth of axons. This feature is taken into account by a<br>more sophisticated elaboration of the mass action model<br>presented by Honda (1998). In his description the growth of axons. This feature is taken into account by a<br>more sophisticated elaboration of the mass action model<br>presented by Honda (1998). In his description the growth<br>cone at every position on its way towards the target presented by Honda (1998). In his description the growth cone at every position on its way towards the target deterpresented by Honda (1998). In his description the growth<br>cone at every position on its way towards the target deter-<br>mines the difference of the input signal and a constant<br>endogenous set-point value. The result of that ca cone at every position on its way towards the target deter-<br>mines the difference of the input signal and a constant<br>endogenous set-point value. The result of that calculation<br>is compared with the result of a corresponding endogenous set-point value. The result of that calculation<br>is compared with the result of a corresponding calculation at a new position probed by the growth cone. The is compared with the result of a corresponding calculation<br>at a new position probed by the growth cone. The<br>outcome of the comparison is fed into a servomechanism<br>to bias the probability of changing the direction of at a new position probed by the growth cone. The<br>outcome of the comparison is fed into a servomechanism<br>to bias the probability of changing the direction of<br>growth The model not only implements the directionality outcome of the comparison is fed into a servomechanism<br>to bias the probability of changing the direction of<br>growth. The model not only implements the directionality<br>of axon guidance. By virtue of its comparative nature it to bias the probability of changing the direction of growth. The model not only implements the directionality<br>of axon guidance. By virtue of its comparative nature it<br>also fits experimental observations gained in Poo's labora-<br>tory (Ming *et al.* 1997: Song *et al.* 1997) t of axon guidance. By virtue of its comparative nature it<br>also fits experimental observations gained in Poo's labora-<br>tory (Ming *et al.* 1997; Song *et al.* 1997) that at least some<br>guiding molecules are not inherently att also fits experimental observations gained in Poo's laboratory (Ming *et al.* 1997; Song *et al.* 1997) that at least some guiding molecules are not inherently attractive or repul-<br>sive but that their attractive or repulsi tory (Ming *et al.* 1997; Song *et al.* 1997) that at least some guiding molecules are not inherently attractive or repulsive, but that their attractive or repulsive behaviour is a mere consequence of internal computation guiding molecules are not inherently attractive or repulsive, but that their attractive or repulsive behaviour is a mere consequence of internal computation of the growth cone. These features provide explanations for some sive, but that their attractive or repulsive behaviour is a<br>mere consequence of internal computation of the growth<br>cone. These features provide explanations for some of the<br>most puzzling results gained with retinotectal ax mere consequence of internal computation of the growth<br>cone. These features provide explanations for some of the<br>most puzzling results gained with retinotectal axons in<br>the *in vitro* stripe assay used to analyse their dec cone. These features provide explanations for some of the<br>most puzzling results gained with retinotectal axons in<br>the *in vitro* stripe assay used to analyse their decision<br>behaviour (non-decision of nasal axons and sten-l most puzzling results gained with retinotectal axons in<br>the *in vitro* stripe assay used to analyse their decision<br>behaviour (non-decision of nasal axons and step-like<br>transition between nasal and temporal behaviours) behaviour (non-decision of nasal axons and step-like transition between nasal and temporal behaviours).

These published models do not consider the most transition between nasal and temporal behaviours).<br>These published models do not consider the most<br>recent observation that the guiding ligand is expressed<br>not only as a gradient on the tectal target but also in a These published models do not consider the most<br>recent observation that the guiding ligand is expressed<br>not only as a gradient on the tectal target but also in a<br>matching gradient on the retina (Hornberger et al. 1999) recent observation that the guiding ligand is expressed<br>not only as a gradient on the tectal target but also in a<br>matching gradient on the retina (Hornberger *et al.* 1999)<br>and that a disturbance of the retinal expression not only as a gradient on the tectal target but also in a matching gradient on the retinal (Hornberger *et al.* 1999) and that a disturbance of the retinal expression affects the establishment of topography. These results matching gradient on the retina (Hornberger *et al.* 1999) and that a disturbance of the retinal expression affects the establishment of topography. These results suggest a novel concent which we call the imprint-matching and that a disturbance of the retinal expression affects the establishment of topography. These results suggest a novel concept, which we call the imprint-matching concept. It states that retinal axons might grow into the establishment of topography. These results suggest a novel<br>concept, which we call the imprint-matching concept. It<br>states that retinal axons might grow into the tectum until<br>they encounter a concentration of the guidance c concept, which we call the imprint-matching concept. It<br>states that retinal axons might grow into the tectum until<br>they encounter a concentration of the guidance cue<br>corresponding to that at their respective site of origin states that retinal axons might grow into the tectum un<br>they encounter a concentration of the guidance c<br>corresponding to that at their respective site of origin.<br>In the final part we presented two extended versions they encounter a concentration of the guidance cue<br>corresponding to that at their respective site of origin.<br>In the final part we presented two extended versions of

