DOI 10.1007/s11055-014-0015-2
Neuroscience and Behavioral Physiology, Vol. 44, No. 9, November, 2014

Genetic Determinants of Rhythmic Movements Motor Pattern Generation in *Drosophila Melanogaster*

S. A. Fedotov, Yu. V. Bragina, N. G. Besedina, L. V. Danilenkova, E. A. Kamysheva, and N. G. Kamyshev

Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 99, No. 1, pp. 120–130, January, 2013. Original article submitted October 23, 2012.

Our previous studies of the molecular and cellular mechanisms underlying the generation of rhythmic movements motor patterns were based on use of a selection of candidate genes in which mutations cause impairments to the motor activity of *Drosophila melanogaster*. We report here testing of the locomotor behavior of *Drosophila* strains with decreases in the expression of 12 candidate genes in the nervous system. Target genes were suppressed by synthesizing interfering RNA using the GAL4/UAS system under the control of the *elav*, *nrv2*, *appl*, and *tsh* gene promoters (drivers). These experiments showed that RNA interference of virtually all the candidate genes was accompanied by changes in one or more locomotor parameters. The nature of the abnormalities occurring under the control the various drivers allowed us to identify those genes whose activity in nervous system cells is required for the normal functioning of the central motor pattern generator for locomotor acts.

Keywords: locomotion, *Drosophila*, RNA interference, central motor pattern generators.

Formation of the motor output in animal nervous systems is determined by regulatory and triggering impulses from sensory and integrative structures, as well as the intrinsic properties of motoneurons and their interactions [6]. The motor output produced in response to single stimuli can be in the form of transient bursts of activity (for example, the knee-jerk reflex) or as a prolonged, rhythmically organized sequence of impulses (for example, wing-flapping in insects). This latter type of response is associated with the existence of central motor pattern generators (CMPG) in the nervous system, these consisting of single pacemakers or groups of neurons which interact in such a way as to produce a rhythmic output. Rhythmic pacemaker activity is defined by oscillations in membrane permeability to sodium, potassium, and calcium ions, though the molecular and cellular mechanisms underlying these oscillations have received very little study [4].

I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, 6 Makarov Bank, 199034 St. Petersburg, Russia; e-mail: serg900@yandex.ru.

Our previous studies used genetic methods to identify more than 20 candidate genes whose products are presumptively involved in the CMPG in *Drosophila* [2]. Mutations in these genes are accompanied by impairments in locomotor behavior and male mating songs. We report here studies based on testing locomotor activity in *Drosophila* strains with reduced expression of 12 candidate genes in the nervous system of flies. Expression was suppressed by synthesizing interfering RNA using the GAL4/UAS system. Localization of RNA interference in the nervous system was obtained by placing the GAL4 activator under the control of the *elav*, *nrv2*, *appl*, and *tsh* promoters, which are expressed exclusively or predominantly in nervous structures [8]. Suppression of the activity of each of these candidate genes (with the exception of *kermit*) in nervous system cells was found to lead to specific changes in different parameters of locomotor activity. The nature of these motor deviations, with consideration of the patterns of expression of the driver, allowed the possible roles of each candidate gene in the processes forming and operating motor pattern generators to be identified. Of the 12 candidate genes stud-

ied, the *CG15630* gene is of particular interest; this is expressed mainly in the nervous system, where is it presumptively a component of neuron cell surfaces and is involved in remodeling the cell cytoskeleton. The product of the CG15630 gene is an obligate component of neuronal circuits involved in mediating locomotor activities, and the functional importance of CG15630 depends on the nervous structure in which it is expressed.

Methods

Testing of motor activity. Flies were reared and kept on standard raisin-yeast medium at 25°C with a 12-h light regime. Locomotor activity was recorded as described previously [2] in 20 individuals simultaneously using two webcams and the *Drosophila tracks* program (by N. G. Kamyshev) to determine the coordinates of each fly at a frequency of 10 Hz, with subsequent processing of the data using the analysis module of the program [1]. Statistical analysis of motor activity parameters was performed using a randomization test [11].

Local candidate gene knockdown system. The expression of candidate genes in the nervous systems of the test strains was suppressed using interfering RNA synthesized in transgenic flies using the GAL4/UAS system [10]. Flies with the GAL4/UAS system were prepared by crossing two transgenic strains, one containing the GAL4 transcriptional activator gene (maternal strain) and the other the regulatory UAS sequence. Binding of GAL4 with the UAS switches on transcription of the element located in the UAS and encoding an interfering RNA (the UAS/RNA transgene). Tissue-specific interference was obtained by placing the GAL4 activator gene under control of the *elav*, *appl*, *nrv2*, and *tsh* gene promoters, which are expressed predominantly in the nervous system in *Drosophila*.

