Drosophila nervous system as a target of aging and anti-aging interventions

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Introduction

Nervous system regulates homeostasis and adaptation to environmental changes of a whole organism, thus deregulation of nervous processes accelerates aging (Alcedo et al., 2013a,b). The aging process in different models is associated with progressive degeneration of the nervous system (Lee et al., 2000) and progression of age-related neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (Boerrigter et al., 1992; Coppede and Migliore, 2010). The neurodegeneration also characterizes the progeroid syndromes, including Hutchinson-Gilford syndrome and Werner's syndrome (Coppede and Migliore, 2010).

Drosophila melanogaster is a good model organism to study age-related neurodegenerative changes (Lu and Vogel, 2009). Enrichment in mutants with neurodegeneration among flies with shortened lifespan has been reported (Buchanan and Benzer, 1993; Kretzschmar et al., 1997). The brain from old flies demonstrates the ultrastructural neurodegenerative changes such as reduction in the number of synapses, defects in mitochondria, and increase in neuronal apoptosis (Hadadi et al., 2014). However, anti-aging interventions may postpone the neurodegeneration (Bumatova et al., 2015).

Here we consider molecular genetic changes in the Drosophila aging brain and the bases for applying the brain as a target for anti-aging intervention.

Aging of the Nervous System

The study of age-related gene transcriptional levels changes in Drosophila showed that in different organs (including the brain) there are two critical time points—30 and 60 day of age (Zhan et al., 2007). Comparing those points with Drosophila mortality curve it could be mentioned that the 30 day time point can be potentially attributed to the age when almost "linear" part of survival curve is followed by the "exponential" part, reflecting more rapid decrease the amount of live flies. These data are in good agreement with the shape of Gompertz curve, which describes the probability of age-related mortality in Drosophila. Gompertz curve has two parameters: R describes background mortality and α—exponential growth of mortality. At the initial 30 day of age Gompertz curve is close to the linear dependence with the R slope, at later 60 day of age the curve decrease exponentially. Our study of normal expression of D-GADD45 gene during aging showed that D-GADD45 brain expression is vanishing at critical point of 30 day old (Bumatova et al., 2015).
What are the genes that change the expression level during brain aging? As it is shown in (Girardot et al., 2006) the main effect is down regulation of genes involved in synaptic transmission at different levels divided into three subgroups. The first one includes genes that play a role in neurotransmitter metabolism such as the choline acetyltransferase (Cha) and the dopamine N acetyltransferase (Dat) genes. In the second subgroup many genes involved in various steps of neurotransmitter secretion: priming for synaptic vesicle fusion (γ-SNAP, unc13, comatose and tomosyn), fusion with presynaptic membrane (Csp, Syx1A and rab3-GAP) and reformation of vesicles through endocytosis (liquid facets, AP-50 and AP-2α). The third subgroup includes several neurotransmitter receptor ion channels. Among these channels, two nicotinic acetylcholine receptors (nAcRβ96A and nAcRα18C) and three ionotropic glutamate receptors (Nmrd1, GluClα, and CG11155) mediate excitatory synaptic transmission. Moreover, three inhibitory GABAergic channels (Lch3, GABA-B-R2, and Rd) are also down regulated in aging Drosophila brain. Up regulated genes in aging Drosophila brain mostly present signatures similar to those observed in whole flies: genes associated with immune response and amino acid metabolism are over-represented. Based upon those whole genomic data it is possible to develop a set of Gal4 reporters that would permit to determine “biological” brain age markers for a given individual and to understand are there a “schedule” of aging at the gene level or is partially “stochastic” process.

Nervous System as a Target for Anti-Aging Interventions

Genetic manipulations with a single gene expression that extend life span are important tools for discovering mechanisms underlying aging. Mutations in the Indy (I’mNotDeadYet) gene dramatically extend the lifespan of the fruit fly, Drosophila melanogaster (Rogina et al., 2000). In the past we had identified an allele Indy-P115, which shows the same life span extension as the first allele (Bulgakova et al., 2002, 2004). Since we had the P(lArB) insertion, we studied the pattern of expression of the gene in the larval tissue. It occurs that the larval brain has clear pattern of expression and we put forward a hypothesis that Drosophila brain can be the main target of aging.

