INITIAL PATTERNING OF THE CENTRAL NERVOUS SYSTEM: HOW MANY ORGANIZERS?

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For three-quarters of a century, developmental biologists have been asking how the nervous system is specified as distinct from the rest of the ectoderm during early development, and how it becomes subdivided initially into distinct regions such as forebrain, midbrain, hindbrain and spinal cord. The two events of 'neural induction' and 'early neural patterning' seem to be intertwined, and many models have been put forward to explain how these processes work at a molecular level. Here I consider early neural patterning and discuss the evidence for and against the two most popular models proposed for its explanation: the idea that multiple signalling centres (organizers) are responsible for inducing different regions of the nervous system, and a model first articulated by Nieuwkoop that invokes two steps (activation/transformation) necessary for neural patterning. As recent evidence from several systems challenges both models, I propose a modification of Nieuwkoop's model that most easily accommodates both classical and more recent data, and end by outlining some possible directions for future research.

GASTRULA
Embryonic stage at which the embryo becomes three-layered, forming the mesoderm and definitive (gut) endoderm.

SENSORY PLACODE
A thickened region of ectoderm that will later give rise to a sensory organ.

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When Hilde Mangold and Hans Spemann performed their famous 'organizer experiment' (BOX 1) in 1924 (REFS 1,2), they were immediately struck by the observation that the second axis generated by transplanting the dorsal lip of an embryo at the GASTRULA stage to the ventral side of a host generated a complete, coherent axis extending from the tip of the nose to the tip of the tail. How can a single cell population generate this complexity? Spemann himself initially favoured a vitalistic explanation (implying a living, quasi-conscious activity rather than the action of inert physical substances emitted by the organizer); but a flurry of investigations (TIMELINE), and almost as many models, immediately followed to explain the physical basis of how this phenomenon might happen.

One of the earliest ideas was that the region of the dorsal lip is not homogeneous, but that different cell populations exist within it, each capable of inducing one part of the axis. Furthermore, as descendants of each of these populations exit the dorsal lip after gastrulation, each set of descendants retains the ability to induce specific regions of the axis (FIG. 1). Equally striking were the findings that certain heterologous inducers (including killed tissues, pieces of adult organs from the same or different species, or particular chemical substances[3–7]) can induce specific regions of the axis. How many distinct inducing tissues and substances are required to generate the full complexity of the axis? Holtfreter, Mangold and their contemporaries went as far as suggesting that even smaller parts of the organizer territory might induce individual SENSORY PLACODES and other small head structures[8–10].

Experiments done mainly by ter Horst and Sala in the late 1940s and 1950s (reviewed in REFS 9,10) confirmed the findings of Mangold and his colleagues, but relatively little light was thrown on the underlying mechanism until 1970. In this year, Eyal-Giladi and Wolk[11] pioneered the idea that forebrain-inducing signals might come not from the organizer, but from tissue that only has extra-embryonic fate — the hypoblast of

the chick. This hypothesis was not followed up at that time, but findings that apparently supported it started to sprout in quick succession some 25 years later, following two separate observations.

First, when transplanted to a host embryo, the mouse organizer — Hensen's node, often called simply 'the node' — generates an axis that lacks the forebrain[12]. Second, formation of the forebrain requires the presence of the anterior visceral endoderm (AVE), a layer of cells with extra-embryonic fate that is never part of the node[13]. Soon, several groups discovered that wild-type function of a number of genes including *Nodal*, *Otx2*, *Lim1*, *Hesx1*/*Rpx* and *Hex* is required within the AVE for normal forebrain formation[13–24]. At about the same time, amphibian experiments had uncovered genes whose early misexpression causes the formation of either an ectopic head[25–27], or more caudal parts of the axis.

The connection between these findings and the concept of a 'head organizer' was only circumstantial, but very tempting. Although most of the laboratories using mouse embryos were careful to avoid this logical leap, other groups interpreted the data as suggesting that at least two separate organizers exist, one responsible for inducing the forebrain and another for the rest of the axis[28–31]. Indeed, many researchers started to assume that the head organizer is a physically separate entity from the original, and that, at the early gastrula stage, it resides outside of the classical territory defined as the organizer by Spemann and his followers.

