Gene *CG15630* (*fipi*) participates in determination of the interpulse interval in *Drosophila* courtship song

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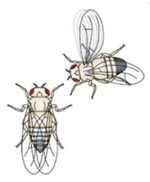
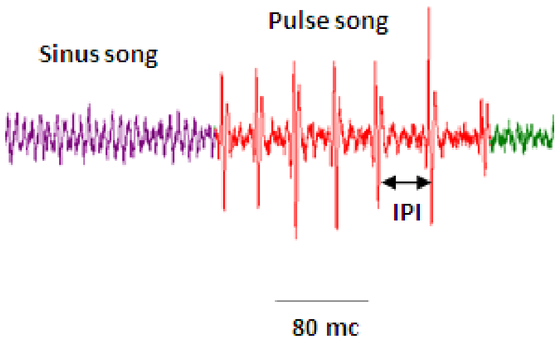
Introduction

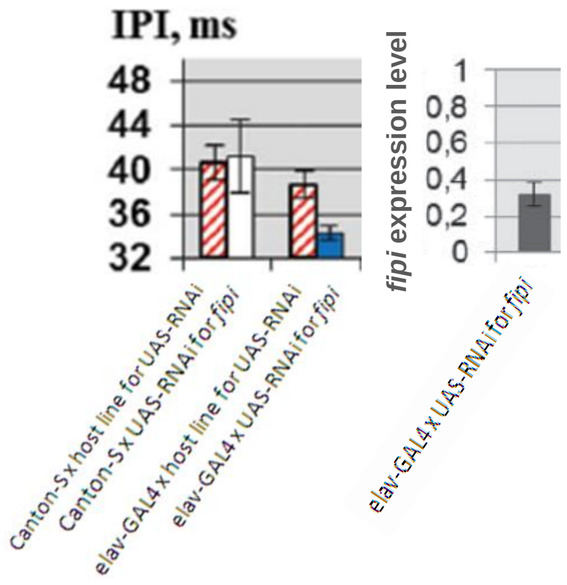
In order to study the mechanisms of functioning of the central pattern generator of rhythmic activity we have previously identified genes, the knockdown of which is accompanied by deviations in the pulse song produced by *D. melanogaster* males during courtship (Fedotov et al., 2014).



Pulse song produced by rhythmic strokes of a wing at frequency specific for each *Drosophila* species.



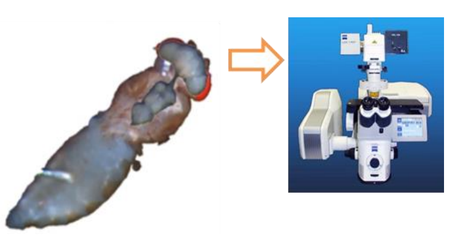
Reduced expression of the gene *CG15630*, which we have called ***fipi*** (*factor of interpulse interval*), resulted from its neurospecific knockdown causes a decrease in the interpulse interval (IPI). The level of *fipi* expression in the knockdown, assessed using real-time RT-PCR, is about one third of the value for corresponding control.



Methods

To identify specific neural networks, where reduced expression of *fipi* causes changes in the IPI, we tested flies with local *fipi* knockdown in the sensory, motor, monoaminergic, cholinergic and other types of neurons. Knockdown was created by crossing Gal4 driver lines (BDSC) and UAS-RNAi line with interfering RNA for *fipi* (VDRC, KK107797). Crosses of the same Gal4 drivers with the host line for VDRC UAS-RNAi KK library (# 60100) were used as controls.

Distribution of the polypeptide coded by *fipi* (FIPI) and FAS2 protein was examined in the CNS on the confocal microscope LSM710. FAS2 is a factor of the axonal growth and was shown to interact physically with the protein FIPI (Ozkan et al., 2013). Whole-mount samples of dissected and fixed CNS were stained with antibodies to FIPI (31-2) and FAS2 (DSHB 1D4). FIPI antibodies originated from rabbits injected with unique peptide coded by a sequence from the *fipi* second exon. The preimmune serum was used as a control for antibody specificity.



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Research aim

Identification of neuronal networks, where reduced expression of the gene *fipi* causes changes in the interpulse interval (IPI) of courtship song

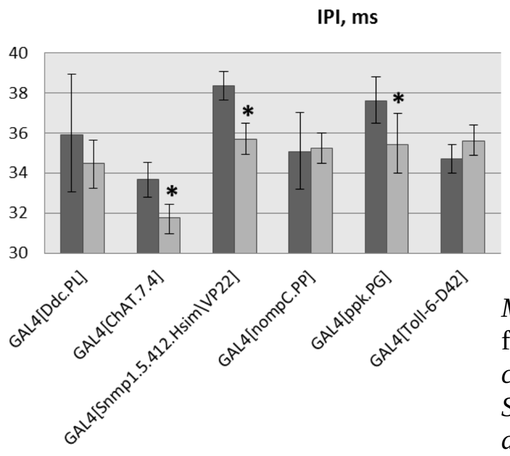
Conclusion

The polypeptide coded by *fipi* (FIPI) functions in cooperation with NCAM protein FAS2 in determination of IPI through processing information from chemosensory organs

Results

fipi knockdown in distinct functional subsets of neurons decreases IPI

We found that *fipi* knockdown in the antennal olfactory sensory neurons (*Snmp-GAL4* driver), which are responsible for reception of pheromones (including cis-vaccenyl acetate) during courtship, reduces the IPI. The same effects were shown for cholinergic neurons (*Chat-GAL4* driver). It is the acetylcholine that is the main neurotransmitter in the sensory and projection neurons of the olfactory system.

