

need to do:

- dye in otic vesicle w. sesame oil

What I know:

if actually, that's not true, I have seen a 17.5 that has no aff.

That the afferents + efferents have already left/ entered the hind brain in all of the stages of embryos, I have injected so far. * In order to analyse that exact point of contact when the eff. are just about to turn and leave I need to go earlier.

Whether this is technically possible is the first Q. Whether it is crucial I go this early / whether it's the only way I can answer my question is another.

I know too that there are differences between the trigeminal nerve and the 7th/8th complex. Proposed in the CUX et al. article → in Zebrafish trigeminal afferents enter + make way for trigeminal efferents. but diff. for 7th/8th → I could try and see if there is a stage where eff. haven't yet exited or have but afferents aren't in yet.

Information I can gather from the photos so far.
- the idea being that I keep going with those and try and get that as consistent as possible.

→ Growth cone interaction
+ trajectory of turning efferents (can be measured)
+ lateral position of that turn.

I feel comfortable assessing the embryo's anatomy + locating nerves.

I'm starting to get a lot better G injections as the cell 2 have worked well and on stage 18-19.

Afferents enter after efferents leave in VII/VIII nerve.

What I don't know:

→ is whole mount antibody labelling a viable method for us?

→ why do afferents only travel a certain distance along edge of hindbrain and then stop.

see: Chang, Fan. 1992



do they extend + then retract or what?

do they continue or retract, why would afferents turn up in a direction that there is nothing to synapse with yet?

What are they even synapsing with a or what will they be synapsing with? Nuclei?

Servey cortex?

- Could I be imaging Glia? (glial sheath?)

- how to use imageJ well.

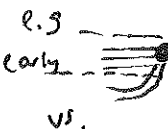
Came from neural crest + epiblastical placodes

- Where do afferent cell bodies initially arise? (Gillend bahu)
ganglia? or do they migrate into periphery or grow there in the first place?

- What could potentially explain the phenomenon I see that series vs turn are abt

- haven't yet tried dying 1st nerve. then later stages?

- Why the development of the Vth nerve would be different to that of the VII, IX, X?



is it to do with the time at which they develop
- Rostrally.

- whether the observation of the dev of Vth nerve in Zebra fish (separate entry/exit points) can be at all translated (applied to the chicken where the 7th + 8th have the same entry/exit point?)

- what if 8 aff. acted in a way that V aff. did + used the way for 7 efferents but I couldn't distinguish between the two so I could never find the answer?

Diverse Mechanisms for assembly of Branchiomeric nerves

VIIIth = motor and sensory limbs are independent in a Zebrafish.

IX, X = efferents appear crucial for afferent direction / formation.

mechanisms of assembly & Differ based on axial position.

interesting

r2 = Vth

r4 = VIIIth

r6 = IX

r7/8 = Xth

In the VIIIth nerve, aff + efferent fascicles formed distinct bundles which entered/exited the hindbrain at different positions. → does this happen in a chicken?

in VIIIth - efferents leave before afferents enter.

BMN formation occurs Rostro-caudally e.g. V first then VII then IX etc.

no aff = VII, IX + X still showed normal pathfinding.

no efferent = in IX + X = affected the rate of the afferents but not in VII as aff + efferent enter/exit through different locations.

Vestibulocochlear afferents may act in a similar role when the 2 ganglia are fixed. - Butler + Hodos. 2005

Evo. patterns of Cranial Nerve Efferent Nuclei in Vertebrates.

Hox genes responsible for differentiation axially

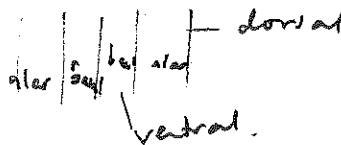
all Motoneuron cell types (visceral, BMN, octavolaterals) originate from ventral basal plate (primitive motor column)

- lateral origin of Locomotor neurons and medial for other 3 types.

- Serotonergic neurons near the floor plate?

Gradient of Sonic hedgehog protein secreted by floor plate / notochord to derive different ventral neuronal classes.

lateral migration of BMN? after extending axon to reach dorsally located sensory-motor root, they migrate laterally



"V - lacks visceromotor + gustatory components + primitively has 2 sensory ganglia. - despite these 'unusual' features, V MN appears to match expectations of proper BMN development" - Reference please???

aff. first but not the case in other BMN so this seems contradictory

Photos I have so far:

HK005 - Stage 17 VIII nerve.

- no afferents visible. very fine dye.

notes: not as organised, turning not as direct. 2 ganglia no afferents.

HK009 - 24/25, very strong bundle of afferents.
VIII

HK010 look for pic on other comp.

HK019 - basal plate injection - afferents are a bundle.
st 23 needed clearer labelling of images re: where I was in basal plate etc.

How can I differentiate between motor neurons and support cells?

HK023 → Afferents are v. & easily individually identified.
stage 19.5 Some aff. appear to be joining other ganglia

- MY BEST, CLEAREST ONE

HK024 = stage 18 - v. few afferents - eventually aren't there for st VIII.

not yet clear aff. turning.

To try:

→ Dye a stage 13 - try a stage 16 today.

→ Prove that afferents haven't entered yet in 7th/8th but test to see whether they have in trigeminal. i.e. get both dye needles in both sets of nerves.

to ever see how insane that would be if it would truly be out of question.
the

Thoughts of "where to from here".

→ So the proposal is that for CN V, afferents must pierce the hindbrain, before eff. can exit. → in both zebrafish + chicken embryos.

→ not much research done about VIIth cranial nerve.

In zebrafish, suggested that because they have separate roots of exit/entry there is no relationship between the two.

in chicken = same entry/exit + shared in fact with VIIIth nerve.

go back in time to see if I can consistently label VIIth / VIIIth + show eff. leave before aff. enter.
perhaps directly as late as stage 17

hopeful!

Pathfinding by cranial nerve VII motoneurons in the chick hindbrain.
- (1992)

- it's only in R5 that axons/efferents turn?"

the turning could be rhombomere directed.

There is turning in R3, R5 also, 6 & 6.7 (1X)

⑧ Stage 13, cranial motoneurons arise abn. midline.

'facial motoneurons are the first motoneurons in the hindbrain to extend axonal processes' - apparently (no ref.)

''Contradictory to the idea of Rostro-caudal temporal development''

HH stage 14⁺ = eff. have reached exit site.

? doesn't mention when afferents arrive though.

in R4 ablated embryos → never did R5 motoneurons keep growing laterally + cross the lateral motor boundary (something about that boundary preventing growth).

'since facial motoneuron somas are located adjacent to floorplate cells yet do not cross the midline?'

Motorneuron pathfinding following rhombomere reversal in the chick embryo hindbrain.

geniculate

Superior sensory ganglia } are these the 'nerve' we
are injecting? the specific
name of their ganglia

- Stage 15 : rhombomere 4 has fan like structure.