Embryonic asymmetry and development of functional neuronal connectivity

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Introduction

Animals display anatomic bilateral asymmetry in their internal organs e.g. different number of lobes in each of the human lungs, as well as functional asymmetry e.g. the two cerebral hemispheres perform different roles. Additionally, the control of our limbs is contralateral i.e. the left hemisphere controls our right hand and vice-versa. The vast majority of people are right handed (>90%) with less than 10% that are left handed or ambidextrous. However, non-right handers can have reversed hemisphere dominance for language processing. They are also disproportionately represented (close to 50 percent) in people suffering from Schizophrenia as well as other psychoses.

We use the *C.elegans* research model to understand the fundamental biology of laterality variations and their neural consequences. This microscopic invertebrate shows predominantly bilaterally symmetric external anatomy, and it also displays clear internal left/right visceral asymmetry. *C.elegans* only has 959 cells and 302 neurons whose connectivity has been fully established. Most neurons are arranged in left-right pairs with some pairs having functional asymmetry. Left-right laterality is established during early embryogenesis, and mutations in a G-alpha subunit coded by *gpa-16* show increased reversal. We hypothesize that reversed laterality will result in atypical neural connectivity and thereby affect behavior, and present results supporting the above.

Objective

- Examine the division pattern of non-dextral embryos and determine whether or not embryonic division patterns affect adult neural connectivity
- Study the functional effects of reversed asymmetry on associative and non-associative learning.
- Investigate the neuronal laterality variation and atypical synaptic connectivity behind the *gpa-16* mutants which effect the learning behavior.

Methods

- Behavioral assays were done using synchronized L4 worms. Animals were conditioned to isoamyl alcohol in the absence of food before being tested. For habituation, the worms were gently tapped on the head until they stop responding to touches.
- Neuronal connection mode was tested on ASE Right (ASEr) chemo-sensory neuron and AIY interneuron.

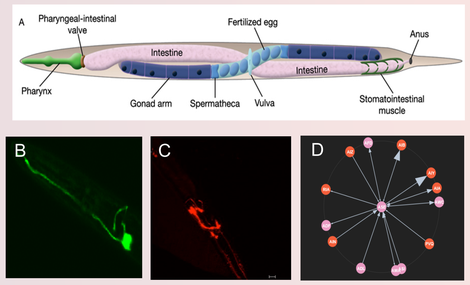

Figure 1: (A) Left-Right asymmetry in *C.elegans* adult hermaphrodite. (B) GFP was fused with gcy-5 to mark the ASEr neuron. (C) mCherry was fused with *ttx-3* promoter to mark the AIY interneuron. (D) Connectivity of ASE neuron. (*Wood et.al., 1996*).

Results

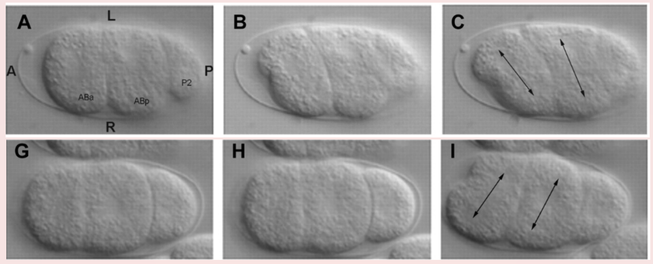

Figure 2 Spindle orientations during cleavage of ABa and ABp in wild-type and *it143* mutant embryos. (A-C) Normal spindle orientations in a wild-type embryo. (G-I) The aberrant spindle orientations in three abnormal *it143* mutant embryos at 20° C. (From Bergmann DC, Lee M, Robertson B, Tsou MF, Rose LS, Wood WB, 2003)

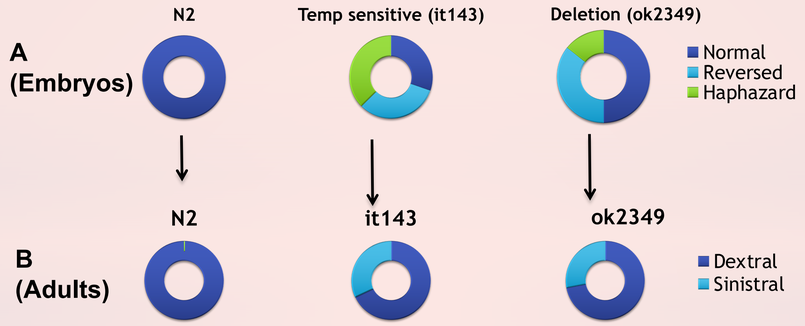

Figure 3: *gpa-16* mutants show increased rate of reversal. (A) All wild-type N2 embryos divided normally while about 1/3rd of both *t.s gpa-16 (it143)* and *del gpa-16 (ok2349)* divided in a reversed fashion. In addition, both strains of *gpa-16* mutants had embryos that divided randomly (n=67). (B) Of the surviving *gpa-16* embryos, about 30% of them develop to be sinistral worms but 100% of the N2 embryos become dextral (n=67).