Transgenic strains carrying the GAL4 activator gene were prepared from the Bloomington *Drosophila* Stock Center (BDSC) at Indiana University (USA) and had the following genotypes:

a) *w**; P{GAL4-elav.L}2/CyO;

b) p{Appl-GAL4.G1a}1, y^1 w*; $Mlf^{\Delta 10}$/CyO;

c) *w**; P{nrv2-GAL4.S}8 P{UAS-GFP.S65T}eg^{T10};

d) y^1 w^{1118}; P{GawB}tsh^{md621}/CyO; P{UAS-y.C}MC1/TM2.

Strains containing a UAS/RNA transgene and strains genetically identical to them but lacking the UAS/RNA transgene and marker gene (parental strain controls) were provided by The Vienna *Drosophila* RNAi Center (VDRC) at the Campus Science Support Facility (Austria). Nine strains with UAS/RNA transgenes had the genotype y w^{1118}; P{attP y^+ $w^{3'}$} and three strains (for the *mesr4*, *Treh*, and *jumu genes)* had the genotype w^{1118}.

Operational efficiency of the GAL4/UAS system. The efficiency of the GAL4/UAS system was assessed in terms of the presence and location of the fluorescent protein green fluorescent protein (GFP) in *Drosophila* embryos at the late stages of development before hatching of larvae. Crossings with driver strains (elav-GAL4, appl-GAL4, tsh-GAL4) used strains containing the *GFP* transgene under control of the UAS sequence. This strain was obtained from the BDSC and had the genotye *w**; P{10XUAS-IVS-GFP-WPRE}attP2. *Drosophila* eggs were dechorionized [25] and placed on a slide in 50% phosphate buffered saline in glycerol for analysis of GFP fluorescence using a MikMed2 luminescence microscope (FITC filter). Photographs of embryos were taken at a magnification of · 20 using a CX05 video camera (Baumer Optronic). The parental strains were used as controls.

In the case of driver strain nrv2-GAL4, whose genome already contains the UAS/*GFP* transgene, the efficiency of the GAL4/UAS system was also assessed by crossing with a strain containing a β-galactosidase transgene under control of a UAS (genotype y^1 w^{1118}; P{UAS-lacZ.Exe1}2, BDSC). A protein extract was prepared from the offspring of crossing 10 adult males and this was used to assess the efficiency of GAL4/UAS in terms of β-galactosidase activity [22]. Activity was measured as the optical density of samples after running the enzyme reaction for 2 h. Protein contents in samples were made uniform by assay using the Bradford method.

Estimation of candidate gene expression levels. Levels of candidate gene expression in test strains were assessed by quantitative polymerase chain reaction (PCR) using the fluorescence dye EvaGreen (Biotium Inc.). RNA was extracted from 20–30 *Drosophila* males [9]. The resulting samples were treated with DNase (Fermentas) and used for cDNA synthesis in a reverse transcription reaction using a protocol and reagents from SibEnzim (http://russia.sibenzyme.com). PCR with cDNA samples was performed in a StepOnePlus thermal cycler (Applied Biosystems) with real-time detection of the quantity of amplified product. The internal standard was the *RpL32* gene. The negative control was a sample subjected to a sham reverse transcriptase reaction, i.e., without transcriptase. PCR was run in the following conditions: 95°C (5 min), 1 cycle; [95°C (30 sec), 63°C (30 sec), 72°C (60 sec)] 35 cycles. The following primers were used:

***CG15630* gene:** forward: ATTCGTTGAGATTCTCGCAATGCG, reverse: CGGCGATTTCCAATGGAGCT;

***sps2* gene:** forward: TGAGAAGGAACGCGACGTTGTG, reverse: GCTGCGCGTTTGACGGTAGTATTAT;

***RpL32* gene:** direct: TATGCTAAGCTGTCGCACAAATGGC, reverse: GTTCTGCATGAGCAGGACCTCCA.

Data were analyzed statistically using the freeware program REST 2009 [18].

