Screening for differentially expressed memory genes on a diabetes model induced by high sugar diet in Drosophila melanogaster: potential players for memory deficits

Julia Sepel Loreto
Universidade Federal de Santa Maria
Sabrina Antunes Ferreira
Universidade Federal de Santa Maria
Pamela de Almeida
Universidade Federal de Santa Maria
João Batista da Rocha
Universidade Federal de Santa Maria
Nilda de Vargas Barbosa (nvbarbosa@yahoo.com.br)
Universidade Federal de Santa Maria https://orcid.org/0000-0002-3661-4253

Research Article

Keywords: D. melanogaster, Diabetes, high-sugar diet, memory, synaptic plasticity

Posted Date: June 7th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3010377/v1

Version of Record: A version of this preprint was published at Molecular Neurobiology on September 12th, 2023. See the published version at https://doi.org/10.1007/s12035-023-03598-z.

Abstract

Type two diabetes mellitus (T2DM) has been shown to affect a series of cognitive processes including memory, increasing the risk for dementia, particularly Alzheimer's disease (AD). Although increasing evidence has supported that both diseases share common features, the pathophysiological mechanisms connecting these two disorders remain to be fully elucidated. Herein, we utilized *Drosophila melanogaster* fed on a high-sugar diet (HSD) to mimic T2DM, and investigate its effects on memory as well as identify potential molecular players associated with the memory deficits induced by HSD. Flies hatched from and reared on HSD for 7 days had a substantial decrease in short-term memory (STM). The screening for memory-related genes using transcriptome data revealed that HSD altered the expression of 33% of memory genes in relation to the control. Among the differentially expressed genes (DEGs) with a fold-change (FC) higher than two, we found five genes, related to synapse and memory trace formation, that could be considered strong candidates to underlie the STM deficits in HSD flies: Abl tyrosine kinase (Abl), Bruchpilot (Brp), Minibrain (Mnb), Skaker (Sh), and Gilgamesh (Gish). We also analyzed genes from the dopamine system, one the most relevant signaling pathways for olfactory memory. Interestingly, the flies fed on HSD presented a decreased expression of the Tyrosine hydroxylase (Ple) and Dopa decarboxylase (Ddc) genes, signals of a possible dopamine deficiency. In this work, we present promising “biomarkers” to investigate molecular networks shared between T2DM and AD.

1. Introduction

Type 2 Diabetes mellitus (T2DM) is the most common form of diabetes, encompassing 90% of the cases worldwide [1, 2]. Of particular importance, T2DM is frequently associated with a series of neuropathological effects, such as impairments in psychomotor speed, executive function, attention, and memory [3]. T2DM shares some similar demographic profiles and risk features with Alzheimer’s disease (AD), the most common cause of dementia [4]. However, there is still little knowledge about the shared molecular markers between the two diseases [5–7]. Alterations in brain insulin signaling seem to be an important factor involved in the pathophysiology of AD, and could be a link between T2DM and cognitive impairments found in AD [8, 9]. In addition to glucose homeostasis, insulin modulates other important pathways in the brain, including the metabolism of the β-amyloid peptide (Aβ) and hyperphosphorylated tau protein [3, 10, 11]. Downstream insulin signaling also regulates the activation of AKT pathway, which modulates different transcription factors and modulators, including CREB and HDAC4 [12–14] Of note, various of these factors regulate the expression of memory associated genes [13, 15].

Experimental organisms have been widely utilized to investigate the potential molecular links between diabetes and memory impairments. One of the most accurate and reproducible ways to mimic T2DM in animal models is by the use of high sugar diets (HSD), where the excess of sugar induces hyperglycemia and insulin resistance in baboons, rodents and alternative organism such as *Drosophila melanogaster* [16–19].

Drosophila melanogaster (*D. melanogaster*) reared on HSD develops several hallmarks of T2DM, including increased levels of glucose/trehalose, obesity-like phenotypes, insulin resistance and changes in insulin-like peptides mRNA levels, renal tubules impairments and even impaired immune responses [19–24]. *D. melanogaster* is also an important model for memory studies, particularly by exhibiting a wide behavioral repertoire and have a brain anatomy already well described in the literature [25–27]. The ample use of transgenic drosophila and genetic screenings have enabled the discovery of several genes involved with different types of memory and learning as well as the mapping of related signaling pathways [28–30].

