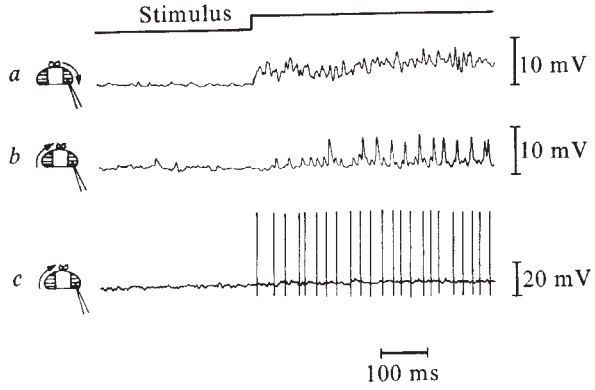

Fig. 2 Intracellular recordings obtained from the H cells shown in Fig. 1 show responses to ipsilaterally presented progressive (*a*) and to contralaterally presented regressive pattern motion (*b*,*c*). Pattern parameters were: spatial wavelength, 20°; contrast, $m = 48\%$; velocity, $w = 5$ deg s^{-1}, luminance 4 cd m^{-2}. Arrows at the schematic horizontal cross section of the head (to the left of the response traces) indicate the direction of pattern movement.

for integrating ipsilateral as well as contralateral inputs. It is not clear whether the spike activity shown by these fibres represents the output of these cells or the contralateral input, but the former seems to be the more plausible interpretation[3].

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Directed outgrowth of optic fibres regenerating *in vitro*

THE way in which growing nerve fibres find their destined sites of termination is largely unknown, although there are supporting arguments and documentation for both local and long-range guidance mechanisms[1–3]. Fibres regenerating following axotomy tend to grow in the direction of the degenerating tracts, apparently guided through channels formed by debris and/or glial cells[4,5]. Here we present evidence that the neuritic outgrowth from goldfish retinal explants is related to the orientation of cut fibres within the explant.

We demonstrated previously that optic nerve crush 10–14 d before retinal explantation will stimulate neurite production in culture[6] and that the neurites originate from ganglion cells[7]. On the day of explantation the retina is cut into square pieces 500 μm across, which are placed on a poly-L-lysine-coated substratum[8]. Several explants are placed in each 35-mm dish (NUNC) without regard to whether it is the vitreal or the photoreceptor surface that contacts the substratum. The ganglion cell axons in these experiments have been cut twice: once, at the time of optic nerve crush, several millimetres from the ganglion cell soma; and again, close to the soma when the retina is cut for explantation.

Examination of explant cultures under phase microscopy indicated that neuritic outgrowth began within a day of explantation. The direction of outgrowth was influenced by several factors. For example, neurites grown on poly-L-lysine-coated planar surfaces (glass or plastic) inevitably curved in a clockwise direction. Experiments designed to reveal the nature of this tendency led us to the conclusion that the directionality reflects an inherent helicity of the fibres[9].

The clockwise directionality of neuritic growth is apparent in Fig. 1, which also indicates another property of the explants: the early outgrowth was usually restricted to one side or corner (Fig. 1*a*). A probable explanation for this asymmetric outgrowth was provided by explants stained with Holmes silver nitrate[10]. An array of parallel fibres could be seen in the optic fibre layer (Fig. 2), and the region of densest neuritic outgrowth was often aligned with this axis. Since the optic fibres in the goldfish retina, as in other vertebrates[11], are arranged in straight, radial lines, all of which converge at the optic disk where the fibres exit the eye as the optic nerve, it seemed probable that the parallel fibres within the explant were the ganglion cell axon tracts. Within the small area of the explant, convergence of the fibres was not apparent. Thus, it was not possible to determine which was the centripetal end of the fibre axis, so we could not establish whether the outgrowing fibres were indeed pointing at the phantom optic disk. In a brief, undocumented report 30 years ago, Vinnikov described directed outgrowth from retinal explants of several vertebrate species which he attributed to the radial pattern of optic fibres[12].

