

Targeted expression of *shibire*^{ts} and *semaphorin 1a* reveals critical periods for synapse formation in the giant fiber of *Drosophila*

R K Murphey, Stephan J Froggett, Phyllis Caruccio, Xiaoliang Shan-Crofts, Toshihiro Kitamoto, Tanja A Godenschwege

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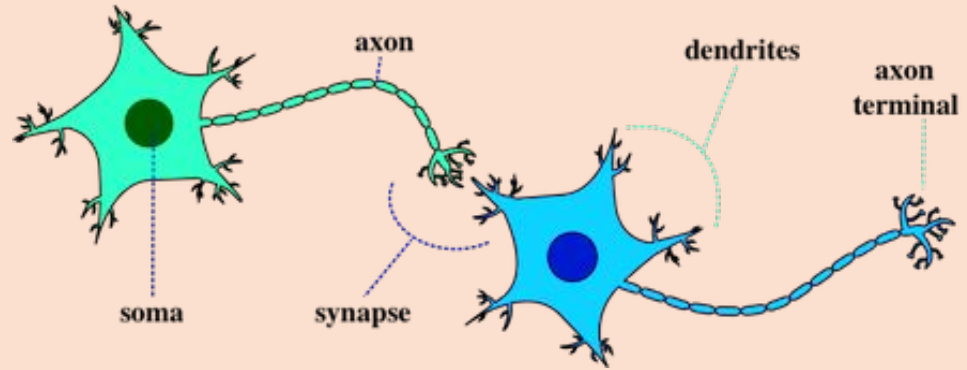
Abstract

In order to determine the timing of events during the assembly of a neural circuit in *Drosophila* we targeted expression of the temperature-sensitive shibire gene to the giant fiber system and then disrupted endocytosis at various times during development. The giant fiber retracted its axon or incipient synapses when endocytosis was blocked at critical times, and we perceived four phases to giant fiber development: an early pathfinding phase, an intermediate phase of synaptogenesis, a late stabilization process and, finally, a mature synapse. By co-expressing *shibire^{ts}* and *semaphorin 1a* we provided evidence that Semaphorin 1a was one of the proteins being regulated by endocytosis and its removal was a necessary part of the program for synaptogenesis. Temporal control of targeted expression of the *semaphorin 1a* gene showed that acute excess Semaphorin 1a had a permanent disruptive effect on synapse formation.

Key Terms

- *Drosophila*
- Synaptogenesis
- Semaphorin
- Endocytosis
- Dynamin

Synaptogenesis: the formation of new synapses between neurons; synapses allow the neurons to communicate with each other using chemical neurotransmitters.



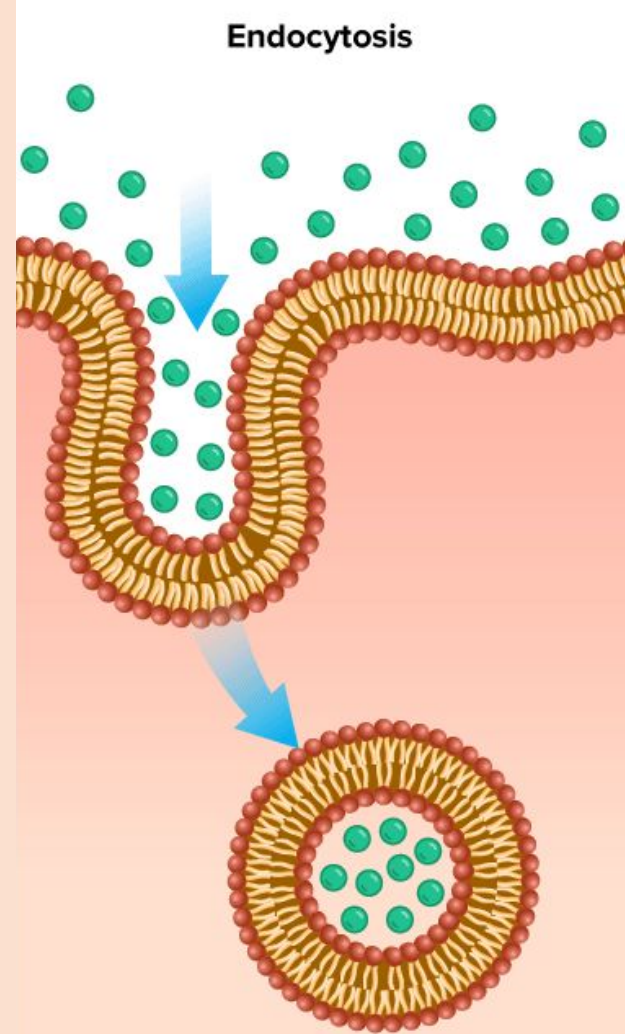
Semaphorin: a family of genes and their resulting proteins; Semaphorin 1a helps guide the direction and amount of growth of projections of cells, such as axons of neurons. The axon is the part of the neuron that sends signals to the next cells.