Amphibian embryos do not have obvious extra-embryonic tissue, and some workers sought to identify an equivalent cell population separate from the organizer at the early gastrula stage that could induce an isolated head. However, no such population could be found[32]. Furthermore, transplantation of the AVE adjacent to (presumably non-neural) ectoderm does not generate an ectopic forebrain in the mouse — this can only be obtained when the AVE is combined with both EPIBLAST and the tip of the early PRIMITIVE STREAK (early gastrula organizer; FIG. 2a)[33].

Another report indicated that the rabbit hypoblast can induce forebrain markers when grafted into a chick host, whereas the chick counterpart cannot[29], an observation coupled to the proposal that mammals had evolved a new way of specifying the forebrain[28–31]. Furthermore, a series of findings in several laboratories indicated that the PRECHORDAL MESENDODERM can specify forebrain fates, at least in cells that have already received neural-inducing signals[21,34–40]. At this point, the only possible conclusion from these findings is that the AVE (and perhaps the hypoblast — its equivalent structure

Box 1 | What is an organizer?

The organizer was defined by Spemann in 1921 as a piece of embryonic tissue that "creates an 'organization field' of a certain [axial] orientation and extent, in the indifferent material in which it is normally located or to which it is transplanted" (translated from REF. 2, p. 45). The concept embodies both induction and patterning: the grafted cells change the fate of the responding tissue, and also generate a coherent ('organized') set of structures. The key word here is 'indifferent', implying that the responding tissue must be, to some extent, naive.

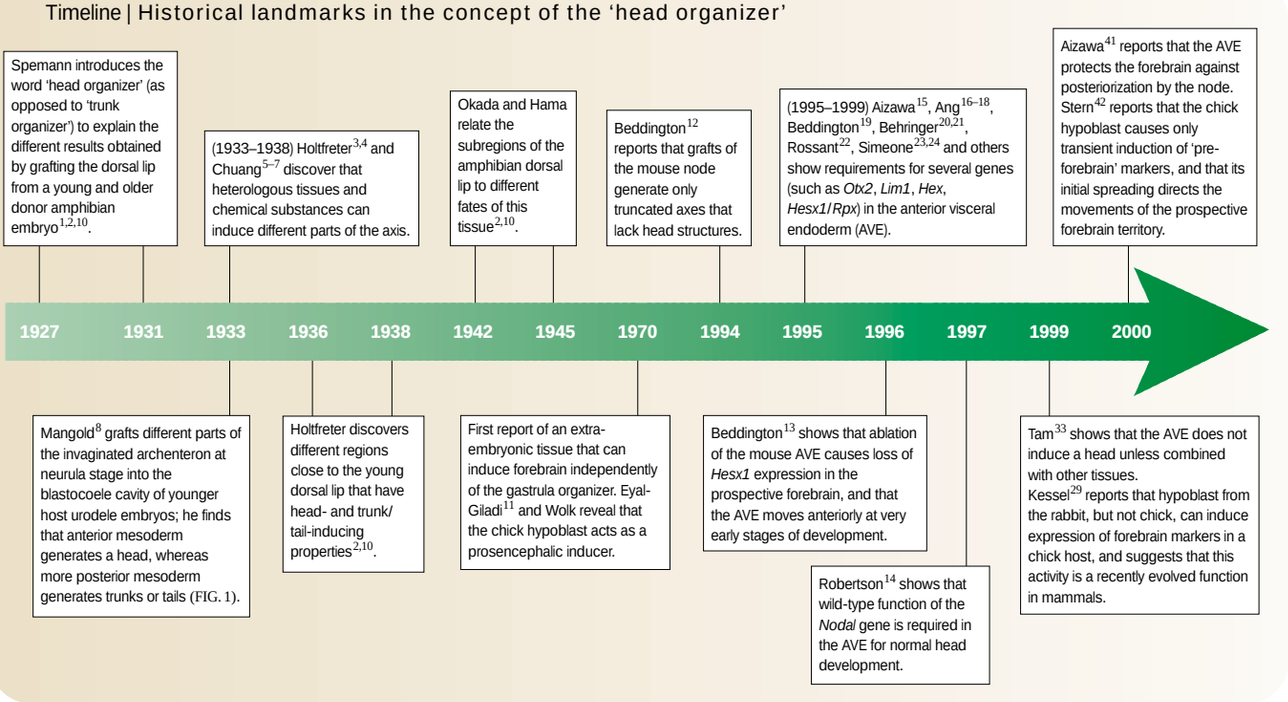
EPIBLAST
One of the layers of cells in the early embryo, which gives rise to the skin and nervous system.