In order to establish definitively whether the neurites were aligned with the pre-existing ganglion cell fibre tracts within the explant, we performed the experiment represented schematically in Fig. 3. A strip of retina approximately 750 μm wide, extending across the full expanse of the retina from one peripheral margin to the other and including the optic disk, was prepared and then cut into thirds. Unlike our usual explants,

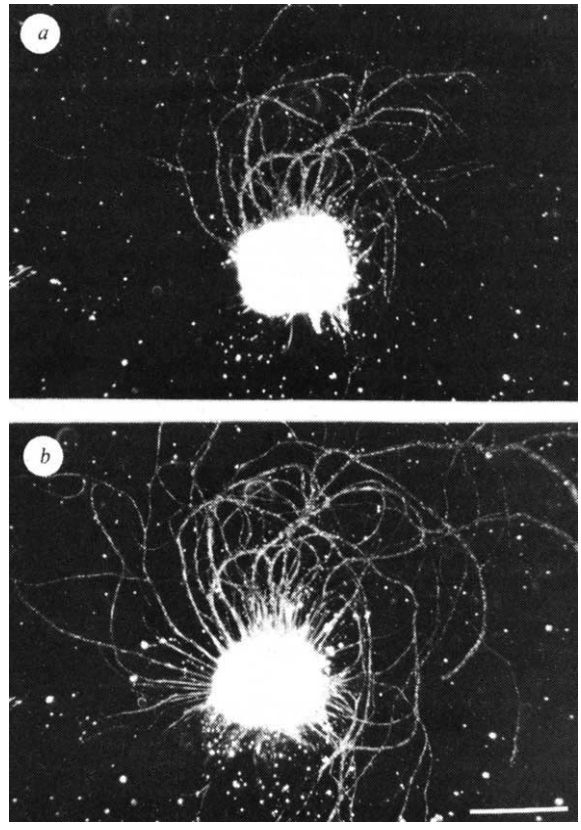

Fig. 1 Retinal explant viewed with darkfield illumination. The majority of neurites were restricted to one edge after 4 d *in vitro* (*a*). Note that most of them curve in a clockwise direction. After 14 d (*b*), neurites had grown out from the other edges as well. Scale bar, 500 μm.

the orientation of each of these pieces was known. We expected no growth from the edges corresponding to the peripheral margins of the retina and very little from the two lengthwise edges, since the latter intersected few of the 'spokes' formed by ganglion cell axons. Further, we anticipated little growth from the central explant, since its ganglion cell axons would be aimed inward. The experimental results shown in Fig. 4 indicate that the neurites grew in the predicted direction. The pattern of outgrowth shown was observed in several replications and was independent of the placement of strips with respect to one another in the dish. The observed asymmetry of early outgrowth was thus a property of each piece of retinal tissue and did not reflect interaction between explants or other extrinsic factors.

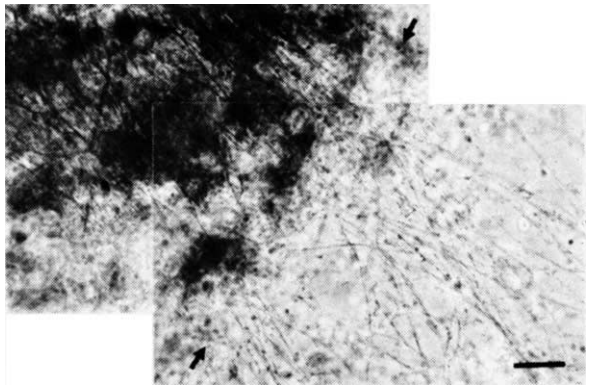

Fig. 2 In this silver-stained explant (6 d *in vitro*), the optic fibre layer can be seen at the upper left; the edge of the explant lies between the two arrows. Neurites aligned or contiguous with fibres in the explant have grown out on to the substratum at the lower right. Scale bar, 25 μm.

After several days in culture, the asymmetry of outgrowth was diminished as fibres emerged from the entire circumference of both the small, square explants (Fig. 1*b*; see also previous studies[7,9]) and from the large strips. Examination of silver-stained explants offered a possible explanation for the eventual appearance of outgrowth from edges which were initially bare. Many of the later-appearing fibres had wandered through the explant or were deflected at the tissue–substratum boundary and grew along the edge of the explant for some distance before emerging on to the dish. In contrast, the first neurites seen probably regenerated from axonal stumps whose cut ends were lined up along the edge of the explant nearest the optic disk, so that their path was unimpeded as they grew out on to the substratum.