Results

Motor activity in strains with local knockdown of candidate genes. Locomotor activity was tested in *Drosophila* strains with decreases in the expression of candidate genes in the nervous system. A total of 12 genes were analyzed: *jumu*, *Treh*, *mesr4*, *CG15630*, *sps2*, *Dgp-1*, *CG34460*, *CG6746*, *CG8708*, *cf2*, *ext2*, and *kermit*. Suppression of one of these genes was produced in each strain using interfering

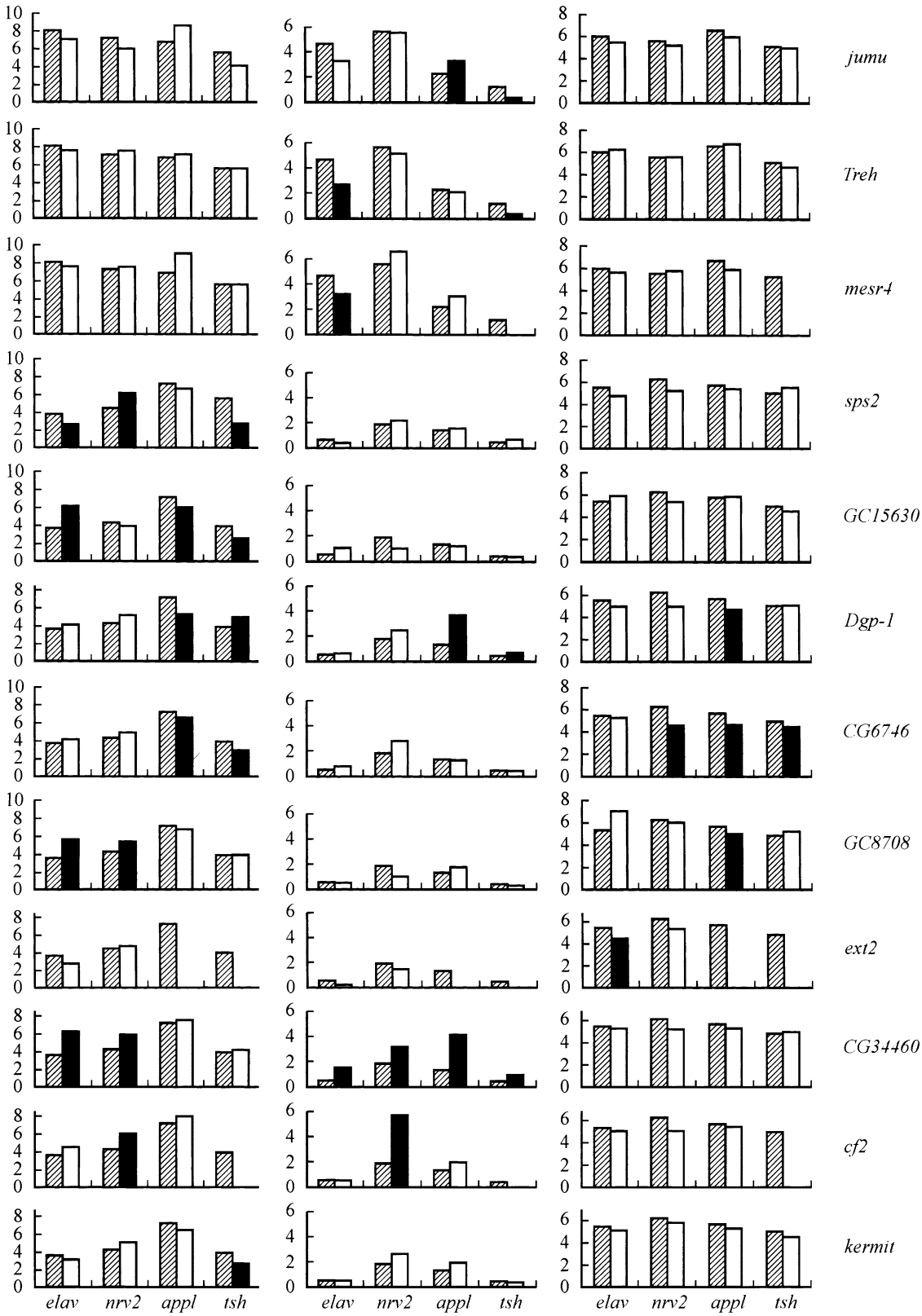

Fig. 1. Measures of motor activity in strains with local knockdown of candidate genes under control of the *elav*, *appl*, *nrv2*, and *tsh* promoters. For each gene, plots from left to right show run duration (sec), run initiation frequency (number per 100 sec), and run speed (mm/sec). Plots show mean values. Shaded columns show controls; significant differences from control are shown in dark columns (randomized test, 10000 iterations, $p < 0.05$).