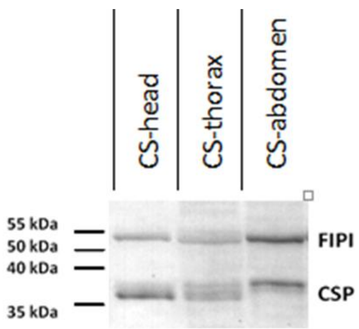


GAL4[Ddc.PL]	dopaminergic and serotonergic neurons
GAL4[ChAT.7.4]	cholinergic neurons
GAL4[Snmp1.5.412.Hsim\VP22]	olfactory sensory neurons of the antenna
GAL4[nompC.PP]	the class III dendritic arborization neurons gentle-touch sensation
GAL4[ppk.PG]	class IV dendritic arborization neurons mechanical nociception
GAL4[Toll-6-D42]	motor neurons

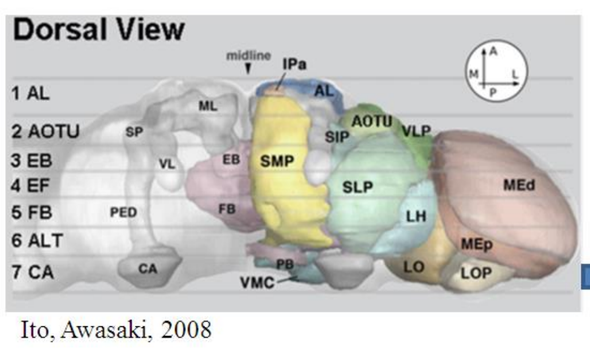
Mean values of IPI (n = 20) in RNAi-mediated knockdown of fipi *under the control of various GAL4 drivers and 95% confidence intervals. The controls are shown with dark bars. Significant differences from control are marked with an asterisk (two-sided randomization test, P <0.05). The list on the right describes the types of cells in which the drivers are expressed.*

fipi encodes a protein of 53 kDa

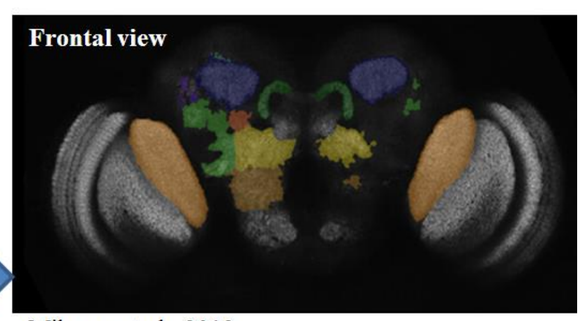
Western blotting with polyclonal antibodies against FIPI detects a protein of approximately 53 kDa. FIPI band was identified in the head, abdomen and slightly in the thorax (Canton-S). Each sample consists of the body part of six flies. RNAi mediated knockdown of *fipi* under Act5c-GAL4 did not reduce FIPI quantity.



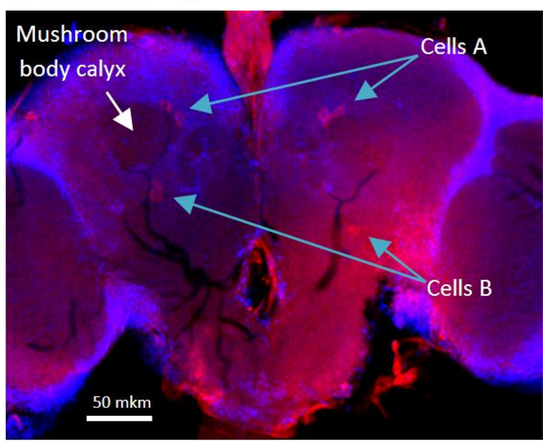
FIPI and FAS2 proteins are colocalized in the two groups of cells in the posterior areas of the brain

