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Investigating the role of EphA/ ephrin-A signalling during trigeminal ganglion axon guidance

A thesis submitted for the degree of Doctor of Philosophy

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December 2004

Table of Contents

Abstract

The ophthalmic, maxillary and mandibular axon branches of the trigeminal ganglion (TG) provide cutaneous sensory innervation to the vertebrate face, and multiple families of guidance cues amalgamate to direct the navigation of these branches. However, target tissue specific guidance cues that discriminately guide the three TG axon branches are unknown. Prior work demonstrated that EphAs and ephrin-As could discriminately direct dorsal versus ventral motor axon projections into the hindlimb. Similarly, do EphA tyrosine kinases and ephrin-A ligands discriminately guide trigeminal ganglion ophthalmic (TGop) lobe versus maxillomandibular (TGmm) axon projections into the chick embryo face? The aims of this work were two-fold: (1) to identify candidate EphA and ephrin-A molecules during TG axon guidance, and (2) to determine the functional significance of TG axon EphA and ephrin-A signalling in *vitro*.

This study identified EphA3, EphA4, *ephrin-A2* and *ephrin-A5* at stages 13, 15 and 20, as putative guidance cues to TG axons. TG-EphA3 and -*ephrin-A5* were identified as putative receptors to guidance cues expressed in the target fields. EphA3 receptor was differentially expressed, with the TGop lobe expressing higher levels compared to the TGmm lobe. However, *ephrin-A5* transcript was not differentially expressed between the two ganglion lobes.

In a substratum choice *in vitro* assay, ephrin-A5-Fc was found to repel approximately 50 % of axons growing from stage 20 whole TG explants. This population of axons was identified to be from the TGop lobe. The *in vitro* data supports the contention that during facial development there may be trigeminal ganglion lobe specific guidance of TGop in comparison to TGmm peripheral sensory axonal projections to target fields coordinated through EphA3 and ephrin-A2/A5 repulsive interactions.

In vitro, EphA4-Fc caused morphological changes to TG growth cones, which is likely mediated through TG ephrin-A5 reverse signaling. Furthermore, this study provided *in vitro* evidence that trigeminal ganglion axons were not responsive to EphA4-Fc, possibly implying that EphAs expressed in the target fields were not repulsive to ganglionic axons during pathfinding.

The data suggests that EphA/ ephrin-A interactions may specifically guide TGop projections into the ophthalmic process similar to lateral motor axon guidance into the hindlimb. For the first time, a model of how EphA/ ephrin-A interactions and other families of guidance cues may act in concert to guide trigeminal ganglion axons is suggested.