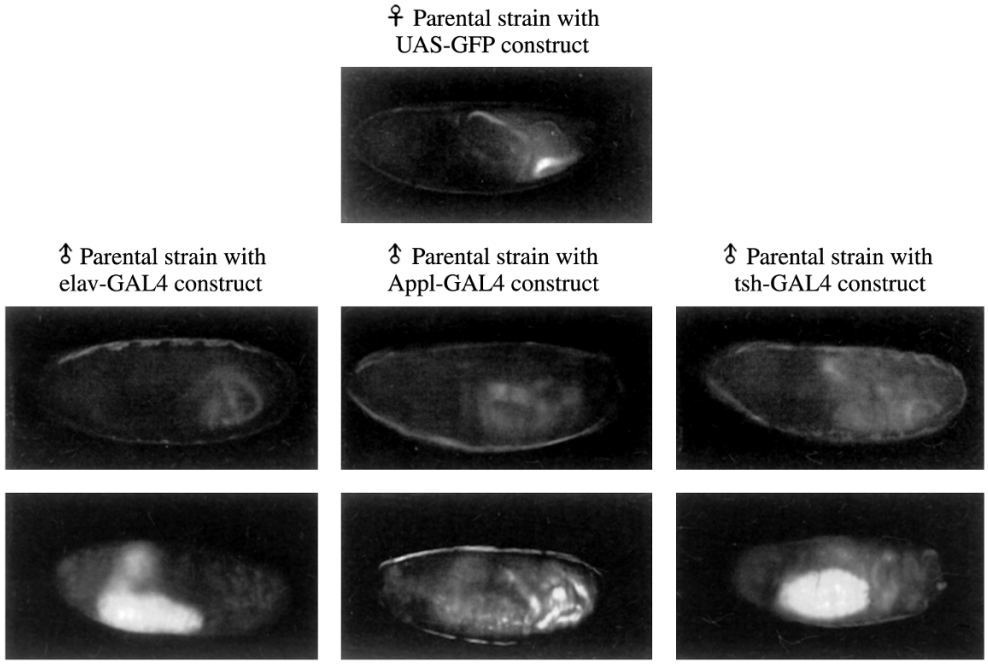

Fig. 2. GFP fluorescence in nervous structures in *Drosophila* embryos at the late stages of development before hatching of larvae (lower row). The anterior part is shown at left and the abdominal part below. Above – an embryo of the parental strain with a genome containing a construct with GFP, which is not expressed in the absence of GAL4. The first row shows embryos of the control parental strains in which GAL4 was expressed under control of promoters (*elav*, *appl*, and *tsh*). In all photographs, different levels of intestinal yolk fluorescence are seen in the posterior part of the egg. Images were taken at a magnification of · 20.

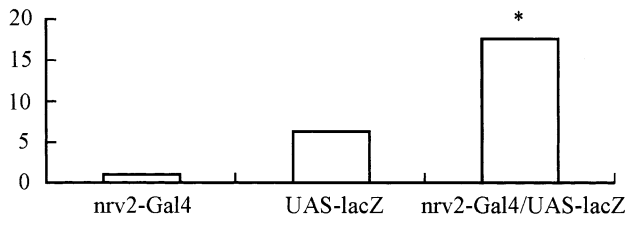

Fig. 3. Levels of β-galactosidase activity in parental (control) strains carrying the GAL4 and LacZ transgenes and in experimental strains with the nrv2-GAL4/UAS-LacZ system. Plots show mean values. Significant differences between experimental samples and controls were identified using Student's t test ($p < 0.01$). The vertical axes show the optical density of samples · 100.

RNA synthesized under control of one of four promoter genes whose expression in the nervous system is maximal (*elav*, *nrv2*, *appl*, and *tsh*). Movement activity was assessed using three main parameters: run duration (sec), run frequency (number of runs per 100 sec), and run speed (mm/sec). Knockdown of each of the study genes led to significant changes in motor activity in terms of one or more parameters (Fig. 1). Controls consisted of offspring from crosses of the driver strain with the corresponding parental strain control (for the *jumu*, *Treh*, and *mesr4* genes the control strain had the w^{1118} genotype; for the other nine genes, the control strain had the w^{1118}; P{attP y^+ $w^{3'}$} genotype). Changes largely affected run duration and frequency, with smaller influences on run speed.