Nomenclature: genes of *Drosophila* are expressed in italics and are not capitalized: *semaphorin 1a*. Protein products of these genes are represented in normal text and have the first letter capitalized: Semaphorin 1a.

Endocytosis: a process by which a cell brings in outside material, like neurotransmitters, other signalling chemicals, proteins, or debris. The cell forms an external pocket open to the outside of the cell, letting in the desired material, then forms a vacuole inside the cell.

Dynamin: a protein that functions in cell division and the formation of vesicles, or bubbles of cell membrane inside the cell itself.

***Drosophila melanogaster*:** a species of fruit/vinegar flies used for genetic research



Introduction and Objective

Development of neurons and the creation of synapses between them isn't well understood in humans. Since it's difficult to experiment or research on humans directly, largely because of ethical concerns and a lack of time, other organisms like *Drosophila* are used instead.

It's known that neuronal formation and development in *Drosophila* requires several steps to be carried out correctly in a particular order, and a couple genes linked to each of these steps have been identified. This paper looks at these steps and when they take place during neurogenesis (development of the central nervous system), and tests effects when certain gene activities are altered.

The goal of this paper is to get a clearer understanding of how the developing organism creates these synapses, and what happens if they occur at the wrong time.

Methodology

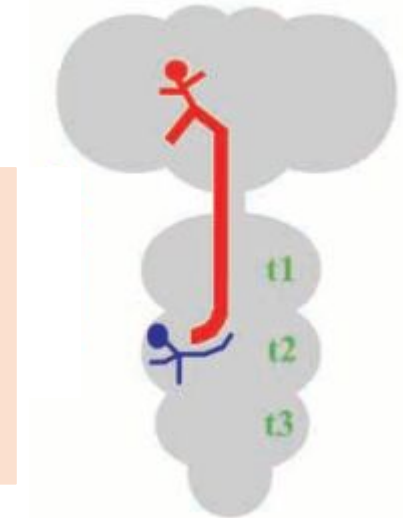
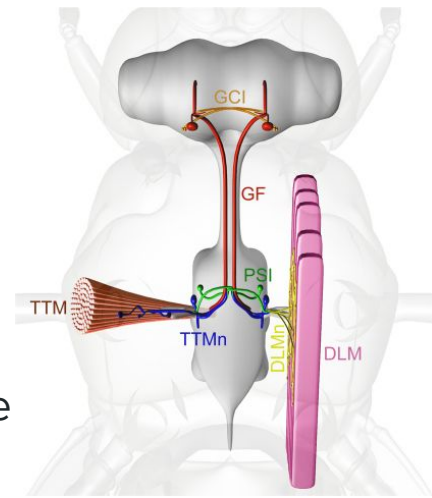
- Driver introduction
- Mutation synthesis
- Heat shocks
- Data collection
- Analysis

This study researched neuron development and synaptogenesis using the Giant Fiber Circuit (GFC), a specific circuit in the fly body used for escape.

The Giant Fibers (GFs) are two long and fast neurons that reach from the brain to the thorax, and are shown in red on the right. They are the main neurons within the GFC, and are very easy to access genetically because a lot of information is known about them.

Drivers are genes that “drive” expression of mutations, and can be expressed in certain kinds of cells.

- P[Gal4]-A307 is expressed in the GF, as well as weakly in other parts of the circuit including the muscles it controls.
- P[Gal4]-c17 is expressed in the GFs but not anything postsynaptic.
- P[shakB-Gal4], only targeted postsynaptic cells (anything downstream of the GFs, including the escape muscles).



