

Feminizing cholinergic neurons in a male *Drosophila* nervous system enhances aggression

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Previous studies in *Drosophila* have demonstrated that whether flies fight like males or females can be switched by selectively manipulating genes of the sex determination hierarchy in male and female nervous systems. Here we extend these studies by demonstrating that changing the sex of cholinergic neurons in male fruit fly nervous systems via expression of the *transformer* gene increases the levels of aggression shown by the flies without altering the way the flies fight. Transformer manipulation in this way does not change phototaxis, geotaxis, locomotion or odor avoidance of the mutant males compared to controls. Cholinergic neurons must be feminized via this route during the late larval/early pupal stages of development to show the enhanced aggression phenotype. Other investigators have shown that this is the same time period during which sexually dimorphic patterns of behavior are specified in flies. Neurons that co-express *fruitless* and choline acetyl transferase are found in varying numbers within different clusters of *fruitless*-expressing neurons: together they make up approximately 10% of the pool of *fruitless*-expressing neurons in the brain and nerve cord.

Supplementary Material

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Supplementary information

Table S1. Counts of the numbers of Fru^M positive and *cha-Gal4*-driver-labeled neurons in various *Fruitless*-neuron clusters in the fly nervous brain and nerve cord.

	Fru ^M			Cholinergic			%
	#	SEM	n	#	SEM	n	Chol
Neuronal Clusters							
Anterior Brain							
1 <i>fru</i> -aSP1	18	2	14	0	0	14	0
2 <i>fru</i> -aSP2	67	14	14	3	1	14	4
3 <i>fru</i> -aSP3	45	7	14	3	2	14	6
4 <i>fru</i> -Lv	22	3	14	1	1	14	3
5 <i>fru</i> -mAL	35	3	14	2	1	14	7
6 <i>fru</i> -AL	71	8	14	8	3	14	11
7 <i>fru</i> -mcAL	52	7	14	3	1	14	5
Anterior and Posterior Brain							
8 <i>fru</i> -SG	33	7	7	15	4	7	44
9 <i>fru</i> -M							
10 <i>fru</i> -Ld							
Posterior Brain							
11 <i>fru</i> -Lo							
12 <i>fru</i> -pSP1	20	3	14	4	2	14	18
13 <i>fru</i> -pSP2	26	9	14	2	1	14	8
14 <i>fru</i> -P	76	12	14	14	3	14	18
15 <i>fru</i> -pL	12	5	14	1	1	14	4

Total Brain		923	40	7	94	9	7	10
Ventral Ganglia								
16	<i>fru-Pr</i>	67	19	13	3	2	13	4
17	<i>fru-PrMs</i>	248	30	13	12	8	13	5
18	<i>fru-MsMt</i>	139	21	13	8	2	13	6
19+20	<i>fru-MtAb & -Ab</i>	313	43	13	51	13	13	16
Total Ventral Nerve Cord		766	91	13	74	17	13	10

Figure S1. Top view of multiwell plate used in behavioral screen (see Methods).