The very low frequencies of runs in controls produced by crossing driver lines with the w^{1118}; P{attP y^+ $w^{3'}$} genotype with control parental strains should be noted. It may be that the consequence of this is the fact that changes in run frequency in the nine relevant experimental strains (henceforth attp strains) were either absent or consisted of increases. Run frequency in the wild-type strain Canton-S (CS) was 5.3, compared with values ranging from 0.2 to 8.5 in P-insertion mutants [2].

Run duration was the most informative parameter in attp strains. Changes in run duration were seen in conditions of RNA interference for eight of the nine candidate genes (the exclusion being *ext2*) under the control of one or several drivers. The magnitudes and directions of changes varied significantly depending on which gene was suppressed and which promoter was used.

The only changes in the three experimental strains for which the parental strains with UAS/RNA transgenes had the w^{1118} genotype (henceforth w strains) affected run frequency. It is interesting to note that run frequency in the control for the w strains was closer to the value in wild-type flies than was the case for the control for the attp strains.

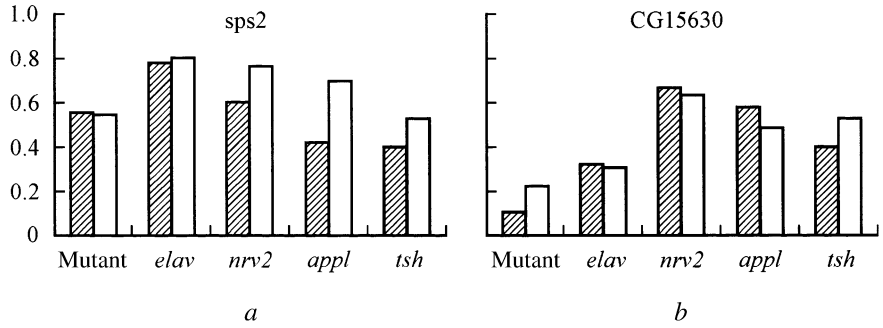

Fig. 4. Levels of expression of CG15630 and sps2 in mutant strains and strains with RNA interference for these genes (as proportions of control values, which were taken as 1). Expression levels in mutants were measured in relation to wild-type CS flies (shaded columns) and in relation to mutants for the other gene analyzed (unshaded columns). Expression in strains with RNA interference was evaluated in relation to the control used for testing motor activity (shaded columns) and in relation to strains with RNA interference for the second gene analyzed (unshaded columns). On the horizontal axis: *elav*, *nrv2*, *appl*, and *tsh* identify the promoters in the GAL4/UAS system used for localization of RNA interference. Significant differences were identified using the randomization test, 10000 iterations, $p < 0.05$.

RNA interference for *ext2* under control of the *appl* and *tsh* promoters, as well as *cf2* under control of the *tsh* promoter, was accompanied by defects in wing morphology. Suppression of the *mesr4* gene under control of *tsh* was lethal.

Expression of GFP and LacZ transgenes via the GAL4/UAS system. The operation of the GAL4/UAS system was confirmed in experiments with activation of the expression of the fluorescent protein GFP under control of the *elav*, *appl*, and *tsh* promoters (Fig. 2) or β-galactosidase under control of the *nrv2* promoter (Fig. 3). It should be noted that the pattern of *GFP* expression varied significantly depending on which promoter was used, but was always located in nervous structures. For the *elav* and *appl* promoters, fluorescence was seen in the brain and abdominal chain; for *tsh*, fluorescence was seen mainly in the abdominal chain.

Quantitative assessment of CG15630 and sps2 gene expression. The expression levels of the *CG15630* and *sps2* genes was quantified in strains with RNA interference for these genes, as well as in strains with mutations (P insertion) of these genes. The *CG15630* and *sps2* genes were selected because of a number of factors. Firstly, mutations in these genes are accompanied by impairments to an important parameter of rhythmic activity – the interimpulse interval in male song impulses [2]. Secondly, changes in run duration in strains with RNA interference for these genes were in different directions depending on the promoter used, and further analysis of these data requires the possibility of ineffective knockdown to be excluded.

All experimental samples showed decreased expression levels, though the magnitudes of the decreases varied significantly depending on which promoter–gene combination was being assessed (Fig. 4). Differences in the extent of suppression were probably associated with the extent of coincidence between the expression patterns of candidate genes and the genes controlling local RNA interference. Thus, suppression under control of the *elav* promoter was maximal for *CG15630*, as this is expressed mainly in the nervous system, and minimal for *sps2*, which has a significant level of expression outside the nervous system [8].