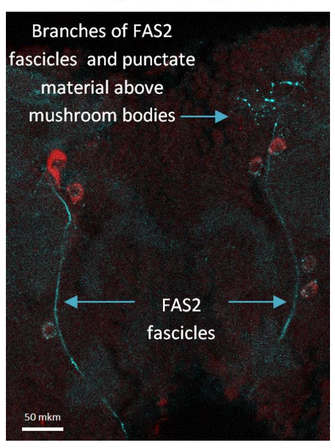


Ito, Awasaki, 2008



Milyaev et al., 2012





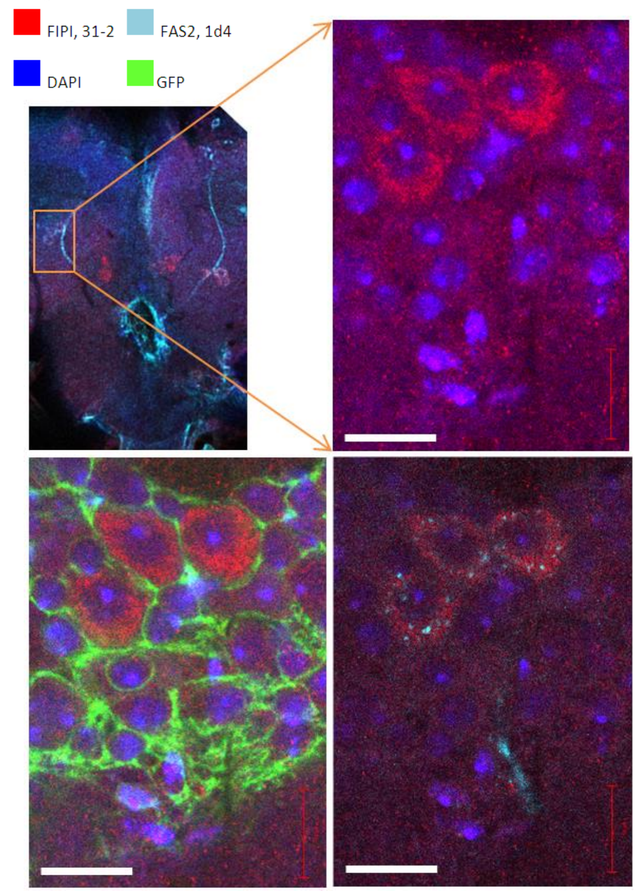
Two samples from crossing Act5C-GAL4 x host line for UAS-RNAi

Confocal slices of the brain at the level of 7 CA. Magnification x10.

FIPI, 31-2
FAS2, 1d4
DAPI

Immunohistochemical analysis of FIPI distribution in the CNS reveals two groups of cells in the posterior brain areas, whose processes join the paths immunostained against the cell adhesion protein FAS2. Each group consist of 1 to 4 cells. Cell bodies in the first group (A) are adjacent to the dorsal-medial surface of the calyces of mushroom bodies. The cells of the second group (B) locate on the rear surface of the ventromedial neuropil. Fascicles containing FAS2 are in close proximity to the cell bodies of both groups .

FIPI and FAS2 are detected in the cytoplasm in the neuron soma

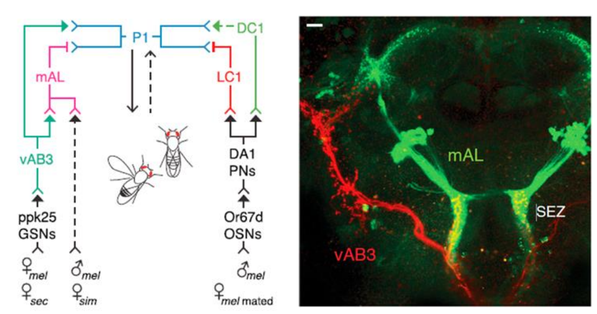


Sample from crossing nrv2-GAL4; UAS-GFP x host line for UAS-RNAi. Confocal slices of the brain at the level of 7 CA. Magnification x100. Scale bars: 10 μm

Immunogenic material to FIPI antibodies is diffusely distributed in the cytoplasm of neurons. Unlike FIPI, FAS2-immunogenic material is located in the cytoplasm in the form of distinct clumps. The cells are wrapped with cortex glia. This confirms our previous conclusion that FIPI functions in neurons rather than in glia (glia-specific *fipi* knockdown under control of *repo-GAL4* had no effect on IPI value, Fedotov et al., 2014).

Discussion

According to the literature data, it is the posterior brain regions where the neural network, which controls the initiation of courtship behavior, is localized (*e.g.*, Philipsborn et al., 2011). The network includes several types of interneurons (mAL, DC1) modulating the activity of P1 interneuron, which triggers courtship in response to signals from chemoreceptors.



Clowney et al., 2015

Location of FIPI-reactive B-cells coincides topographically with the location of inhibitory neurons mAL. Besides, mAL processes go to the neuropil around the esophageal passage, that agrees with the trajectory of FAS2 fascicles in our study. Activity of olfactory sensory neurons, where local knockdown of *fipi* reduces IPI, suppresses P1 neurons. Since inhibition of P1 neurons, in turn, leads to IPI reduction, we have concluded that FIPI functions in cooperation with FAS2 in determination of IPI through processing of inhibitory inputs from chemosensory organs.

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