



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

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Table of Contents

Table of Contents	i
List of Figures and Tables	vi
Declaration	ix
Abstract	xi
Acknowledgements	xiii
Personal bibliography	xv
Chapter 1: Introduction	1
1.1 Axon guidance	3
1.1.1 Types of guidance cues	4
1.1.2 Growth cone machinery and dynamics.....	7
1.1.2.1 Structure of the growth cone	7
1.1.2.2 Guidance cues and the growth cone cytoskeleton	8
1.1.2.3 Rho GTPases and axon guidance	8
1.1.2.4 Overview	8
1.2 The trigeminal ganglion- a model system?	11
1.2.1 Morphology of the trigeminal ganglion	11
1.2.2 Dual embryonic origin of the trigeminal ganglion.....	12
1.2.2.1 Neural crest.....	12
1.2.2.2 Epidermal neurogenic placode.....	12
1.2.2.3 Requirement for both neural crest and placode components.....	17
1.3 The trigeminal ganglion and axon guidance	18
1.3.1 The placode and axon pathfinding.....	18
1.3.2 Trigeminal ganglion guidance cues	19
1.3.3 Trigeminal ganglion lobe specific guidance cues?.....	20
1.4 Ephs and ephrins as candidates	25
1.4.1 Eph receptor structure	26
1.4.2 Signalling mechanisms.....	26
1.4.2.1 Eph kinase “forward” signalling	31
1.4.2.2 ephrin “reverse” / Eph-kinase independent signalling	31
1.4.2.3 Adhesive/ attractive and repulsive Eph/ ephrin interactions.....	37
1.4.2.4 Modulation of Eph signalling by co-expressed ephrins	47
1.4.3 Examples of Eph/ ephrin interactions during development of the nervous system	48
1.4.3.1 Axon pathfinding- roles during optic nerve formation and during commissural axon tract formation	48
1.4.3.2 Axon fasciculation	51
1.4.3.3 Roles during anterior-posterior retinotectal topographic mapping	55
1.5 Summary: EphAs and ephrinAs- possible candidates for lobe specific trigeminal ganglion axon guidance?	65
1.5.1 Project Aims	66

Chapter 2: Materials and methods	71
2.1 Abbreviations	73
2.2 Materials	73
2.1.1 Chemicals	73
2.1.2 Enzymes	74
2.1.3 DNA plasmids	74
2.1.3 RNA in situ hybridisation probes	74
2.1.4 Antibodies/ Fc-fusion chimeras	74
2.1.6 Chick embryos	75
2.3 Methods	76
2.3.1 Chick embryos	76
2.3.2 Bacteriological techniques	76
2.3.2.1 Bacterial culture	76
2.3.2.2 Bacterial stains	76
2.3.2.3 Preparation of electrocompetent DH5 α cells	76
2.3.2.4 Transformation of DH5 α cells	77
2.3.2.5 Plasmid screening for transformed recombinant clones- cracking	77
2.3.2.6 Plasmid screening for transformed recombinant clones- alkaline lysis	78
2.3.3 DNA techniques	79
2.3.3.1 Plasmid preparations	79
2.3.3.2 Electrophoretic separation of DNA	79
2.3.3.3 DNA modifying enzyme reactions	79
2.3.3.4 Preparation of DNA for ligations	79
2.3.3.5 Cleanup of ligation products for transformation into DH5 α cells	79
2.3.3.6 Cleanup of cDNA for real-time PCR	80
2.3.3.7 Real-time PCR	80
2.3.3.8 Automated DNA sequencing	81
2.3.4 RNA techniques	81
2.3.4.1 Total RNA extraction from trigeminal ganglion lobes	81
2.3.4.2 Reverse transcription	82
2.3.4.3 Transcription of RNA probes for <i>in situ</i> hybridisation	83
2.3.5 Embryo/ Trigeminal ganglion harvesting	84
2.3.5.2 Chick embryos	84
2.3.5.1 Trigeminal ganglia and trigeminal ganglion lobes	84
2.3.6 Tissue sectioning	85
2.3.6.1 Vibratome sections	85
2.3.6.2 Cryostat sections	85
2.3.7 Whole-mount RNA in situ hybridisation	85
2.3.8 Antibody/ Fc-Fusion techniques	86
2.3.8.1 Antibody staining	86
2.3.8.2 Eph and ephrin-Fc staining	87
2.3.8.3 Microscopy	87
2.3.8.4 Whole-mount EphA3 trigeminal ganglion intensity readings	88
2.3.8.5 EphA3 growth cone intensity readings	88
2.3.9 Trigeminal ganglion culture	88
2.3.9.1 Antibody/ Fc-fusion staining of cultures	89
2.3.10 <i>In vitro</i> assays, analysis and statistics	89
2.3.10.1 Substratum choice assay and uniform substrate assay	89
2.3.10.2 Analysis of neurite parameters	89