Discussion

We report here testing of movement activity in *Drosophila* in conditions of tissue-specific suppression of the expression of candidate genes previously identified by screening P-inversion mutants with significantly altered locomotion and mating song parameters [2]. Expression was suppressed using RNA interference under the control of the *elav*, *nrv2*, *appl*, and *tsh* gene promoters. Each of these genes, used to obtain tissue-specific RNA interference, was expressed mainly in *Drosophila* nerve cells [8], though each showed its own characteristic features in the pattern of expression in nervous tissues. Differences in the pattern of expression, along with the nature of the motor abnormalities, suggested mechanisms for the involvement of the candidate genes in generating the motor pattern for locomotor acts.

RNA interference of the candidate genes *jumu*, *Treh*, and *mesr4* (w strains) was accompanied by changes only in run frequency. There were no changes in strains with suppression of these genes under control of *nrv2*, which, in contrast to *elav* and *appl*, is not expressed in sensory neurons, including photoreceptors in the imaginal disks of the eyes, at the larval stage [24]. The *jumu* and *mesr4* genes encode transcription factors which have been shown to be involved in the development of the peripheral nervous system [3, 17]. Thus, the most likely cause of locomotor abnormalities on interference of *jumu* and *mesr4* may consist of impairments to the development of the sensory organs of *Drosophila*. The *Treh* gene encodes an enzyme (NC-IUBMB classification: Trehalase, EC 3.2.1.28), which cleaves trehalose, a disaccharide utilized for the transport of hemolymph glucose to various organs and tissues [7]. Motor abnormalities associated

with suppression of this gene are probably associated with nonspecific impairments to nerve cell energy metabolism.

The group of genes consisting of *CG15630*, *sps2*, and *Dgp-1* is interesting, and differently directed changes in run duration with different promoters were seen. Quantitative analysis of expression levels of *CG15630* and *sps2* confirmed the effectiveness of RNA interference for all promoters. Suppression of the *CG15630* gene controlled by *elav* was accompanied by increased run duration, while this parameter decreased under the control of *appl* and *tsh*. On the one hand, the nature of the motor abnormalities, as in the cases of *jumu* and *mesr4*, suggests impairments to the development of the sensory organs. However, this suggestion does not explain the differently directed nature of changes or the function of *CG15630* in the central nervous system (CNS) of animals, where the level of expression of this gene is quite high. On the other hand, *appl* powerfully and *tsh* mainly are expressed in the thoracic ganglia, in contrast to *elav*, whose maximum level of expression and, thus, interference of *CG15630*, is in the brain [8]. Thus, the effect of RNA interference at *CG15630* may depend on the ratio of the extent of suppression in different parts of the nervous system. This leads to the conclusion that the product of the *CG15630* gene is an obligate component of neuronal circuits involved in mediating locomotor acts, the functional significance of *CG15630* being determined by the nervous structures in which it is expressed. This conclusion is in good agreement with bioinformatic data on the probable location of the product of *CG15630* on the neuron cell surface, where it is involved in remodeling of the cell cytoskeleton [2].

Suppression of the *sps2* gene led, on the one hand, to decreases in run duration using the *elav* and *tsh* promoters and, on the other, to increases under control of *nrv2*, which, like *appl*, is expressed identically strongly in all parts of the *Drosophila* CNS [8]. These data are difficult to interpret, considering that the product of *sps2* is an enzyme (NC-IUBMB classification: selenide, water dikinase 2, EC 2.7.9.3), which is involved in selenocysteine synthesis [21] and is synthesized mainly outside the CNS [8]. In addition, the similarity between *sps2* and *CG15630* in terms of the complex nature of motor abnormalities in RNA interference, as well as the similarity in previously established changes in the parameters of song impulses in mutants [2], points to the occurrence of an interaction between these genes and the processes in which they are involved. This may indicate that *nrv2* is presumptively expressed in glial cells [24], while *elav* and *appl* have been shown not to be expressed in several types of glial cells [14, 19]. It is possible that the effects of RNA interference of *sps2* are associated with impairments to the development and functioning of glial cells in the nervous system.