In a previous work using a transcriptomic approach, we found that adult *D. melanogaster* hatched and reared on HSD, for 7 days, presented significant alterations in the transcription of genes involved with ribosomal biogenesis, energetic processes and muscle development; features with a tight correlation with T2DM responses [19]. Herein, we intend to find out whether HSD affects the short-term memory (STM) of flies, and through transcriptome to identify potential players associated with the memory deficits generated by the T2DM model. In general, we found that HSD diet caused STM loss and affected the expression of genes that orchestrate synapses and memory formation, neurogenesis and neurodegeneration. We believe that these genes, namely Abl, Brp, Mnb, Sh and Gish as well as their products and related pathways are promising targets to explore the relationship between Diabetes and AD.

2. Methods

2.1 Fly Stock and experimental design

Flies of the Oregon-R strain were kept in 2.5 x 6.5 cm bottles containing 10 mL standard corn medium, with relative humidity of 60% and light/dark cycle of 12 h at a constant temperature of 24 ± 1°C. Corn medium contained 44% coarse and 35% medium corn flour, 11% wheat germ, 8% sucrose, 0.5% milk powder, 0.5% salt, 0.5% soybean flour, 0.5% rye flour, a pinch of methyl p-hydroxybenzoate antifungal (Nipagin®) and lyophilized yeast. For experiments, flies were raised from eggs until adult phase on corn medium containing or not sucrose 30% (HSD 30%). The choice of sucrose concentration was based in previous studies from our research group [19, 20]. After hatching in control and HSD 30%, the flies (0 day old) from viable larvae were placed in flasks with the respective diets until 7 days. Flies were transferred to fresh medium every 2 days. Memory and transcriptome analyses were performed in flies hatching from and reared on HSD for 7 days (Scheme 1).

2.2 Assays

2.2.1. Aversive Memory Test by association

The aversive association memory test was performed according the methodology described by Préat, T. (1998) [31], with some modifications. The test was carried out with flies hatched from and reared on control and HSD 30% diet for 7 days. In ‘Training Phase’, a group of 20 flies was presented to a

conditional stimulus in the form of an odor A (3-octanol diluted in mineral oil in a proportion of 1:100), while received a negative unconditioned stimulus (electric shocks of approximately 75V) for four minutes. After ten-minutes, the flies were placed in an apparatus where they could choose to move to the side with the presence of the previous odor A or with a new odor B (4-methylcyclohexanol diluted in mineral oil in a proportion of 1:50) in the 'Testing Phase'. The test was done in the dark, with a red light, which did not influence the distribution of the flies inside the apparatus. Data were analyzed by a performance index that takes into account the number of flies that selected either the side of the apparatus previously associated with the aversive stimulus (odor A) or the side with the odor B. The performance index indicates the percentage of flies from the groups able to achieve a memory of association between the electric shock and odor A. To note, before the trials, we carried out tests with the odors A and B to ensure that the flies had no preference for one of these specific odors (data not shown).

2.2.2. Transcriptome analyses

Data of memory genes were taken from a transcriptome previously published by our group (See Loreto et al., 2021) [19], where the whole-body of 7 days-old adult flies hatched from and reared on control diet or HDS-30% were analyzed, using the available *Drosophila melanogaster* genome (ID:47 in NCBI) as guide. Through this transcriptome, herein we performed a screening for all genes related to memory in flies. We used a list of interest genes (all genes marked as 'learning or memory' with GO:0007611 in the FlyBase dataset) and searched for those that were considered differentially expressed (DEG) in the HSD libraries compared to the control. The gene transcription quantification was obtained for each RNA-seq by the RPKM (Reads Per Kilobase Million) method, and RPKM values were used to establish a transcriptional fold-change (FC) or direct comparison between group libraries. A memory gene was considered DEG when the HSD group had a change in RPKM values greater than FC = 1 in relation to control. The DEG was considered 'up-regulated' (up) when there was an increase in expression and 'down-regulated' (down) when there was a decrease in expression. We also considered those genes that had no expression values in control libraries and were expressed in HSD libraries as 'activated' genes (Activ.), and the opposite situation, expression on control and no expression on HSD, as 'repressed' genes (Repr.). Besides, we analyzed some specific genes for discussion that were not marked as 'learning or memory' in FlyBase's dataset. A table with information of the specific genes (Name, symbol, FlyBase ID and Annotation symbol) is found in the supplemental materials (Supplemental Table 1). DEGs with FC > 2 were analyzed individually, and classified according to the type of memory they are related in the literature: learning (LRN), short-term memory (STM), middle-term memory (MTM), long- term memory (LTM), anesthesia-resistant memory (ARM), spatial orientation memory or neurodegeneration.

2.3. Statistical analysis

The analyses were performed using unpaired t-test for parametric data, and Mann-Whitney's test for nonparametric data. Results were expressed as mean ± standard deviation and analyzed using the Graph Pad Prism software version 8.0. Significant levels were considered when $P \leq 0.05$.