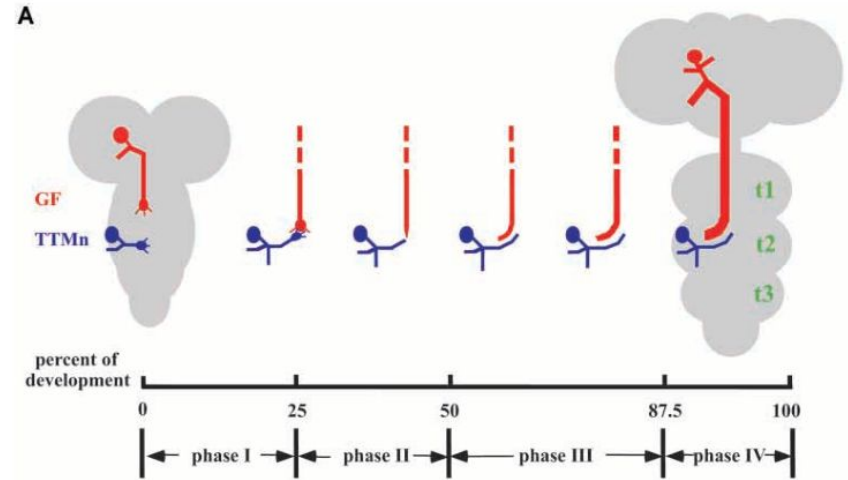
Using the drivers, it was possible to target expression of *shibire*^{ts} to certain neurons within the circuit. This mutated gene affects cell development negatively by making dynamin unstable, inhibiting the cells' ability to carry out endocytosis.

The gene is temperature sensitive, so the mutations only occur if the fly is at 30° C or above; otherwise, the mutations don't happen and the cells it's expressed in behave normally.

The expression of *semaphorin 1a* was also altered using the drivers; it was either increased or reduced during development.

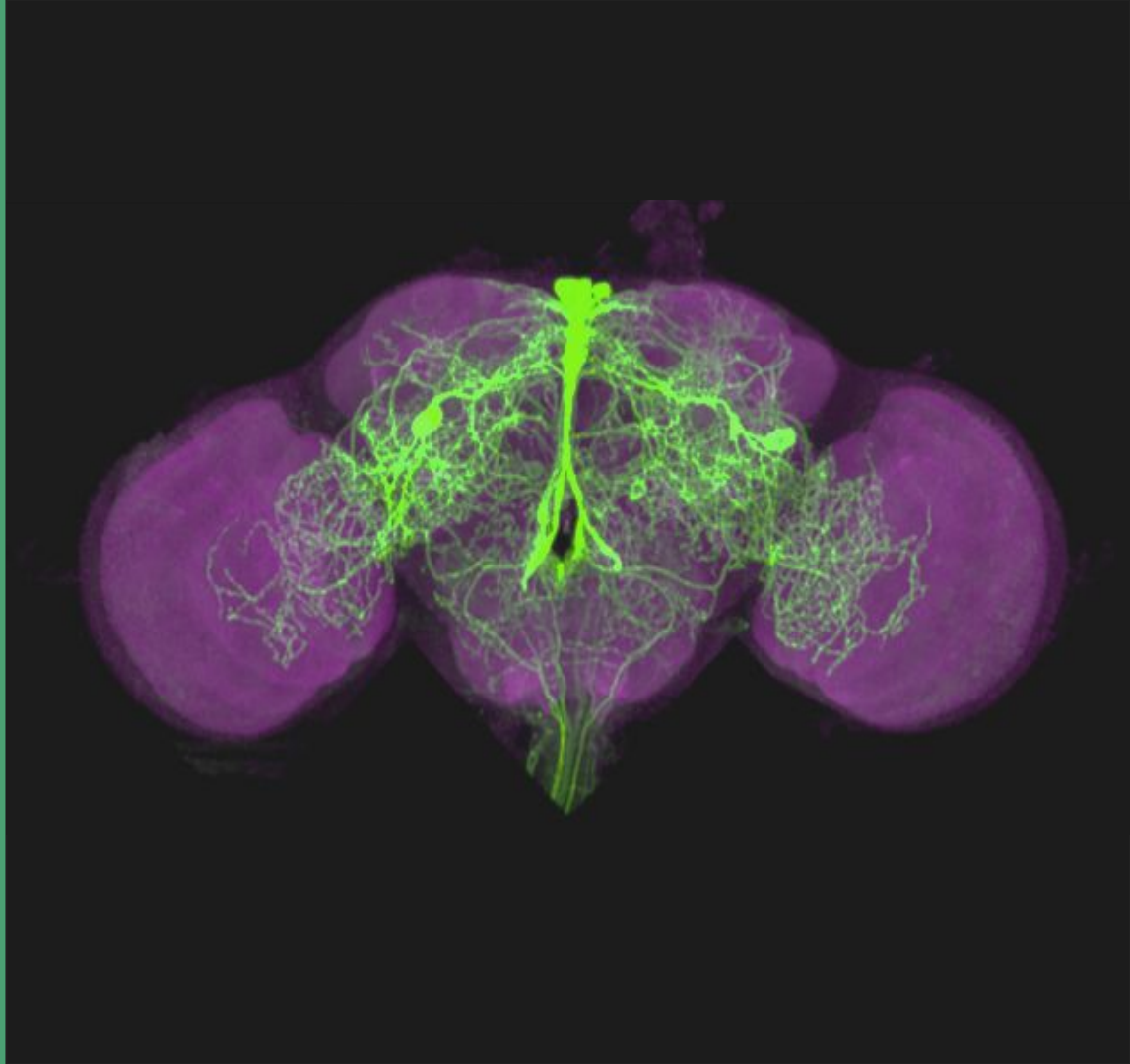
The effects of these mutations were tested independently and together, and at different times during development.