Studies on other models confirm our assumption. For example, mutations in daf-2 disrupting an insulin-like signaling pathway dramatically extend the adult C. elegans life span (Guarente and Kenyon, 2000). The study of cell-specificity of daf-2 action reveals that the neurons are responsible for the effect (Wolkow et al., 2000). The lit mutant mouse strain, which has a mutation disrupting the hypothalamic GH releasing hormone (GHRH), lives longer. Homozygous lit/lit mice live up to 25% longer than wild-type mice (Flurkey et al., 2001).

The creation of Gene-Switch Gal4 drivers (Osterwalder et al., 2001) now permits to identify the genes, whose ectopic activation/suppression can prolong Drosophila life span when overexpressed in adults. In particular Elav-GS driver directs conditional RU486 expression in the nervous system. With this approach it was shown, that overexpression of Cbs, Eip71CD, G6PD, GCLc, hep, Jafracl, p53, Sir2 and the silencing of CG9172, CG18809, l(3)neo18, Namm in the adult brain leads to increased life span (Table 1). It is also necessary to mention that similar data was published for D-Gadd45 (Plyusnina et al., 2011). Those data gave the heavy background to consider adult brain as the target of aging. However, the range of the genes tested with the approach is very small, so we like to analyze how large the range of such genes can be. All the genes mentioned above showed not only the life-span extension induced by Elav-GS driver, but similar extensions were observed also with one of Act-GS-Gal4 or Tub-GS-Gal4 drivers, showing ubiquitous over-expression also results in the life extension. So in Table 1 we made an attempt to correlate the list of the genes already studied by Gene-Switch approach with the level of their expression in development and tissues (modENCODE Tissue Expression Data). It can be seen that 30 genes studied within the da-Gal4, tub-GS-Gal4, Act-GS-Gal4, hs-Gal4 UAS-geneX system are heterogeneous group including high and low expression genes. Among those only AGBE, CalpA, Men, wdb demonstrate evident preponderance of head expression level. It is very probable that those genes, preferentially expressed in the head, also affects adult life-span by targeting the brain.

It was discovered cases when ubiquitous drivers: da-GS-Gal4 and tub-GS-Gal4 can extend life-span when inducing RNAi-geneX constructs (Table 1). Among those only CG17856, ms(3)272Dt have very low level of expression in the head.

Recent investigations shown, that the nervous system may be a target for anti-aging pharmacological interventions also. For example, serotonin antagonists (272N18, mianserin, mirtazapine, methiothepin and cyproheptadine), some of which are used clinically, extend the lifespan of adult C. elegans by 20–33% (Petrascheck et al., 2007). Screening of a library of compounds with known mammalian pharmacology revealed 60 compounds that increase longevity in C. elegans (Ye et al., 2014). The 33 compounds increased resistance to oxidative stress, and enhanced resistance to oxidative stress was associated primarily with compounds that target receptors for biogenic amines, such as dopamine or serotonin (Ye et al., 2014).

Conclusion

Now the thesis “Drosophila nervous system as a target of aging and anti-aging interventions” has been proved for some cases. On the one side of the nervous system is one of the targets of aging process and the state of nervous system may be regarded as a marker of aging. In this context, intervention aimed to combat the aging should lead to postponement of neurodegeneration. On the other hand, many pharmacological and genetic aging-suppressive interventions act through the nervous system. Therefore, it can be considered as one of the targets of anti-aging therapy. However, conditional expression approach reveals also other essential targets. We think that now days, when a large list of longevity genes already become known, it needs to put some efforts for complete longevity targets determination for every case. For example, current studies of the Indy mutations
## TABLE 1 | Tissue expression data of longevity genes in normal conditions.