3. Results

3.1 Aversive memory

The effect of HSD 30% intake on memory by association was analyzed by an aversive memory test, in which flies were trained to associate a specific odor with electric shock. The time interval between 'Training' and 'Testing' phase was 10 minutes, characterizing a STM, which has at most 60 minutes of duration [13, 26, 32, 33]. We found that flies hatched from and reared on HSD 30% presented an impaired STM (Fig. 1), with a decrease of 50% of performance index in comparison to control values.

3.2 Transcriptomic screening of memory genes

The HSD changed the expression levels of 33,13% of all genes that were reported as 'learning or memory' in the FlyBase (Fig. 2), where 57 DEG had a FC > 1 and 14 DEG a FC > 2. A table containing all genes DEG in HSD reared flies is found in supplemental Materials (Supplemental Table 2). After, we analyzed only those with a FC > 2, establishing for each gene the FC level, the type of regulation displayed in relation to the control and the type of related-memory. Then, we found 6 DEG related to LRN -mushroom body miniature (mbm), Abl tyrosine kinase (Abl), shaggy (sgg or GSK3), Shaker (Sh), minibrain (mnb) – 3 DEG related to STM - Sh, bruchpilot (brp) and gilgamesh (gish) – 2 DEG related to MTM- CASK and Cyclic-AMP response element binding protein B (CrebB) – 5 DEG related to LTM – hopscotch (hop), CASK, Histone deacetylase 4 (HDAC4), Mob2 and CrebB – 2 DEG related to ARM – CASK and brp - and 2 DEG related to spatial orientation – Ribosomal protein S6 kinase II (S6kII) and ellipsoid body open (ebo) - (Table 1).

3.3 Learning and STM genes

To better understand the impact of DEG on STM deficits in HSD treated flies, we further analyzed the role of the 14 DEG with a FC > 2, along with the expression levels of genes from related signaling pathways or regulators of these memory genes. We found that HSD caused an up-regulation of gene Abl (FC = + 3,974, Fig. 3a), and also a similar up-regulation of genes involved with the Wnt signaling, namely: Cyclin-dependent kinase 5 (FC = + 1,616), Frizzled (FC = + 3,616), Van Gogh (FC = + 2,1230), Disabled (FC = 2,1556) and Dishevelled (FC = + 1,9662) (Fig. 3b-f). The expression level of genes Huntingtin and β-amyloid protein precursor-like did not differ from control (Fig. 3g-h).

Differently, HSD caused a general down-regulation in LRN and/or STM genes related with synaptic plasticity, such as Bruchpilot (FC=-2,202, Fig. 4a), Minibrain (FC=-2,361, Fig. 4b), Gilgamesh (FC=-2,152, Fig. 4c), and Shaker (FC=-3,370, Fig. 4d). The Hyperkinectic, a Shaker subunit, was also down-regulated in HSD flies when compared to the control (FC=-1,568, Fig. 4e). HSD also up-regulated the Elongater complex protein 3 (FC = + 1,970) and Histone deacetylase 6 (FC = + 2,716), regulatory genes of Bruchpilot acetylation (Fig. 4g-h).

3.4 Genes from Dopamine System

Given importance of dopamine signaling in olfactory memory formation, we decided to analyze the expression of genes involved with dopamine synthesis, binding and transport, namely Ddc (Dopa descarboxylase), Dop1R1(Dopamine Receptor 1), Dop1R2 (Dopamine Receptor 2), Ple (Tyrosine Kinase),

and DAT (Dopamine Transporter). Some of these genes had already been listed in our previous screening, since they were marked as ‘memory or learning’ in FlyBase dataset: the Ddc, and Dop1R1. However, most of them were not annotated as memory genes (Ple, Dop1R2 and DAT). Now looking for these specific genes, we found that HSD flies had a down regulation of two genes involved in dopamine synthesis in relation to the control: the Ple with a FC= -9,81 (Fig. 5a), and Ddc with a FC= -1,5 (Fig. 5b). The genes involved with binding and transport of dopamine like Dop1R1 (Fig. 5c), Dop1R2 (Fig. 5d) and DAT isoforms (Fig. 5e-f) were identified as EEG.

4. Discussion

In the last decades a strong connection between diabetes and Alzheimer’s disease (AD) has been established, with T2DM patients presenting increased risk of developing AD. Although impaired metabolism, inflammation, and defective insulin signaling are known as key pathological features of both diseases, the understanding about the molecular mechanisms shared by the diseases is still elusive. In this sense, the transcriptomic approaches have contributed successfully to identify molecular components involved in these signaling networks. With this in mind, here we performed a transcriptomic screening for genes that could be behind the memory impairments induced by HSD-induced T2DM model.