PRIMITIVE STREAK
An elongated depression of reptile, bird and mammalian embryos, through which mesodermal and endodermal cells migrate into the interior of the embryo. The most anterior tip of the primitive streak forms Hensen's node. The streak is functionally homologous to the amphibian blastopore.

PRECHORDAL MESENDODERM
A tissue derived from the node, lying at the rostral tip of the head process (notochord). The mesodermal component will give rise to some eye muscles.

URODELE
Order of amphibians, including salamanders and newts, in which the larval tail persists in the adult.

Timeline | Historical landmarks in the concept of the 'head organizer'



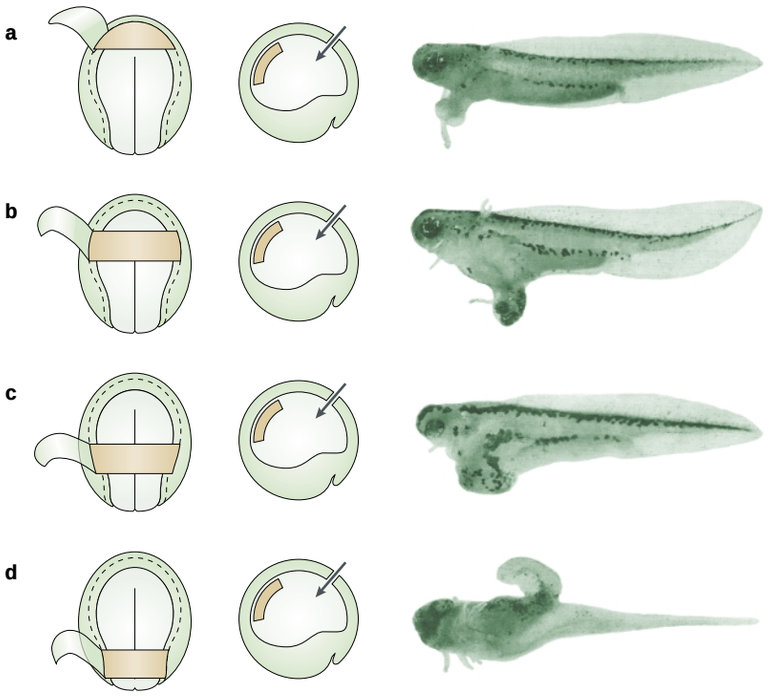


Figure 1 | **Otto Mangold's experiment**[8]**.** Different regions of the invaginated archenteron induce different parts of the axis. **a, b** | More anterior pieces of archenteron induce more rostral regions when introduced into the blastocoele of a host embryo, whereas **c, d** | grafts of more posterior archenteron induce more caudal regions of the axis.

in the chick[11]; see FIG. 2b) might indeed be required for forebrain development, but that its role is unlikely to be as simple as the direct induction of forebrain or head character.

Two recent studies have further strengthened this conclusion. Matsuo, Aizawa and their colleagues[41] showed that the mouse AVE might act protectively against 'caudalization' of the nervous system by the organizer, and that its anterior movements might regulate the movement of the overlying epiblast (including the prospective forebrain) in the same direction. In the second study[42], it was shown that the chick hypoblast does direct the movements of the overlying forebrain territory in the epiblast. In addition, whereas in naive cells the hypoblast is capable of transient induction of markers that define forebrain at a later stage (for example, *Otx2*, *Sox3*), the expression is unstable and the interaction is not sufficient to generate a recognizable forebrain or the expression of definitive PROSENCEPHALIC markers[42].

In fact, as in amphibians, the chick node is able to induce a complete axis including the forebrain even when grafted into a region that has never been in contact with hypoblast. But for this to occur the node must be obtained from an embryo at the full primitive-streak stage but before the emergence of the head process[43–47]. All of this evidence strongly argues against the existence of a true 'head organizer' that resides outside the 'classical' organizer region (the node).