corresponding to that at their respective site of origin.<br>In the final part we presented two extended versions of<br>Honda's model, which implement the imprint-matching<br>concent and which we call the set-point variation and th In the final part we presented two extended versions of<br>Honda's model, which implement the imprint-matching<br>concept and which we call the set-point variation and the<br>gradient-sensitive adaptation model. In the set-point Honda's model, which implement the imprint-matching<br>concept and which we call the set-point variation and the<br>gradient-sensitive adaptation model. In the set-point<br>variation model the retinal ligand is used to calculate th concept and which we call the set-point variation and the gradient-sensitive adaptation model. In the set-point variation model the retinal ligand is used to calculate the set-point signal according to mass action kinetics thereby variation model the retinal ligand is used to calculate the<br>set-point signal according to mass action kinetics thereby<br>providing a position-specific imprint to the projecting<br>neurons. The searching growth cone attempts to set-point signal according to mass action kinetics thereby<br>providing a position-specific imprint to the projecting<br>neurons. The searching growth cone attempts to exactly<br>halance this set-point signal by the input signal de providing a position-specific imprint to the projecting<br>neurons. The searching growth cone attempts to exactly<br>balance this set-point signal by the input signal derived<br>from the tectal gradient. Complete balancing is achie neurons. The searching growth cone attempts to exactly<br>balance this set-point signal by the input signal derived<br>from the tectal gradient. Complete balancing is achieved<br>when the ligand concentration at the position of the balance this set-point signal by the input signal derived<br>from the tectal gradient. Complete balancing is achieved<br>when the ligand concentration at the position of the<br>growth cone just matches the ligand concentration at t from the tectal gradient. Complete balancing is achieved when the ligand concentration at the position of the growth cone just matches the ligand concentration at the axons site of origin. Axon guidance in the set-point varia-<br>tion model is therefore a match-to-sample task. Due growth cone just matches the ligand concentration at the<br>axons site of origin. Axon guidance in the set-point varia-<br>tion model is therefore a match-to-sample task. Due to<br>the fact that the model crucially relies on a comp axons site of origin. Axon guidance in the set-point variation model is therefore a match-to-sample task. Due to<br>the fact that the model crucially relies on a comparison of<br>concentrations of the same ligand on the projecti tion model is therefore a match-to-sample task. Due to the fact that the model crucially relies on a comparison of concentrations of the same ligand on the projecting and

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target area, which are measured by the same receptor, it<br>is independent of the absolute concentration of the ligand target area, which are measured by the same receptor, it<br>is independent of the absolute concentration of the ligand<br>and the concentration of the receptor as long as saturation target area, which are measured by the same receptor, it<br>is independent of the absolute concentration of the ligand<br>and the concentration of the receptor as long as saturation<br>is not reached. These features lend superior r is independent of the absolute concentration of the ligand<br>and the concentration of the receptor as long as saturation<br>is not reached. These features lend superior robustness to<br>the model. To enable the growth cone to dist and the concentration of the receptor as long as saturation<br>is not reached. These features lend superior robustness to<br>the model. To enable the growth cone to distinguish<br>hetween retinal set-point and tectal input signals is not reached. These features lend superior robustness to<br>the model. To enable the growth cone to distinguish<br>between retinal set-point and tectal input signals we<br>postulate two independent signal-transduction pathways the model. To enable the growth cone to distinguish<br>between retinal set-point and tectal input signals we<br>postulate two independent signal-transduction pathways<br>through the guidance receptor depending on whether the between retinal set-point and tectal input signals we<br>postulate two independent signal-transduction pathways<br>through the guidance receptor depending on whether the<br>ligand is presented *cis* (on the same cell as in the ret postulate two independent signal-transduction pathways through the guidance receptor depending on whether the ligand is presented *cis* (on the same cell, as in the retina) or *trans* (on other cells, as on the tectum).