| GeneX | Expression pattern according to modENCODE Tissue Expression Data | References |
|-------|---------------------------------------------------------------|------------|
| *da-Gal4, tub-Gal4, Act-Gal4, hs-Gal4 > UAS-GeneX* | | |
| Atg8a | Very high expression almost in all organs including nervous system | Simonsen et al., 2008 |
| AGBE | Moderately high, high in the head. Expression is lower in the other organs | Paik et al., 2012 |
| CalpA | Moderate expression in the head. Expression is lower in the other organs | Paik et al., 2012 |
| CG8155 | Low expression in the head. Expression in other organs also low | Paik et al., 2012 |
| CG10383 | Low expression in the head. Expression in other organs also low | Paik et al., 2012 |
| CG10916 | Low expression in the head. Expression in other organs also low | Paik et al., 2012 |
| CG30427 | Low expression in the head. Expression in other organs also low | Paik et al., 2012 |
| CG42666 | Low expression in the head. Expression in other organs also low | Paik et al., 2012 |
| dPh | Very low and low expression | Runko et al., 2008 |
| Dtc90F | Moderately high, moderate expression in the head. Expression in other organs also high | Paik et al., 2012 |
| dPnx5 | Very high in testis, high and moderately high in other organs except salivary gland and fat body | Radyuk et al., 2009 |
| dTsc1 | High and moderately high in imaginal discs, ovary, and testis, moderate in almost all other organs | Gao et al., 2002 |
| GCLm | High and very high expression in many organs, moderate expression in nervous system of larvae and pupae | Orr et al., 2005 |
| Hsp22 | Low expression in the head. Expression in other organs stronger | Kim et al., 2010 |
| Hsp26 | Low expression in the head. Expression in other organs stronger | Wang et al., 2004 |
| Hsp27 | Low expression in the head. Expression in other organs stronger | Wang et al., 2004 |
| ImpL2 | Moderately high, moderate expression in the head. Expression in other organs also high. | Paik et al., 2012 |
| Men | High expression in the head. High expression in some other organs | Paik et al., 2012 |
| Nlaz | High expression in the head. High expression in some other organs | Hull-Thompson et al., 2009 |
| Pcmt | High expression in imaginal discs and testis, moderate expression in other organs including nervous system | Chavous et al., 2001 |
| PGRP-LF | Weak expression everywhere | Paik et al., 2012 |
| Pnx5 | High and moderately high in the head. High in other organs | Radyuk et al., 2009 |
| S6k | Moderate, moderately high in the head and other organs | Kapahi et al., 2004 |
| SifaR | Low expression everywhere except pupae nervous system | Paik et al., 2012 |
| Sin3A | Moderate in the head and some other organs | Paik et al., 2012 |
| sm | Moderately high, moderate in the head. Expression higher in some organs | Paik et al., 2012 |
| Sod2 | High and very high in the head and other organs | Curtis et al., 2007 |
| Tor | Low expression everywhere | Kapahi et al., 2004 |
| Trx-2 | Moderately high, moderate in the head and other organs | Seong et al., 2001 |
| Tsc1 | Moderate in the head and other organs | Kapahi et al., 2004 |
| wdb | Moderately high, moderate in the head and some other organs | Funakoshi et al., 2011 |

| GeneX | Expression pattern according to modENCODE Tissue Expression Data | References |
|-------|---------------------------------------------------------------|------------|
| *da-Gal4, tub-Gal4 > RNAi-GeneX* | | |
| CG17856 | Very low everywhere | Copeland et al., 2009 |
| ms(3)202t | Very low everywhere | Copeland et al., 2009 |
| *Elav-Gal4 > UAS-GeneX* | | |
| Cbs | Low everywhere | Kabil et al., 2011 |
| Ep171CD | High, moderately high in the head and some other organs | Chung et al., 2010 |
| G6PD | Moderate expression in testis and head of 20 day male, moderate and low expression in other organs. Expression in nervous system of larvae and pupae—very low | Legan et al., 2008 |
| GCLc | High expression in digestive system and salivary glands | Orr et al., 2005 |
| Hep | Moderate in the head and other organs | Biteau et al., 2010 |
| Jafrac1 | Very high, high, and moderately high almost everywhere, except salivary gland fat body | Lee et al., 2009 |
| pS3 | Very low everywhere | Shen et al., 2009 |
| Sir2 | Moderate in the head and other organs | Whitaker et al., 2013 |
| *Elav-Gal4 > RNAi-GeneX* | | |
| CG9172 | High, moderately high in the head and some other organs | Copeland et al., 2009 |
| CG18809 | Moderately high, moderate in the head. Expression lower in other organs | Copeland et al., 2009 |
| l(3)neo18 | Very high in the head and some other organs | Copeland et al., 2009 |
| Naam | Very low in the head and other organs | Balan et al., 2008 |
extending life-span are concentrated on the gene function in the gut (Rogina et al., 2014). However, for the most of the longevity genes the target organs are poorly studied. We suggest that the brain is one of the main aging targets.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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