PROSENCEPHALON
The most rostral of the primary vesicles present in the early neural tube, which later gives rise to two secondary vesicles: telencephalon (prospective cerebral hemispheres) and diencephalon (prospective thalamus and hypothalamus).

NEURULA
Stage of development following gastrulation, when the neural plate starts to form from the ectoderm.

Alternative models

If the concept of separate organizers for different regions of the axis is incorrect, do any of several alternative models proposed over the past 50 years provide a better view of the mechanisms by which the head is specified? One of the most widely considered alternatives was proposed by Nieuwkoop[48–50] (FIG. 3), who postulated that induction and patterning of the nervous system occurs in two steps. During a first step ('activation'), neural fate is induced and the forebrain is specified. Later, during the second step ('transformation', also called 'posteriorization' or 'caudalization'), some of these cells receive other signals from the organizer, which gradually cause them to acquire a more caudal character.

This model has received some support, but there are also some data that are difficult to explain with this proposal. One finding in favour of the model is that when prospective forebrain territories are combined with the tail regions that contain the remnants of the organizer at the NEURULA stage, the intermediate regions are produced from the rostral piece[51]. Three candidate molecules have been proposed as mediators of the transformation process: fibroblast growth factors (FGFs)[51,52], retinoic acid[53–68] and Wnt3A[69], all of which can generate posterior from anterior structures to different extents.

But this model seems particularly difficult to reconcile with the finding that grafts of older organizers in amphibians, chicks or mice generate a truncated nervous system that lacks a forebrain (for example, REFS 8,12,43–47). The concept of multiple (head and trunk/tail) organizers is formally incompatible with the activation/transformation model; however, several studies (particularly in amphibians) have been interpreted in terms of a mixture of both models by assuming, for example, that the head organizer emits activating signals, and the trunk/tail organizer emits both neuralizing and transforming signals.

As early as 1937, and on the basis of experiments designed to study the mechanisms that establish the initial polarity of the entire embryo, Dalcq and Pasteels (reviewed in REFS 9,10) proposed that different levels of two morphogens might specify positional identity. Subsequently, several other groups (notably Yamada, Okada, and a Finnish group led by Toivonen and Saxen; reviewed in REFS 9,10,70,71) extended these ideas to the patterning of the nervous system, by proposing that cells define their identity according to local levels of one or two morphogens or 'potentials'. Interestingly, they considered that cell movements might contribute to positional diversity by transiently exposing cells to different conditions, an idea that received relatively little experimental attention. Another model that was proposed for generating rostrocaudal patterning in the nervous system was the evocation–individuation model of Waddington and Needham[72]. This model argued that the initial neural-inducing signals generate neural tissue that lacks regional characteristics, which are subsequently imparted by one or more signals at a later stage. None of these models ever received overwhelming support, but it is also true and rather curious that none has been tested enough to dismiss it completely.

