Segmentation and neural development in vertebrates

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A prominent feature of the development of most higher organisms is the subdivision of the embryo into a series of repeating elements, or segments. In vertebrates, the degree to which the nervous system is involved in this process is uncertain, and has received little attention recently. It may be relevant, however, to an understanding of the mechanisms underlying neural development

Segmentation in the vertebrate embryo is most obvious in the repeating pattern of the somites, and this is reflected in the adult by the serial arrangement of the vertebrae and their associated muscles, nerves, ribs and blood vessels It is also visible in the nervous system. Morphological segments in the neural tube were first noticed by von Baer in 1828[1], and came to be called neuromeres Subsequently some authors took them as evidence, additional to the existence of somites, that vertebrates evolved from a primitive segmented ancestor, and that the vertebrate head has a segmental origin[2]. However, this was not generally accepted[3,4] In particular, it was never agreed whether neuromeres, most obvious in the region of the developing hindbrain (Fig 1), extend the full rostro-caudal extent of the neural tube In a review on the subject in 1918, Neal[3] pointed out that 'there is not the slightest evidence that the neuromeres of the spinal cord are other than the passive result of the mechanical pressure of the adjacent mesodermic somites'. Kallen[5] later produced some evidence that they represent localised regions of mitotic activity While Neal's statement continues to be valid, the possibility that neuromeres have rather more developmental significance remains.

In the earliest experimental studies on neural segmentation, Lehmann and Detwiler wanted to know how the peripheral nerves become segmentally arranged. Specifically, they wondered whether this is because of external constraints imposed on the outgrowing axons by the somites, or because the neural tube is intrinsically segmented with respect to the position of outgrowth. Lehmann found that removal of several consecutive somites in urodele embryos leads to a loss of segmentation of sensory ganglia in the operated region Detwiler then went on to show that grafting an additional somite produces an additional spinal nerve and ganglion He concluded, as had Lehmann earlier, that 'segmentation of the spinal cord and peripheral nerves is entirely subservient to mesodermic segmentation and that an intrinsic segmentation is non-existent '

Peripheral nerve segmentation in the chick embryo

In re-examining these phenomena in the chick embryo, we were interested first to know how motor and sensory axons growing from the neural tube region are related to the somites, which lie in longitudinal series adjacent to the neural tube Twentieth century textbooks of embryology describe the spinal nerves of higher vertebrates as developing either opposite the middle of each somite, or between somites. It was therefore surprising to find, in zinc iodide-osmium tetroxide stained, whole-mounted embryos, that axons actually traverse the anterior (rostral) half of the sclerotome of each somite[8] (and it was less surprising to find that this had been described in 1855 by Remak[9]) (Figs 2, 3) To test whether this segmented outgrowth occurs because of intrinsic neural tube segmentation or because of some difference between anterior and posterior sclerotome halves, rotation experiments were carried out[8]. First, a portion of neural tube opposite 2 or 3 somites was rotated 180° antero-posteriorly (A–P) prior to axon outgrowth, so that neural tube previously opposite anterior half-somite came to lie opposite posterior half After 2 days of further development axons had still grown out through the anterior halves of those somites opposite the rotated neural tube Second, a portion of segmental plate mesoderm, 2–4 presumptive somites long, was rotated 180° A–P, this time, after further development, axons had traversed the posterior (original anterior) halves of the grafted somites

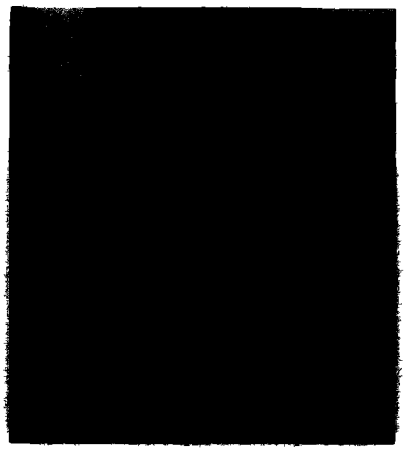

Fig. 1. *Neuromeres in an unfixed, stage 21 chick embryo The hindbrain region of the neural tube was opened out by a dorsal cut along the midline, and several segments (rhombomeres) are visible on each side Photographed with reflected light, × 200*