RNA interference of *Dgp-1* produced abnormalities in locomotor activity only when the *tsh* and *appl* promoters were used, though changes affected all three analysis parameters. This pattern may be the result of impaired functioning of a small, scattered group of neurons in the *Drosophila* nervous system in which *Dgp-1* under the control of *appl* is suppressed significantly more effectively than it is when under control of *elav* and *nrv2*. This group of neurons must be part of or directly connected with those structures of the central complex of the fly brain which determine run speed [23]. The proposed role of the product of the *Dgp-1* gene is to counter impairments to intracellular protein metabolism [2]. It is possible that the motor defects seen on RNA interference of *Dgp-1* result from neurodegenerative processes in the structural elements of the central complex. It should be noted that *Dgp-1* is the only gene whose suppression under the control of *tsh* led to increases in run duration, though the cause of this change may be associated with the functioning of *Dgp-1* outside the nervous system, as *tsh*, which has a high level of expression in the abdominal chain, displays its activity in many other tissues [12].

RNA interference was accompanied by changes in run speed with a further three candidate genes: *CG6746*, *CG8708*, and *ext2*. Two of these genes – *CG8708* and *ext2* – encode sugar transferases and their derivatives [15, 16] (NC-IUBMB classification: core1-b1.3-galactosyltransferase, EC 2.4.1.122, and exostosin-2, EC 2.4.1.224, E.C. 2.4.1.225). The general physiological significance of these enzymes, along with the uniform directionality of all changes in run speed regardless of which gene was suppressed and which promoter was used, suggests nonspecific mechanisms of action of interference of these three genes on the development and operation of CMPG.

The increases in motor activity on suppression of the *cf2* and *CG34460* genes were also interesting. He *cf2* gene encodes a transcription factor which has been studied in detail in relation to its involvement in the muscular organs of *Drosophila* [5]. CF2, along with MEF2, is an activator of muscle protein genes at the embryonic and larval stages. Our data point to the involvement of *cf2* in nervous processes affecting animal's motor activity, and these processes must be have a specific localization which in some way intersects the pattern of *nrv2* expression. The product of the *CG34460* gene belongs to the SCV protein family (single von Willibrand factor C domain) and it is expressed exclusively in the testes [8, 20]. The products of SVC genes are presumptively secreted into the hemolymph in response to environmental stressors. Nothing has as yet been discovered regarding the role of *CG34460* in the *Drosophila* nervous system, so further studies are required to obtain an understanding of the marked effects of RNA interference of this gene on motor activity in *Drosophila* seen here.

Our list of candidate genes included the *kermit* gene, as previous studies have demonstrated that mutations in this gene decrease motor activity [13]. However, results obtained on RNA interference of this gene in the CNS make its involvement in the processes generating the motor pattern seem unlikely. The changes in run duration seen in the

present study on suppression of this gene under control of *tsh* are probably induced by impairments in other *Drosophila* tissues in which *tsh* is expressed [12].

We would like to thank the Vienna *Drosophila* RNAi Center (VDRC) at the Campus Science Support Facility (Austria), the Bloomington *Drosophila* Stock Center (BDSC) at Indiana University (USA), and Dr Konstantin Iliadi (Hospital for Sick Children, Toronto, Canada) for providing and sending transgenic strains for RNA interference experiments.

This work was supported by the "Russian innovatory scientific and scientific-pedagogical groups" Federal Targeted Program 2009–2013 and the "Living nature: current state and problems in development" and "Mechanisms of the integration of molecular systems on execution of physiological functions" programs of the Presidium of the Russian Academy of Sciences.