Murphey et al. constructed a 4-stage development plan for the circuit. They blocked different cells' development through *shibire^{ts}* and the GFC-specific drivers, and altered *semaphorin 1a* at different stages, resulting in different effects on the circuit.



These effects were documented for several samples, and a few flies' CNSs were dissected and stained to show the morphological differences in the development of the Giant Fiber.

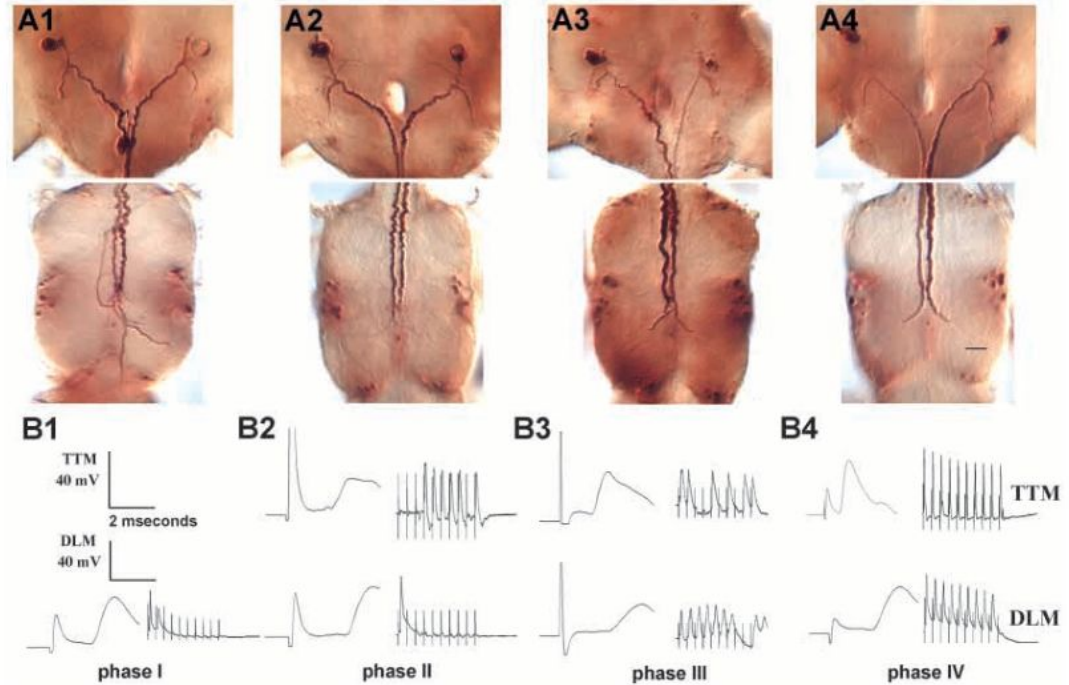
Results



This figure shows alteration of the Giant Fiber's shape and length based on which phase in development *shibire*^{ts} was activated.