The set-point variation model fails to explain adaptation processes in the growth cone. The gradient-sensitive The set-point variation model fails to explain adaptation processes in the growth cone. The gradient-sensitive adaptation mechanism is introduced in order to implement the *in vitro* observation that growth cones seem to tion processes in the growth cone. The gradient-sensitive adaptation mechanism is introduced in order to implement<br>the *in vitro* observation that growth cones seem to read<br>concentration increments, thereby indicating an a adaptation mechanism is introduced in order to implement<br>the *in vitro* observation that growth cones seem to read<br>concentration increments, thereby indicating an adapta-<br>tion to constant basal levels of the guidance cue B the *in vitro* observation that growth cones seem to read<br>concentration increments, thereby indicating an adapta-<br>tion to constant basal levels of the guidance cue. Besides<br>strictly exclusive *cis*- and *trans*-interaction concentration increments, thereby indicating an adaptation to constant basal levels of the guidance cue. Besides strictly exclusive *cis*- and *trans*-interactions (leading, however to the same intracellular signal) the gr tion to constant basal levels of the guidance cue. Besides<br>strictly exclusive *cis*- and *trans*-interactions (leading,<br>however, to the same intracellular signal) the gradient-<br>sensitive adaptation mechanism requires a dif strictly exclusive *cis*- and *trans*-interactions (leading, however, to the same intracellular signal) the gradient-<br>sensitive adaptation mechanism requires a differential sensor for spatial inhomogeneity of the guidance however, to the same intracellular signal) the gradient-<br>sensitive adaptation mechanism requires a differential<br>sensor for spatial inhomogeneity of the guidance signal<br>within the growth cone. The output of that sensor is sensitive adaptation mechanism requires a differential<br>sensor for spatial inhomogeneity of the guidance signal<br>within the growth cone. The output of that sensor is<br>suggested to be inhibitory for a receptor adaptation sensor for spatial inhomogeneity of the guidance signal<br>within the growth cone. The output of that sensor is<br>suggested to be inhibitory for a receptor adaptation<br>machinery. Thereby the mechanism leads to receptor within the growth cone. The output of that sensor is suggested to be inhibitory for a receptor adaptation machinery. Thereby the mechanism leads to receptor suggested to be inhibitory for a receptor adaptation<br>machinery. Thereby the mechanism leads to receptor<br>desensitization on homogeneous substrates but prevents<br>adaptation in the gradient allowing the growth cone to machinery. Thereby the mechanism leads to receptor<br>desensitization on homogeneous substrates but prevents<br>adaptation in the gradient allowing the growth cone to<br>read absolute positional information. Gradient-sensitive desensitization on homogeneous substrates but prevents<br>adaptation in the gradient allowing the growth cone to<br>read absolute positional information. Gradient-sensitive<br>adaptation can be incorporated into either Honda's mode adaptation in the gradient allowing the growth cone to<br>read absolute positional information. Gradient-sensitive<br>adaptation can be incorporated into either Honda's model<br>or the imprint-matching model, the first alternative read absolute positional information. Gradient-sensitive<br>adaptation can be incorporated into either Honda's model<br>or the imprint-matching model, the first alternative being<br>more parsimonious the second being more robust. T adaptation can be incorporated into either Honda's model<br>or the imprint-matching model, the first alternative being<br>more parsimonious, the second being more robust. The<br>two alternatives can be distinguished experimentally or the imprint-matching model, the first alternative be<br>more parsimonious, the second being more robust.<br>two alternatives can be distinguished experimentally. two alternatives can be distinguished experimentally.<br>I.L. and F.W. contributed equally to this work. The authors are

J.L. and F.W. contributed equally to this work. The authors are grateful to T. Bonhoeffer, U. Drescher, R. Friedrich, A. Gierer<br>and B. Müller for stimulating and critical discussions and help-J.L. and F.W. contributed equally to this work. The authors are grateful to T. Bonhoeffer, U. Drescher, R. Friedrich, A. Gierer and B. Müller for stimulating and critical discussions and help-ful comments on the manuscript grateful to T. Bonhoeffer, U. Dres<br>and B. Müller for stimulating and<br>ful comments on the manuscript.

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