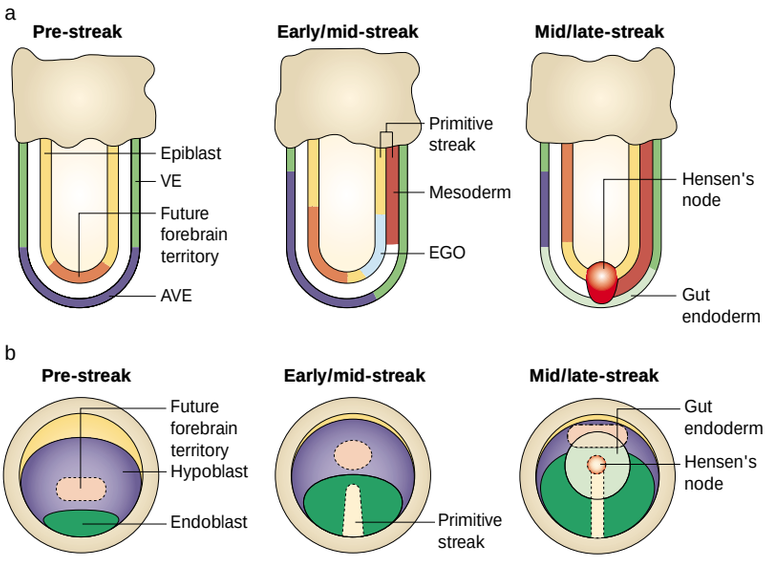


Figure 2 | **Mouse and chick structures with proposed inducing functions. a** | Diagrams of mouse embryos at pre-streak (about embryonic day (E) 5.5), early to mid-streak (about E6.0) and mid- to late-streak (E6.5–7.0) stages showing the anterior visceral endoderm (AVE), the node, the prospective forebrain and the early gastrula organizer (EGO). The embryos are shown in mid-sagittal section, anterior to the left. Gaps have been introduced between the various layers for clarity; in reality the tissues are apposed more closely. **b** | Diagrams of chick embryos at equivalent stages to the mouse embryos in **a**. The embryos are viewed from the ventral (hypoblast/yolk) side, anterior to the top. Note that the primitive streak, Hensen's node and the prospective forebrain territory should only be visible from the dorsal (opposite) side, but their positions are shown here as if the endodermal layer were transparent. (VE, visceral endoderm.)

Nieuwkoop's model modified

If, as Nieuwkoop proposed[48,49], there are two steps to neural induction, when do these steps take place? It has been widely assumed that neural induction begins during gastrulation, when the organizer can first be identified morphologically and mesoderm induction has probably ended[73–75]. Recent results indicate, however, that an early step could occur much earlier, before gastrulation begins. In both chick and mouse embryos, expression of early neural markers (for example, Sox3 and ERNI in the chick, and Sox1 in the mouse) begins before the appearance of the primitive streak in a broad domain of the epiblast[76]. In the chick, the expression of these markers coincides with the spread of the hypoblast layer, indicating that the hypoblast might be responsible for inducing their expression.

Indeed, this conclusion is supported by the finding that the hypoblast transiently induces the expression of Sox3 and Otx2 (REF. 42). At later stages of development, Otx2 is a marker for the forebrain and anterior midbrain[54]. Before primitive-streak formation, however, it is expressed very broadly in the epiblast, throughout the prospective neural plate in both chick and mouse embryos. These observations led my laboratory to propose[42,76] that the broad, early co-expression of markers, which later in development identify all neural tissue (Sox3), as well as forebrain and midbrain (Otx2), marks cells that have been 'activated', as in Nieuwkoop's model. However, this activation is not sufficient for the cells to acquire a forebrain or even a neural phenotype because, when the inducing tissue (hypoblast) is allowed to remain in contact for a longer period, expression of both markers is lost and no neural or forebrain structures develop[42].

What are the early activating signals? Similar to grafts of the hypoblast or of organizer-precursor cells, FGF-8 can induce Sox3, ERNI and Otx2 expression. In addition, loss-of-function experiments indicate that FGF signalling is necessary for this induction[76,77]. It is therefore conceivable that FGF signalling represents both the activation signal and a transforming step; however, FGF signalling is not sufficient for either activation or for neural induction, because when either FGF, precursors of the organizer, or hypoblast grafts are allowed to stay in place they do not elicit expression of later markers, even when combined with BONE MORPHOGENETIC PROTEIN (BMP) antagonists[76,78]. So it is likely that additional, maintenance signals are also required.

Moreover, FGF is probably not the only transforming signal because injection of a DOMINANT-NEGATIVE FGF receptor in *Xenopus* does not cause the complete absence of a posterior nervous system[79,80]. We therefore proposed[42,76] that the activation step of Nieuwkoop's model generates a transient, 'pre-forebrain'/'pre-neural' state, on which maintenance signals must act to allow cells to acquire both neural and prosencephalic fate. A subset of the cells that were initially activated will later receive caudalizing signals, which also stabilize their neural state but generate more posterior parts of the central nervous system (FIG. 4).