These images show dissections of adult central nervous systems, stained to show the Giant Fiber.

Blocking during stage 4 resulted in a morphologically normal GF, but earlier blocking had negative effects on axon growth and Termination.



This figure shows the measured alterations in the circuit under effects of *shibire^{ts}* and an increase of *semaphorin 1a* expression.

This shows that the Semaphorin 1a protein, while critical in earlier stages of neuron development, must be removed later; otherwise, the axon doesn't develop fully.

Phase	Genotype*	Stage [†] of TS (dissection)	n	Anatomy				n	Physiology				
				Wild type (%)	Bendless [‡] (vesicles) (%)	t1 (%)	Brain (%)		TTM wild type [§] (%)	TTM latency in mseconds	TTM FF (%)	TTM dis- connection (%)	TTM or DLM connection (%)
I	shi ^{ts}	12.5 (adult)	25	88	8	0	4	11	81	1.05	90.7	0	100
I	sema	12.5 (adult)	31	58	26	13	3	19	33	1.14 [¶]	58 [¶]	5	100
II	shi ^{ts}	37.5 (adult)	32	94	6	0	0	16	88	1.01	91.3	0	100
II	sema1a	37.5 (adult)	13	15	73	12	0	12	0	1.67 [¶]	30.4 [¶]	8	92
II	shi ^{ts} +sema	37.5 (adult)	18	6	44 (44)	6	44	13	0	1.61 [¶]	14.9 [¶]	46	54
II	shi ^{ts} +sema	37.5 (after TS)	33	0	46 (0)	23	31	nd	nd	nd	nd	nd	nd
II	shi ^{ts}	50 (adult)	36	83	17	0	0	16	56	1.09	79 [¶]	0	100
II	sema	50 (adult)	26	38	62	0	0	14	7	1.18 [¶]	54.8 [¶]	7	93
II	shi ^{ts} +sema	50 (adult)	29	21	59 (24)	7	14	17	0	1.59 [¶]	42.6 [¶]	35	82
II	shi ^{ts} +sema	50 (after TS)	11	0	55 (0)	27	18	nd	nd	nd	nd	nd	nd
III	shi ^{ts}	62.5 (adult)	19	95	5	0	0	18	44	1.08	95.9	0	100
III	sema	62.5 (adult)	18	83	17	0	0	19	58	1.03	83.6	5	95
III	shi ^{ts} +sema	62.5 (adult)	18	33	61 (17)	0	6	14	0	1.85 [¶]	10.9	21	93
III	shi ^{ts} +sema	62.5 (after TS)	21	0	33 (0)	0	14	nd	nd	nd	nd	nd	nd
	shi ^{ts}	No TS (adult)	31	90	10	0	0	21	90	0.92	99.8	0	100
	sema	No TS (adult)	18	83	11	6	0	21	67	0.96	81.3	5	100
	shi ^{ts} +sema	No TS (adult)	58	50	40	5	5	22	45	1.08	74.2	0	100

Discussion

Through alteration of cell functionality with *shibire*^{ts}, this paper shows critical periods for development of the Giant Fiber. If interruption happens too early, the neuron won't form at all; if it happens later in development, the neuron will appear normal but won't synapse to its targets and therefore won't electrically function.

Through extended expression of *semaphorin 1a*, this paper shows that development takes place through not only a specific order, but a specific place in time; if this development is out of sync with the rest of the fly's body, such as muscular development or exoskeleton formation, the circuit won't function properly with the rest of the body.

These results can be extrapolated to other circuits in *Drosophila* besides the GFC; this therefore creates a model for circuit development over time during neurogenesis.

Application

This paper gives a basis for neuron development and circuit development over time, which is a model that can be extended to other organisms. If the mechanism is retained in mammals like humans, this could provide insight into neurodegenerative diseases and congenital neuronal disorders.

Understanding how the nervous system develops over time is important for research into disease treatment, and important for further research in related fields.

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