Axons therefore grow through anterior half-sclerotome, regardless of its position relative to the neural tube or to the A–P axis of the whole embryo These experiments confirm Lehmann's and Detwiler's conclusion that segmented axonal outgrowth is due to the somites. In addition, they show that in the chick segmentation is due to a difference between anterior and posterior sclerotome cells Axons do not grow out simultaneously along the length of the neural tube and then become secondarily segmented by the developing sclerotome. Rather, they grow out in a punctuated manner, first axons exit opposite anterior half-sclerotome, while later axons do exit opposite posterior half-sclerotome but fasciculate on previously outgrown axons so as to diverge towards anterior half-sclerotome on either side[8]

Neural crest cells

Weston[10], using [^{3}H]thymidine autoradiography, originally described the ventral pathway of migrating truncal neural crest cells as being through sclerotome, but later studies using the quail-chick chimaera system[11], or a monoclonal antibody[12], put the major pathway between adjacent somites. More recently, immunohistochemical studies have confirmed Weston's results Furthermore, they have shown that neural crest cells share the pathway of motor axons by migrating primarily through the anterior half of each sclerotome (Rickmann, M , Duband, J L , Fawcett, J W , Keynes, R. J and Thiery, J P , unpub-

Fig. 2. *Transverse semi-thin section of a late stage-16 chick embryo, at the level of the wing somites, stained with toluidine blue By the stage of motor axon outgrowth (arrow), the somite has developed into dermatome, myotome (D, M, respectively presumptive dermis and skeletal muscle) and sclerotome (S, presumptive vertebral column) NT, neural tube, NC, notochord Scale bar, 100 μm* Reproduced with permission from Ref 8)

lished observations) (Fig 4) Fibronectin, which has been suggested as being the controlling factor in the guidance of crest cells, is localized mainly at the somite borders, as previously described[13], there was no detectable variation in the A–P distribution of this molecule within the somite This implies that fibronectin does not play a critical role in determining the route taken by crest cells or axons.

Since crest cells precede motor axons in the anterior half-sclerotome, one possibility would be that axons grow on crest cells However, surgical removal of the neural crest does not alter the segmented outgrowth of motor axons. It would appear, then, that whatever differences exist between anterior and posterior sclerotome cells and/or their extracellular matrices, they can be detected independently by axons and neural crest cells.

Comparisons with other species

Does an A–P subdivision of the somite exist in all vertebrate classes? The answer is probably yes. Since Remak's original description of the development of the vertebral column in the chick embryo[9], it has been confirmed in all vertebrate classes that the sclerotome subdivides into anterior and posterior halves which subsequently differ in cell density[8] In the chick, a boundary, first described by von Ebner[14], can be seen separating the two halves of the sclerotome in the middle of each somite. This 'von Ebner's fissure' is never crossed by axons In order to explain the overlap which occurs between axial muscles and vertebrae, both of which develop from the somites, Remak introduced the concept of 'neugliederung', or re-segmentation, whereby on each side of the embryo the anterior half of one sclerotome merges rostrally with the posterior half of the next sclerotome to form a vertebra That cells from one somite can contribute to two adjacent vertebrae has been confirmed recently using the quail-chick chimaera technique[15]. However, the original descriptions of re-segmentation are open to criticism[16], and further experiments will be needed to examine this phenomenon in more detail

Whether the A–P subdivision determines axonal segmentation in all vertebrates is less certain In fishes and amphibia, axons normally grow out at a stage when few or no sclerotome cells are present, motor axons have been described as being either between myotomes *(Xenopus)* or opposite the middle of the myotome At later stages, though, both motor and sensory axons and sensory ganglia are found in the anterior half-sclerotome. In apodan amphibia*, reptiles, birds and mammals, axons grow out at a stage by which the sclerotome is well developed, and it is likely that in these higher vertebrate classes the A–P subdivision simultaneously determines axonal segmentation[8]

One interesting feature of the A–P subdivision of the vertebrate somite is its parallel with the insect segment Insect epidermal segments can be subdivided into anterior and posterior 'compartments'[17] A compartment in this sense has been defined as comprising all the surviving descendants of a small group of founder cells[17,18] We do not know whether the anterior and posterior sclerotome halves are also developmental compartments, and the similarity is, at best, a superficial one In *Drosophila,* a number of 'homoeotic' genes have been identified[19,20] which are believed to play a controlling role in the specification of segment identity and polarity. For example, the homoeotic gene *engrailed* is involved in determining the distinction between posterior and anterior compartments in *Drosophila* epidermal segments[20]. A short DNA sequence associated with several of these genes, which is conserved in the vertebrate genome,

* The order Apoda comprises a group of legless amphibians living in South America, tropical Africa, the Seychelles and south east Asia