In addition to providing an early activation similar to that proposed by Nieuwkoop, the chick hypoblast and the mouse AVE seem to have a separate role: directing the movement of at least some of the pre-forebrain cells anteriorly, away from the influence of the node and its transforming/caudalizing influence[41,42]. In fact, this property might not be exclusive to the AVE/hypoblast, but may also be shared by the remaining visceral endoderm/endoblast[23,42]. This mechanism would explain how the forebrain is ever induced, as in both chick and mouse embryos it never lies close to the node[42]. But what, then, provides the maintenance signals? One possibility is that the anterior head process[81] and/or the prechordal mesendoderm[21,34–40] provide this maintenance function, as well as some degree of further protection against caudalizing signals, because grafts of prechordal mesendoderm placed adjacent to prospective hindbrain can elicit expression of forebrain markers[34–40].

An important, additional observation is that grafts of Hensen's node into regions of a host chick embryo (for example, the peripheral AREA OPACA) that have never been exposed to the hypoblast do induce a complete nervous system[46,47]. This indicates that Hensen's node emits signals capable of initiating the neural induction and neural patterning cascades, which can substitute for those normally emitted by the hypoblast.

So, does this modification of Nieuwkoop's model account for the early findings that led to the concept of separate organizers for the head and for more caudal parts of the axis? To answer this question, it is important first to separate clearly the concepts of neural induction

BONE MORPHOGENETIC PROTEINS
Secreted proteins of the transforming growth factor-β superfamily. In the early embryo, they participate in dorsoventral patterning.

DOMINANT-NEGATIVE PROTEIN
A mutant molecule that forms heteromeric complexes with the wild type to yield a non-functional complex.

AREA OPACA
A peripheral ring of the early avian blastoderm, which only gives rise to extra-embryonic tissues.

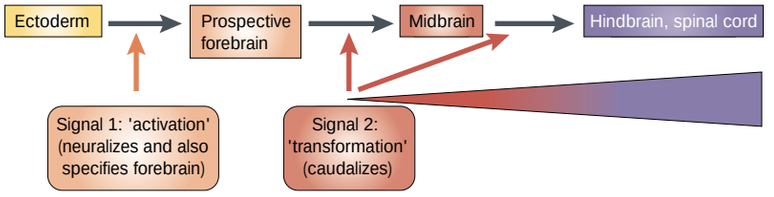


Figure 3 | **Nieuwkoop's activation–transformation model[48].** Induction of the nervous system is proposed to occur in two steps: initial induction of forebrain, and subsequent caudalization of part of it. According to this model, the organizer emits both the activating and the transforming signals. The gradient represents either increasing strength of the transforming signal, or increasing time of exposure to this signal, which results in portions of the nervous system becoming progressively more caudal.

(meaning the conversion of cells from a non-neural to a neural fate) from those of patterning (the acquisition of regional traits and markers). In the chick, one manipulation that reveals this distinction is a comparison between grafts into the peripheral (area opaca) region, which is only fated to give rise to extra-embryonic tissues, and into the more central epiblast, adjacent to or within the neural plate of the host. When the most anterior axial (prechordal) mesendoderm is grafted into the peripheral region, it does not induce neural tissue[37]. In contrast, when it is grafted into either the prospective neural plate or very close to it, it generates ectopic neural structures that are partially fused with the host axis and express prosencephalic markers[37,38]. The rest of the axial mesoderm can, however, induce neural tissue even in the area opaca, although this ability appears to decrease sharply in a rostral-to-caudal direction[81].

With these results in mind, I propose that some of the experiments of Mangold[8] and his followers can be re-interpreted as follows. Grafts of the axial mesendoderm can 'capture' tissue from the nearby neural plate, stabilize its neural fate, and endow it with regional character according to the level (or molecular nature?) of caudalizing factors emitted by the graft. When tissue is isolated from the peripheral part of the prospective neural plate at an early stage, however, it will not give rise to neural tissue; this is not because it has not received the early signals, but because it has not been exposed to any stabilizing signals. One could therefore view most of the amphibian ANIMAL CAP (at least its dorsal half) at the beginning of gastrulation as having been activated but not yet stabilized.

Similar arguments can be used to explain why a node graft to a lateral region of the mouse embryo generates truncated axes, which share cephalic structures with the host but have a separate trunk and tail[12]. In this case, the grafted node would capture cells from the host neural plate, and then would elongate away from the host axis, progressively caudalizing cells of the adjacent neural plate. In addition, as discussed elsewhere[41,42], the modified model can also account for the phenotype of all the mouse mutations described to date that affect development of the rostral regions of the neuraxis.