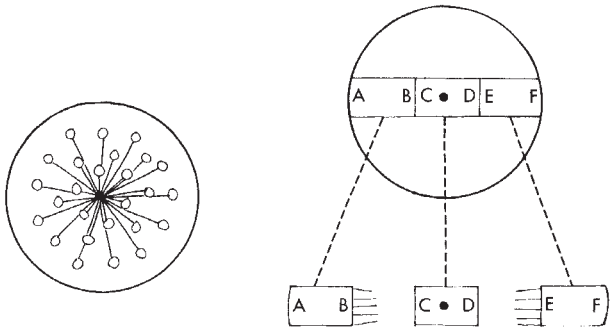

Fig. 3 The schematic diagram on the left shows the radial pattern of optic fibres in the retina. Ganglion cell somata are represented by small, open circles and their axons by line segments which converge at the optic disk (filled circle). At the upper right, the retina is shown as it was prepared for explantation. A strip of retina that included the optic disk was divided longitudinally into three approximately equal pieces and then explanted. If the neurites maintained their centripetal orientation (towards the disk), they would grow out, as shown at the bottom right, from the ends labelled B and E.

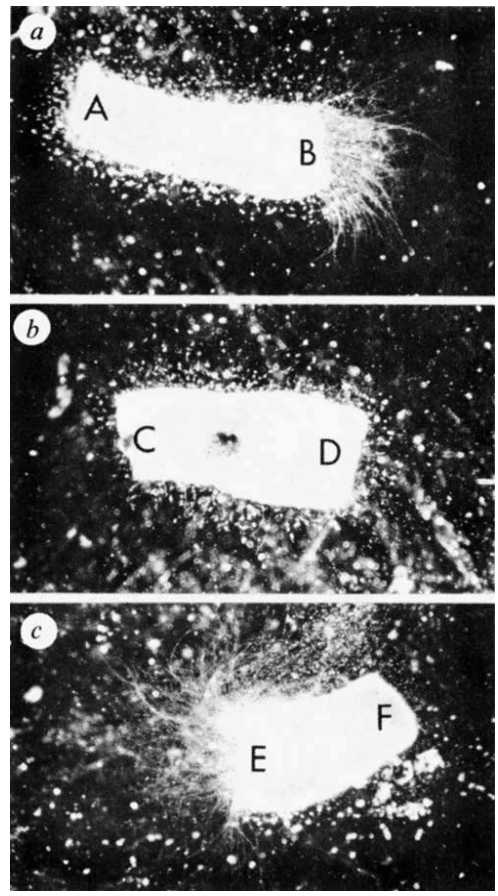

Fig. 4 The results of an experiment as described in Fig. 3 are illustrated. After 6 d *in vitro*, most of the neurites have grown out as predicted from ends B (*a*) and E (*c*). The optic disk appears as a dark spot in *b*. Scale bar, 500 μm.

The explants in Fig. 4 each contain approximately 5,000 ganglion cells[13] from which axons regenerated in a direction predictable from their pre-existing pathways. While it is well known that growing axons can be guided both *in vivo* and *in vitro*[1,14,15], in these retinal explants a great many fibres have been channelled in the same direction in the absence of extrinsic influences. This preparation thus seems to provide a unique opportunity to directly examine retinal fibre–fibre interactions and their proposed role in neuronal recognition[16,17].

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Neuromuscular transmission is adequate in identified abnormal dystrophic muscle fibres

It has been suggested that 'functional denervation', a failure of structurally intact neuromuscular junctions to evoke a muscle fibre action potential following nerve stimulation, plays an important part in the pathogenesis of murine muscular dystrophy[1,2]. Other workers, however, have been unable to confirm the existence of 'denervated' muscle fibres in dystrophic mouse muscle[3]. Moreover, the demonstration that transmitter release in response to nerve stimulation is normal in such muscle[4] seems to preclude the possibility that 'denervation' may arise in an unpredictable or spasmodic way as a result of changes in transmitter store size or mobilisation during or after the repetitive discharge of the motoneurone. It has been considered possible, however, that the microelectrode techniques used in many of these studies do not always sample diseased muscle fibres, but select only a hypothetical group of 'normal' or 'healthy' muscle fibres. We have therefore applied a technique of intracellular staining[5,6] and demonstrated that structurally abnormal muscle fibres in dystrophic muscle display normal neuromuscular transmission.