REFERENCES

1. N. G. Kamyshev, "*Drosophila* as a model system for discovering the molecular mechanisms of motor and cognitive dysfunctions and the search for pharmacological means for correcting them: a quantitative approach to assessing changes in locomotor behavior," in: *Measurement and Information Technologies in Healthcare: Coll. Sci. Works*, St. Petersburg (2011), pp. 152–160.
2. S. A. Fedotov, Yu. V. Bragina, N. G. Besedina, et al., "Genetic studies of motor functions in *Drosophila melanogaster*," *Ekol. Genetika*, **10**, No. 1, 51–61 (2012).
3. S. Abdelilah-Seyfried, Y. M. Chan, C. Zeng, et al., "A gain-of-function screen for genes that affect the development of the *Drosophila* adult external sensory organ," *Genetics*, **155**, No. 2, 733–752 (2000).
4. Y. I. Arshavsky, T. G. Deliagina, and G. N. Orlovsky, "Pattern generation," *Curr. Opin. Neurobiol.*, **7**, No. 6, 781–789 (1997).
5. C. Bagni, S. Bray, J. A. Gogos, et al., "The *Drosophila* zinc finger transcription factor CF2 is a myogenic marker downstream of MEF2 during muscle development," *Mech. Dev.*, **117**, No. 1–2, 265–268 (2002).
6. K. L. Briggman and W. B. Kristan, "Multifunctional pattern-generating circuits," *Annu. Rev. Neurosci.*, **31**, 271–294 (2008).
7. R. S. Burton and A. la Spada, "Trehalase polymorphism in *Drosophila melanogaster*," *Biochem. Genet.*, **24**, No. 9–10, 715–719 (1986).
8. V. R. Chintapalli, J. Wang, and J. A. Dow, "Using FlyAtlas to identify better *Drosophila* models of human disease," *Nat. Genetics*, **39**, No. 6, 715–720 (2007).
9. P. Chomczynski and N. Sacchi, "The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on," *Nat. Protoc.*, **1**, No. 2, 581–585 (2006).
10. J. B. Duffy, "GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife," *Genesis*, **34**, No. 1–2, 1–15 (2002).
11. E. S. Edgington, *Randomization Tests*, Marcel Dekker, New York (1995), 3rd ed.
12. L. Fasano, L. Roder, N. Core, et al., "The gene teashirt is required for the development of *Drosophila* embryonic trunk segments and encodes a protein with widely spaced zinc finger motifs," *Cell*, **64**, No. 1, 63–79 (1991).
13. J. Kim, S. Lee, S. Ko, and J. Kim-Ha, "dGIPC is required for the locomotive activity and longevity in *Drosophila*," *Biochem. Biophys. Res. Commun.*, **402**, No. 3, 565–570 (2010).
14. L. E. Martin-Morris and K. White, "The *Drosophila* transcript encoded by the beta-amyloid protein precursor-like gene is restricted to the nervous system," *Development*, **110**, No. 1, 185–195 (1990).
15. C. McCormick, G. Duncan, K. T. Goutsos, and F. Tufaro, "The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparin sulfate," *Proc. Natl. Acad. Sci. USA*, **97**, No. 2, 668–673 (2000).
16. R. Muller, A. J. Hulsmeier, F. Altmann, et al., "Characterization of mucin-type core-1 β1-3 galactosyltransferase homologous enzymes in *Drosophila melanogaster*," *FEBS. J.*, **272**, No. 17, 4295–4305 (2005).
17. J. Z. Parrish, M. D. Kim, L. Y. Jan, and Y. N. Jan, "Genome-wide analyses identify transcription factors required for proper morphogenesis of *Drosophila* sensory neuron dendrites," *Genes Dev.*, **20**, No. 7, 820–835 (2000).
18. M. W. Pfaffl, G. W. Horgan, and L. Dempfle, "Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR," *Nucl. Acids Res.*, **30**, No. 9, e36 (2002).
19. S. Robinow and k. White, "Characterization and spatial distribution of the ELAV protein during *Drosophila melanogaster* development," *J. Neurobiol.*, **22**, No. 5, 443–461 (1991).
20. T. J. Sheldon, I. Miguel-Aliaga, A. P. Gould, et al., "A novel family of single VWC-domain proteins in invertebrates," *FEBS Lett.*, **581**, No. 27, 5268–5274 (2007).
21. M. S. Shim, J. Y. Kim, H. K. Jung, et al., "Elevation of glutamine level by selenophosphate synthetase I knockdown induces metamitochondrial formation in *Drosophila* cells," *J. Biol. Chem.*, **284**, No. 47, 32,881–32,894 (2009).
22. J. A. Simon and J. T. Lis, "A germline transformation analysis reveals flexibility in the organization of heat shock consensus elements," *Nucl. Acids Res.*, **15**, No. 7, 2971–2988 (1987).
23. R. Strauss, "The central complex and the genetic dissociation of locomotor behaviour," *Curr. Opin. Neurobiol.*, **12**, No. 6, 633–638 (2002).
24. B. Sun, P. Xu, and P. M. Salvaterra, "Dynamic visualization of nervous system in live *Drosophila*," *Proc. Natl. Acad. Sci. USA*, **96**, No. 18, 10,438–10,443 (1999).
25. W. Supatto, A. McMahon, S. E. Fraser, and A. Stathopoulos, "Quantitative imaging of collective migration during *Drosophila* gastrulation: multiphoton microscopy and computational analysis," *Nat. Protoc.*, **4**, No. 10, 1397–1412 (2009).