Linking transcription factors to neuronal connectivity in the Central Complex of *Drosophila*

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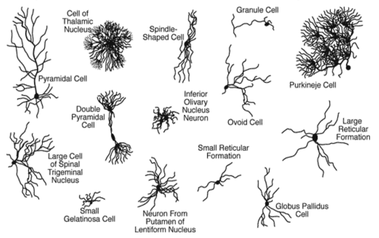
Abstract

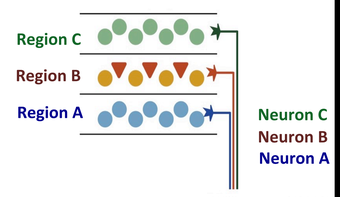
Every neuron in the brain has a specific identity including a key feature called neuronal connectivity, which is defined as how neurons form synaptic pairs. Neurons innervate certain regions of the brain through an attribute of neuronal connectivity known as target specificity. We hypothesize that proteins called Transcription Factors (TFs) bind to DNA to regulate gene expression that determines target specificity. More than 750 TFs are present in the fruit flies, *Drosophila melanogaster*, but antibodies for only 200 TFs are available. The study was done in the Central Complex (CX) of adult *Drosophila*. Located in the midbrain, the CX is an ancient conserved brain region critical for insect navigation. The purpose of our study was to identify the post-mitotic TF profile of neurons innervating the adult central complex using antibody screening in flies driven by the binary CX GAL4-UAS system to relate to neuronal connectivity in the CX. Here, we screened 85 TF antibodies in the Central Complex of adult brains. Our results show that 22 TFs from 85 antibodies express in differential densities in the CX. We additionally discovered at least two specific TFs that label single cell-types innervating the central complex. This will provide a primary basis for further research on the relationship between specifying neuronal identity and establishing neuronal connectivity. Thus, the results could enhance our understanding of the underlying causes of neuropathological disease.

Background

Each neuron has a specific identity including a key feature: neuronal connectivity

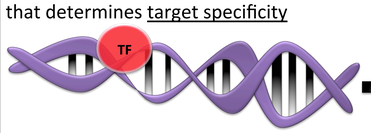


Neurons innervate stereotyped regions of the brain (Target Specificity)



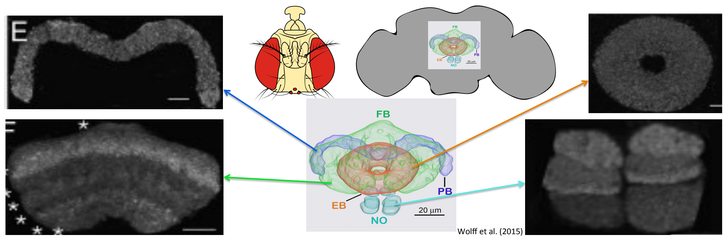
Williams *et al* (2010)

Hypothesis: Transcription Factors (TFs) bind to DNA to regulate gene expression that determines target specificity



Regulate genes that determine target specificity

The Central Complex is an ancient conserved brain region critical for insect navigation



Wolff et al. (2015)

My Aims

Primary goal of the project: Identify TFs that determine target specificity

Aim 1: To identify the TFs expressed in the adult Central Complex (CX)

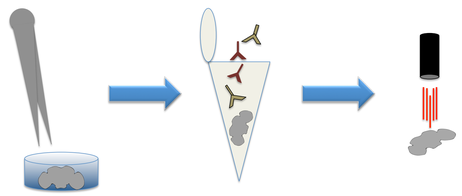
Aim 2: To relate the identified TFs to single cell-types in the CX

Final aim: To relate the identified TFs to neuronal connectivity for single cell-types innervating the CX

Methods

Aim 1: To identify the TFs expressed in the adult CX

- **Screening the TFs**

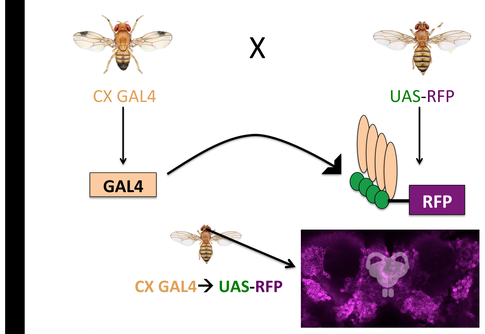


Dissect adult *Drosophila* brains

Stain with Primary and Secondary antibodies

Image on the Confocal

CX GAL4-UAS system labels cells innervating the CX



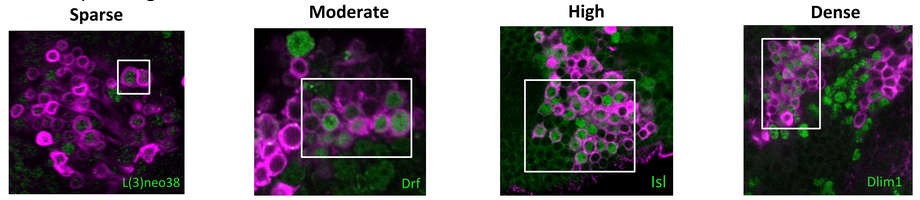
RFP: Red Fluorescent Protein

Cell bodies of neurons in the CX

Results

Aim 1 results:

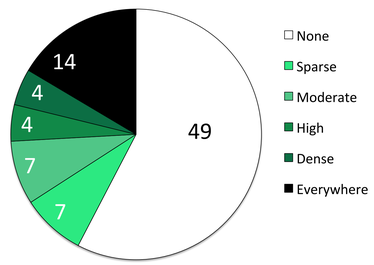
- 22 out of 85 TFs tested are expressed in the adult *Drosophila* Central Complex
- Positively-identified TFs grouped into 4 categories: Sparse, Moderate, High, and Dense according number of cells expressing the labeled TF



22 TFs (from 85 antibodies) express in differential densities in the CX

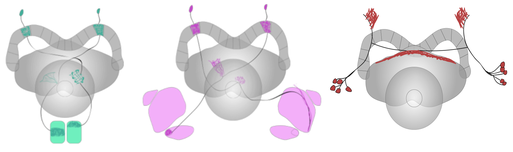
Abd-A		CG9932		eya		knirps		seq	
abrupt		CG9932		eya		Kr		slp1	
acj6		chm		fkh		l(3)neo38		slp1	
bigmax		crp		FoxD		Lim3		slp1	
BtbVII		cut		ftz1		lz		slp2	
cas		dar1		gce		mab		slp2	
CG10267		Dfd		gem		maf-S		Sox-15	
CG12071		DHR4		Hb9		maf-S		Ssdp	
CG12391		dlim1		HmgZ		mbl		Ssdp	
CG12391		Dll		hth		mid		Su(H)	
CG12565		Drf		inv		MTA-1 like		svp	
CG12605		E93		inv		odd		tai	
CG15715		eagle		lsl		opa		ttl	
CG15715		Eip75B		jim		otd		trio	
CG18619		Eip75B		jing		pdm3		Unc-4	
CG18619		ems		kni		RunxB		Vsx2	
CG31224		eve		kni		Scr		vvl	

Distribution of TF's expression



Aim 2: To relate the identified TFs to single cell-types innervating the CX

Single cell-types innervating the CX



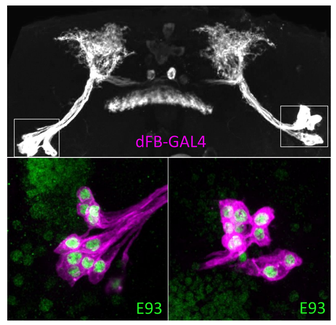
Intrinsic Neurons *Noduli*

Extrinsic Neurons *LAL*

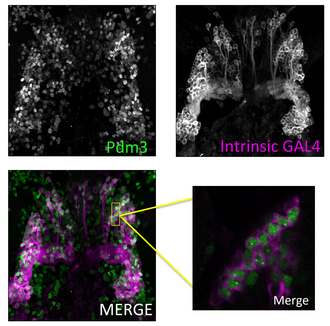
Dorsal Fanshaped body Neurons (dFB) *FB*

Tested for TFs expressed in the CX: **E93, Pdm3,** and **Lz**

E93 is expressed in all dFB neurons



Pdm3 is expressed in all CX-intrinsic neurons



Next step:
Confirm with single cell-type driver

Aim 2 Results:
Relating TFs to single cell-types innervating the CX

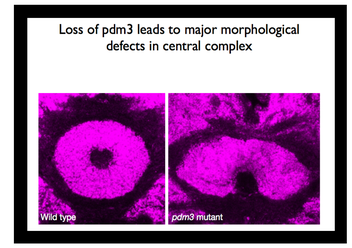
	CX-Intrinsic	CX-Extrinsic	dFB neurons
• E93	?	?	All+
• Pdm3	+	-	?
• Lozenge	?	-	?

Conclusion

- 22 out of 85 TF antibodies are expressed with varying density in the CX
- E93 is expressed in all dFB neurons in the CX
  - Functions to regulate sleep behavior
- Pdm3 is expressed in intrinsic neurons in the CX
  - Functions to mediate navigation during flight

Future Directions

- Vary the degree of antibody concentration for TFs expressed everywhere and not expressed at all in this project
- Continue to relate the identified TFs to single cell-types innervating the Central Complex
- Relate the identified TFs to neuronal connectivity for single cell-types innervating the CX
  - Loss of Function neuroanatomical analyses in the adult CX



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Acknowledgments

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