Table of contents

2.3.10.3 Analysis of growth cone parameters	89
2.3.10.4 Data processing and statistics	90
Chapter 3: EphA and ephrin-A expression analysis in the trigeminal ganglion peripheral targets	91
3.1 Introduction	92
3.2 Results	97
3.2.1 EphA- and ephrin-A-Fc staining during trigeminal ganglion axon guidance	97
3.2.1.1 Complementary and overlapping EphA and ephrin-A expression in the trigeminal ganglion target fields	97
3.2.1.2 Differential Eph/ ephrin-A expression in the trigeminal ganglion.....	98
3.2.2 EphA expression in the trigeminal ganglion peripheral target fields	103
3.2.2.1 EphA3 is expressed in the ophthalmic process and is expressed in the trigeminal ganglion.....	103
3.2.2.2 EphA4 is expressed in the ophthalmic process during ophthalmic trigeminal ganglion axon growth at stage 13.....	109
3.2.2.3 EphA5, EphA7 and EphA9 are not candidate guidance cues for trigeminal ganglion axons	112
3.2.3 ephrin-A expression in the trigeminal ganglion peripheral target fields...	117
3.2.3.1 <i>ephrin-A2</i> is expressed in the maxillary and mandibular processes.	117
3.2.3.2 <i>ephrin-A5</i> is expressed in the maxillary and mandibular processes and in the trigeminal ganglion	119
3.3 Summary and discussion.....	125
3.3.1 Complementary expression of EphA3/A4 and ephrin-A2/A5 at stages 13 and 15 when trigeminal axons are pathfinding	125
3.3.2 Similar EphA/ ephrin-A expression patterns are observed for mouse and chick	126
3.3.3 Conclusion	127
Chapter 4: EphA and ephrin-A expression in the trigeminal ganglion	131
4.1 Introduction	132
4.2 Results	133
4.2.1 EphA4, A5, A7, A9 and ephrin-A2 are not expressed in the trigeminal ganglion.....	133
4.2.2 EphA3 is differentially expressed in the trigeminal ganglion	133
4.2.2.1 EphA3 localises to the ophthalmic placode and trigeminal ganglion neurons at stages 13 and 15.....	133
4.2.2.2 The ophthalmic lobe expresses high levels EphA3 transcript and protein at stage 20	135
4.2.3 <i>ephrin-A5</i> is not differentially expressed in the trigeminal ganglion	149
4.2.3.1 <i>ephrin-A5</i> localises to the ophthalmic placode and trigeminal ganglion neurons at stages 13 and 15.....	149
4.2.3.2 The trigeminal ganglion at stage 20 non-differentially expresses <i>ephrin-A5</i>	157
4.2.4 Differential co-expression of EphA3 and <i>ephrin-A5</i> in the maxillomandibular lobe	157
4.3 Summary and discussion.....	161

4.3.1 Insights into intra-ganglionic EphA3/ ephrin-A5 interactions.....	161
4.3.2 EphA3 is differentially expressed within the ganglion	162
4.3.3 Significance of EphA3 and ephrin-A5 expression in the placode during axon guidance	162
4.3.4 Conclusion.....	163
Chapter 5: <i>In vitro</i> analysis of trigeminal ganglion EphA3 forward signalling.....	169
5.1 Introduction	170
5.2 Results	175
5.2.1 Trigeminal ganglion explant axons express EphA(s).....	175
5.2.2 A sub-population of trigeminal ganglion axons are sensitive to substratum bound ephrin-A5	175
5.2.3 Trigeminal ganglion ophthalmic lobe axons are sensitive to substratum-bound ephrin-A5	179
5.2.4 Axons and growth cones from Ophthalmic and maxillomandibular lobe explants express EphA3.....	184
5.3 Summary and discussion.....	188
5.3.1 Ephrin-A5 as a guidance cue	188
5.3.2 Ephrin-A5-Fc and the differential guidance of ophthalmic versus maxillomandibular lobe axons	189
5.3.3 EphA3 expressing maxillomandibular axons are not responsive to ephrin-A5-Fc.....	193
5.3.4 Conclusion.....	195
Chapter 6: <i>In vitro</i> analysis of trigeminal ganglion ephrin reverse signalling.....	197
6.1 Introduction	198
6.2 Results	199
6.2.1 Trigeminal ganglion explants express ephrin-A5	199
6.2.2 Trigeminal ganglion axons are not responsive to EphA4-Fc	200
6.2.3 EphA4-Fc does not promote neurite growth	203
6.2.4 EphA4-Fc influences growth cone morphology	207
6.2.5 Trigeminal ganglion explants express ephrin-B2	209
6.3 Summary and discussion.....	211
6.3.1 <i>In vivo</i> EphA3/ A4 expression patterns correlate with <i>in vitro</i> substratum choice assay results	211
6.3.2 ephrin-A5 reverse signalling and the growth cone	212
6.3.3 Is there convergence of ephrin-A5 and ephrin-B2 reverse signalling? ...	214
6.3.4 Is EphA4-Fc permissive or adhesive to trigeminal ganglion growth cones/ axons?	215
6.3.5 EphAs are pathfinding cues to trigeminal ganglion growth cones?	215
6.3.6 Conclusion.....	217
Chapter 7: General discussion and future directions	221
7.1 Similarities and differences between trigeminal ganglion lobe guidance and motor axon hindlimb innervation	227
7.2 Suggested model of trigeminal ganglion axon guidance	230

7.3 <i>In vivo</i> examination of EphA/ ephrin-A interactions during trigeminal ganglion axon guidance	233
7.3.1 Elucidating <i>in vivo</i> trigeminal axonal-EphA3 and first branchial arch-ephrin-A2/A5 interactions in the chick embryo.....	233
7.3.2 Elucidating <i>in vivo</i> EphA/ ephrin-A interactions in the mouse embryo	234
7.4 <i>In vivo</i> elucidation of guidance cue interactions during trigeminal ganglion axon guidance	235
7.5 What signals lie downstream of EphA3 activation in trigeminal ganglion axons/ growth cones?	236
7.6 Other roles for ganglionic EphA3/ ephrin-A5 interactions during trigeminal ganglion development?	237
7.7 Conclusion.....	239
References	240

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.