Experiments were carried out at room temperature on extensor digitorum longus (EDL) muscles removed from 3–6-months-old dystrophic (Bar Harbor 129 ReJ) mice and their clinically normal litter mates. In some muscles, muscle fibre action potentials were generated in response to indirect excitation and were recorded using standard intracellular techniques. In other muscles, endplate potentials (e.p.ps) were recorded following nerve stimulation at frequencies of 3 Hz and 30 Hz in the presence of 0.6–1.2 μM *d*-tubocurarine. The quantum contents of the e.p.ps were estimated from the coefficient of variations of e.p.p. amplitudes[7]. After recording either action potentials or e.p.ps, a second microelectrode filled with a 4% (w/v) aqueous solution of Procion

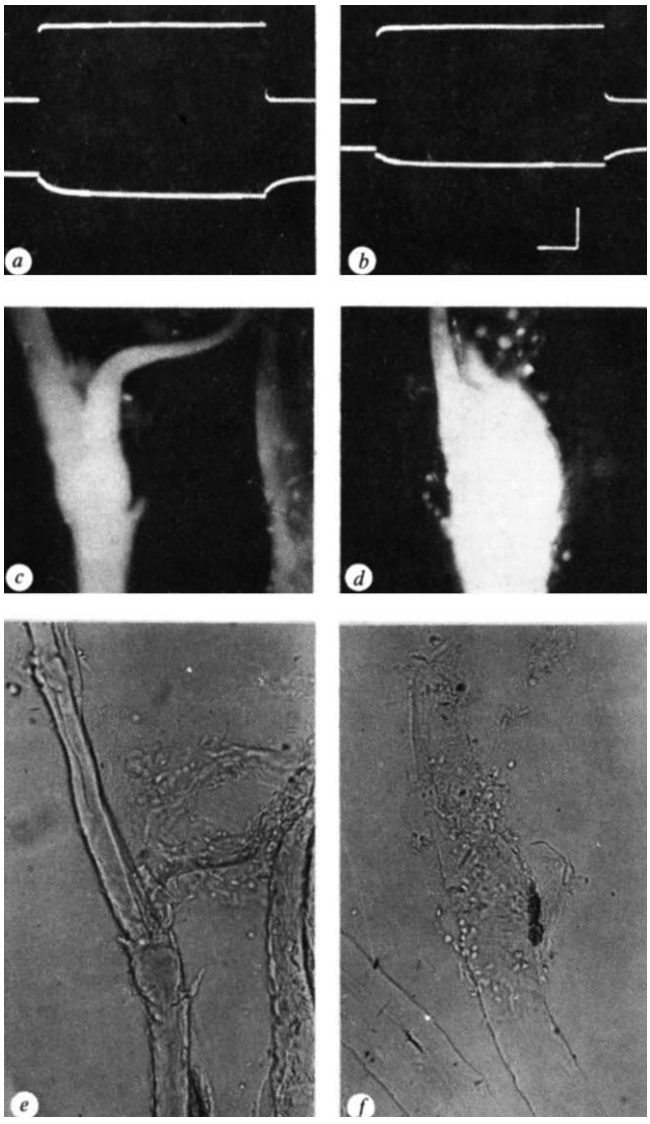

Fig. 1 Two typical experiments in which the input resistance of a muscle fibre was recorded using a standard two microelectrode technique. The current passing electrode was filled with a 4% solution of Procion brilliant red. The hyperpolarisation of the membrane (lower trace) in the two fibres following the passage of current (upper trace) is shown in *a* and *b*. The muscle fibres were then marked with dye by passing steady d.c. current for 10–20 s. The marked fibres were located using fluorescence microscopy (*c*, *d*) stained for cholinesterase and teased from the whole muscle (*e*, *f*). The first fibre (*a*, *c*, *e*) showed longitudinal splitting, and dye injection had taken place at the site of splitting. The second fibre (*b*, *d*, *f*) was 'short' and had probably undergone necrosis. M.e.p.ps were recorded from this fibre (not shown). Calibrations; *a*, *b*, 5 ms horizontal; 20 nA, 20 mV vertical; *e*, *f*, 70 μm.

Fig. 2 An indirect action potential with its first derivative (*b*) was recorded from a dystrophic muscle fibre. The fibre was subsequently marked with Procion brilliant red and located using fluorescence microscopy (*c*). After staining for cholinesterase and teasing (*a*, *d*) the endplate cholinesterase (ep) and the site of the dye injection (PBR) were located. This fibre displayed longitudinal splitting, confirmed by serial section of the embedded fibre (*e*, *f*, *g*). Calibrations; *b*, 50 mV, 500 Vs^{-1} vertical; 5 ms horizontal; *d*, 80 μm.