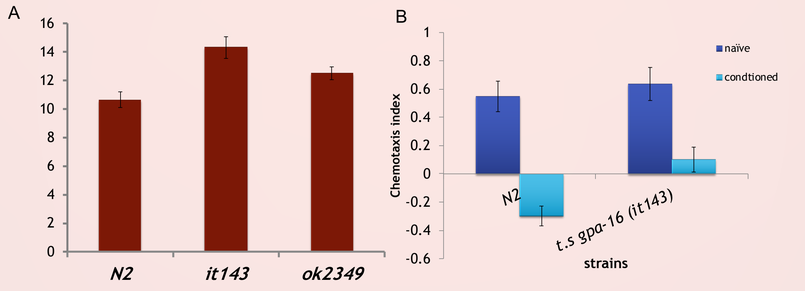

Figure 4: (A) Both strains of *gpa-16* were abnormal in non-associative learning (habituation). Compared to N2, *gpa-16* required more touches to habituate (n=40). (B) *gpa-16* mutants are defective in associative learning. Isoamyl alcohol was used to condition the worms in the absence of food. When transferred to a test plates, N2 worms avoided the isoamyl alcohol while *gpa-16* mutants maintained their attraction (n=7).

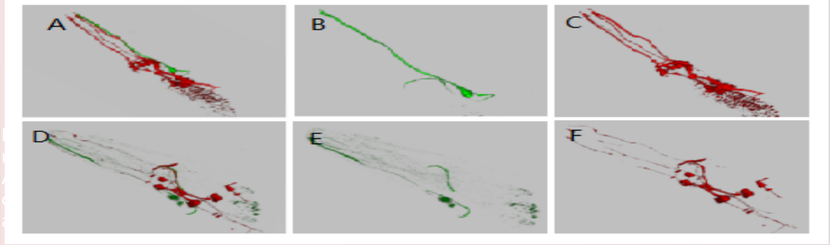

Figure 5: Connectivity of sensory neuron ASEr with interneuron AIY. Wildtype N2 animals show stereotypic ASEr location(A-C) while some of the ***del.gpa-16*** animals showed reversed ASEr neuron location(D-E). ASEr sensory neuron and AIY interneuron in N2 animals (A). ASEr located to the right of AIY, and axon loop projected to left (B). AIY interneurons in N2 animals (C). ASEr sensory neuron and AIY interneuron in ***del.gpa-16*** animals (D). ASEr located to the left of AIY and axon loop projected to right (E). AIY interneurons in ***del.gpa-16*** animals (F).

Discussion and future directions

- Absence of functional ***gpa-16*** results in randomly dividing embryos and reversed Left-Right laterality in adult worms, as compared to wildtype N2 worms.
- ***gpa-16*** mutants showed defects in non-associative learning and associative learning.
- Transgenic strain ***del.gpa-16:gcy-5::GFP:ttx-3::mCherry*** showed anatomically reversed ASEr and atypical neuronal connectivity with AIY interneuron in nerve ring at certain level.

Future work:

- Quantitatively analyze the synaptic transduction efficiency between ASEr and AIY neuron in *gpa-16* deletions and N2 animals.
- Calculating the proportion of ASEr reverse in ***del.gpa-16*** animals and evaluate its contribution to learning defects.
- Collecting and comparing transcriptomes derived from ***del.gpa-16 and N_2***
- Estimating the lateral asymmetry variation on another major chemo-sensory neuron AWC (l/r), and its connection mode to AIY interneuron in ***del.gpa-16*** animals.

Key references

- Bergmann DC, Lee M, Robertson B, Tsou MF, Rose LS, Wood WB. Embryonic handedness choice in *C. elegans* involves the Galpha protein GPA-16. Development. 2003;130(23):5731-40
- Wood WB. Handed asymmetry in nematodes. Seminars in Cell Dev Biol. 1998;9(1):53-60

Acknowledgements

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