Fig. 3. *Whole mount of stage-19 chick embryo, wing region, stained with zinc iodide-osmium tetroxide Motor axons are seen having emerged from the neural tube (inferior), and are confined to the anterior (left in the figure) halves of the somites The somite borders are enclosed by asterisks Scale bar, 50 μm*

has recently been discovered and is known as the homoeo box[21,22]. This has led to the speculation that the developmental mechanisms which underlie segmentation in insects and vertebrates might be similar. Several homoeotic-like mutations affecting the development of body segments have been identified in mouse embryos[23]. However, it is not yet known whether the vertebrate homoeo box is associated with, let alone restricted to, homologous homoeotic genes controlling somite diversification. Moreover, segmentation could have evolved independently in chordates and arthropods, their common ancestor being unsegmented. If so, the underlying mechanisms may turn out to be rather different.

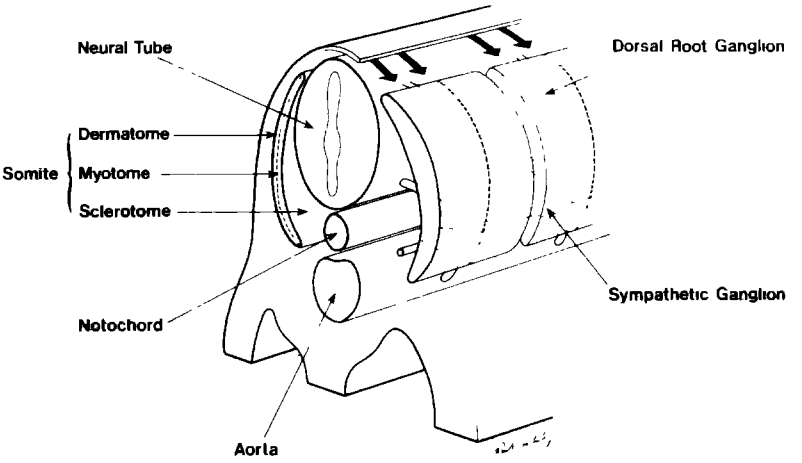

Fig. 4. *Diagram showing the major pathways of truncal crest cell migration and axon growth in the chick embryo. The somite is dispersed into its three components (see Fig. 2). Neural crest cells (heavy arrows) migrate from the dorsal aspect of the neural tube into the anterior (left in the figure) half of each somite. They pass both between the dermomyotome and sclerotome and through the sclerotome itself, before becoming components of the autonomic nervous system. Some remain in the anterior half-sclerotome and develop into dorsal root ganglion cells. Sensory axons, which arise from the dorsal root ganglion cells, are therefore confined to the anterior half-sclerotome, as are the motor axons.*

Segmentation and axonal guidance

The earliest guidance of outgrowing axons in somite regions of the chick embryo is non-specific, in the sense that any axon, whether motor or sensory, will grow through any anterior half-sclerotome. Opposite limb regions, motor axons from different neighbouring motor pools of the spinal cord, destined for different limb muscles, are mixed with each other within each ventral root[24]. Could segmentation be involved in any more specific way in the guidance of axons? It seems possible that it could. Motor axons can be guided by specific cues to their correct limb muscles; for example, after 180° A–P rotation of a length of neural tube opposite 3 to 5 leg somites, motor axons are still able to project to their correct muscles[25]. Lance-Jones and Landmesser[24], using orthograde and retrograde HRP tracing, have shown that motor axons normally sort out at the root of the limb, in the region of the developing nerve plexus. This is also the region where axons sort out following a variety of experimental manipulations[25–27]. As a result of this process, axons from a given muscle's motor pool, which project out in more than one ventral root, are collected together, and they remain together as they grow towards the developing muscle.

In considering possible sources of specific guidance for motor axons it would be interesting to know what cell types axons encounter as they undergo this sorting process. One possibility is that muscle cells provide these cues. Muscle cells migrate into the limb from the ventral edges of those dermomyotomes opposite the limb bud[28]. The process of emigration ends well before axons sort out, for example, in the chick leg, muscle cells cease to leave the somites at about stage 20 (Ref. 29), whilst axon sorting starts after stage 23, or almost 24 h later[24]. Since the site of axon sorting lies adjacent to the ventral edges of the dermomyotomes, guidance cues might be in the form of trails of extracellular matrix molecules provided by the muscle cells that earlier migrated through this region[30]. Alternatively, muscle cells themselves could still be present here, providing cellular trails for axons to follow into the limb muscle masses. Such a muscle cell trail, also somite derived, precedes the outgrowth of axons of the hypoglossal nerve, in what could be an analogous developmental system[31].