ANIMAL CAP
An explant cut from an amphibian embryo at the blastula stage, comprising a 'cap' of about 60° centred on the animal pole.

NOTOCHORD
Rod-like structure of mesodermal origin in vertebrates, which provides rigidity to the early embryo and will later contribute to the vertebral centra and intervertebral disks.

TELEOST
Group of fish with bony skeletons.

Different organizers within the organizer?

Spemann, Mangold and their followers already speculated that within the organizer there might be distinct populations of cells, each emitting distinct signals responsible for specific inducing and/or patterning functions. Many subsequent studies[82–85] provided convincing evidence that signals involved in neural induction and patterning can emanate not only from the mesoderm underlying the prospective neural plate, but also from the ectodermal component of the organizer, from where they appear to flow within the plane of the ectoderm. Indeed, grafts of either layer of the organizer alone can induce neural tissue that expresses regional markers[82–86]. These findings suggest that, rather than there being subsets of cells within the organizer that possess distinct inducing and patterning functions, the organizer properties are distributed through the organizer.

Some future directions

The proposals that constitute the three-step model proposed above were suggested independently by quite different experimental strategies in the mouse[41] and the chick[42,76]. It is therefore likely that this or a similar mechanism might be responsible for generating the nervous system and its initial gross subdivisions in most, if not all, vertebrates. But many questions remain unanswered. For instance, what are the maintenance signals? How are the putative activating and transforming roles of FGF separated? When does the activation step occur in amphibian and TELEOST embryos, and what tissues are responsible for emitting the signals? When and how does the trunk nervous system become further subdivided?

It also seems likely that more complex mechanisms, perhaps including some degree of pre-patterning (or differential competence) of the prospective neural plate[87], and/or a role for differential timing of exposure to organizer-derived signals will be revealed by future research. The next few years will no doubt provide interesting answers to many questions that were first articulated so lucidly three-quarters of a century ago.

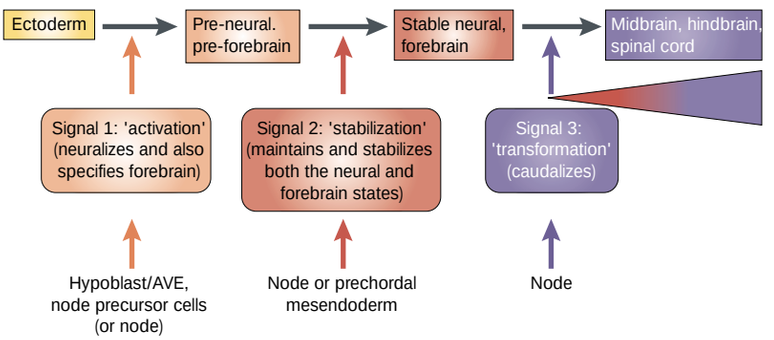


Figure 4 | **A modification of Nieuwkoop's model for neural induction and patterning.** A first step occurs before gastrulation, in which the hypoblast/anterior visceral endoderm (AVE) (perhaps together with organizer precursors) activates a labile, pre-neural and pre-forebrain state. To retain neural fate, cells must also receive stabilizing signals from the organizer and/or its descendants (prechordal mesendoderm, perhaps also the anterior head process/NOTOCHORD). If they remain close to the organizer, however, they become progressively caudalized. The hypoblast/AVE is also responsible for directing movements of the pre-forebrain territory away from the caudalizing organizer.

Links

DATABASE LINKS *Nodal* | *Otx2* | *Lim1* | *Hesx1* | *Hex* | *Sox3* | FGFs | Wnt3A | ERNI | Sox1

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Acknowledgements

This review is dedicated to Rosa Beddington, who inspired so many, including myself, to think deeply about patterning of the nervous system. Although I have tried to provide a balanced view by incorporating data from many different model systems, I am unable to do justice to the work of many researchers, to whom I must apologize. The recent research of my group on this topic was funded by grants from the National Institutes of Mental Health (USA) and the Medical Research Council (UK).