Either way, for muscle cells to provide specific cues requires that they be appropriately labelled. They might, for example, be specified on an A–P basis according to their somite of origin, and carry this label into the limb: motor axons of the same segmental level may then associate with these cells in preference to those of a different segmental origin. This possibility, of continuous segmental matching between motor nerves and myotomes within the tetrapod limb, was first raised by Goodrich[32], arguing by analogy with the innervation of fish fin muscles. Indeed, he felt able to say in 1906 'That in a series of metameric myotomes and nerves each motor nerve remains faithful to its myotome, throughout the vicissitudes of phylogenetic and ontogenetic modification, may surely be considered as established.' The motor plexus of a limb is brought about, not by the nerve deserting one muscle for the sake of another, but by the combination of muscles derived from neighbouring segments.' The experiments of Wigston and Sanes[33] do suggest that the segmentally derived intercostal muscles retain some label, perhaps segmentally determined, which biases the innervation they receive. It is, nevertheless, equally possible that muscle cells are specified after leaving the somites by a system set up within the limb itself, or that non-muscle cells provide the cues instead. It should be possible to distinguish between these alternatives experimentally.

The results of embryonic manipulations[25–27] suggest that motor axons are also labelled to allow them to recognise their guidance cues, but the basis for this is unknown. As far as the A–P axis is concerned there are perhaps two major possibilities. First, that there is a graded continuous rostro-caudal labelling system extending down the neural tube, and second, that there is a discontinuous system, arising on the basis of intrinsic segmentation within the neural tube. While Detwiler concluded that intrinsic segmentation is non-existent, neither his nor our experiments on peripheral nerve outgrowth in fact exclude it with respect to the development of neurons within the neural tube. The existence of neuromeres at least hints at this possibility, and there are descriptions of segmen-

tally arranged neurons in the adult spinal cord of *Amphioxus*[34] and several vertebrates[35,36] In this light it is perhaps worth noting that the neuromeres of the spinal cord lie out of register with the somites by a distance of half a somite

There is, as yet, no evidence that the other elements of the peripheral nervous system (the cranial, autonomic and spinal sensory axons) are positionally specified prior to axon outgrowth in the way that spinal motor axons are A–P selectivity between regenerating pre-ganglionic sympathetic axons and post-ganglionic cells has, however, been demonstrated[37] In addition, in both the sympathetic and parasympathetic systems, the sections of the neural tube which contribute pre- and post-ganglionic cells are broadly equivalent along the A–P axis[11]. These phenomena, and the striking segmented pattern of cutaneous innervation[38,39], could reflect an underlying recognition system based on segmental matching While the process of segmentation certainly influences the earliest axon outgrowths, it remains to be seen whether it continues to play a role in shaping neural development at later stages.

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Books

Theory in Psychopharmacology: Vol. 2

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The second volume of 'Theory in Psychopharmacology' contains six chapters by authors who are well known and active researchers in the field, and is well balanced between contributors with a predominantly psychological, and those with a predominantly pharmacological, background The aim of the volume, and one in which it succeeds admirably, is to review the background to areas of research that are currently prominent, and to relate the empirical data that has been produced to theories about how the effects of a drug on behaviour may be achieved.

The general quality of the contributions is excellent the chapters by Cooper and Sanger are particularly good critical evaluations of current hypotheses about the mechanisms underlying drug effects on feeding and drinking, and the chapter by Rupniak, Jenner and Marsden provides a much-needed criticism of the hypothesis that dysfunction of the brain's dopamine systems underlies schizophrenic disorders, a theory that is still presented as accepted in general pharmacology courses, but against which considerable evidence is amassing The other fields of research covered in the present volume are endogenous modulation of learning and memory, correlations between activity of serotonergic systems in the CNS and behaviour, and the discriminative stimulus properties of drugs acting at opiate systems in the nervous system. Although the contributions are not aimed at presenting new data, they are well-illustrated by figures representing important findings, and a comprehensive bibliography follows each article

Each of the articles stimulates thought and illustrates important questions of theory and methodology, both general and specific, that can be applied to several areas of psychopharmacological research Psychopharmacology (behavioural pharmacology) is a science that is concerned with the interactions between drugs and behaviour, and thus can be approached from at least two standpoints One approach is to study the ways in which drugs can produce changes in behaviour, another approach is to study the way in which behavioural factors (for example, past