First, we investigated whether HSD intake would generate memory deficits in adult flies. Then, a Pavlovian methodology of classical conditioning (negative association) between odor and electric shock was used to test the STM of flies. We found that flies hatched from and reared on HSD diet had memory loss, presenting performance index values significantly lower than control flies. Our work is the first to show how a diabetes-like state induced by HSD can impact on STM of adult flies. A similar work has been done with an obesity-like state induced by High-Fat diet (HFD) in drosophila, although only LTM was impaired [34]. In rodents, the diet impacts on memory have been measured through the use of High-Fat diet (HFD) or Western diets (High-Fat High-sugar diets), with a general concordance that the diets can lead to memory defects [35–38]. In a previous work, our research group investigated the general changes induced by HSD in flies through an ontology analysis, where ‘Memory’, ‘Learning’ or related words did not appear as enrichment terms [19]. We ponder that ‘memory’ was not an enrichment term probably because the list of genes marked as ‘learning or memory’ is relatively short to appear in a wider analysis, even if there was a significant number of DEG among them. However, taking into account the decline of performance induced by HSD in the memory test, we considered pertinent to examine, from this whole-transcriptome, the expression of memory-related genes in the flies. For this, we performed a screening of all genes annotated as 'learning or memory' in FlyBase dataset within our transcriptome. From 172 memory-related genes found, more than 30% were significantly altered by HSD (57 DEG in total), and more than 8% were DEG with FC > 2 (14 DEG) (Fig. 2). Then, we highlighted the 14 DEG with FC > 2 as the most relevant genes from screening, and classified them according to the type of memory to guide the search for genes with potential function to explain the STM deficits in HSD-flies. For a more robust discussion, we focused on genes that had a combination of pattern of transcription and phenotypic changes described in literature in similarity to the ones we observed in HSD flies. This brought to us five

genes that we consider strong candidates to underlie the STM deficits in HSD flies: Abl, Brp, Mnb, Sh and Gish.

One of the most prominent DEGs in our analysis was the Abl, with a high activation profile: control libraries had no detectable expression values for Abl, while HSD exhibited an FC = 3,9. Abl gene encodes a non-receptor tyrosine kinase that, together with CDK5, participates in signaling pathways associated with Tau hyperphosphorylation and neurodegeneration, possibly mediated by Aβ42, a hallmark molecule of Alzheimer's disease [39, 40]. HSD also promoted a significant increase in the CDK5 expression (Fig. 3b), result that strengthens the link with neurodegeneration (Fig. 3i). In addition to its role in neurodegeneration, Abl is involved in the formation of αβ and α'β' lobes in the mushroom bodies (MB): dysregulation of Abl levels by either overexpression or lack of expression causes disorganization of actin structures and compromises the axonal growth [41]. In line with this, we found previously from this transcriptome a remarkable down-regulation for *Actin* (*Act88F*) in HSD flies [19], effect that was associated with a decrease in muscle mass, but that now, as shown here, seems to impact on the correct formation of the nervous system as well.

We also investigated the expression of genes connected with the activation and regulation of Abl: huntingtin (Htt) (Fig. 3g), β amyloid protein precursor-like (APPL) (Fig. 3h), and elements from the Wnt signaling pathway (Fig. 3c-f). Except for APPL and Htt, which were EEG, all elements from the Wnt pathway were up-regulated by HSD (Fig. 3c-f). These results indicate the potential role of this pathway, whose over-activation in HSD flies could be driving the axonal growth defects in MB (Fig. 3i), and possible memory deficits.

Looking for other DEGs with FC > 2, we found that the HSD caused a down-regulation on LRN and STM related genes involved in diverse signaling pathways, but with shared functions on synaptic plasticity and memory trace formation: Brp, Mnb, Gish and Sh (Fig. 4a-d). Brp encodes a structural protein that anchors the presynaptic vesicles forming synaptic buttons that can increase in size when Brp is acetylated by Elp3 or decrease in size when Brp is deacetylated by HDAC6 [42]. In Drosophila, it has been shown that Brp is primarily required for ARM formation, however; Brp knockdown mutants also show a deficit in STM similar to the observed herein in HSD flies [43]. While Brp was down-regulated by HSD (Fig. 4a), phenomenon that can be related to memory deficit, the Brp regulators that function in an antagonistic way (Elp3 and HDAC6) had the expression increased in HSD flies (Fig. 4g-h). Then, we hypothesized that the decreased levels of Brp paired with a possible dysregulation of acetylation caused by the altered expression of both Elp3 and HDAC6 might lead to a decrease in synaptic plasticity (Fig. 4f).

Another HSD down regulated-gene linked to synaptic plasticity was the Mnb (Fig. 4e). Mnb encodes a neurogenesis-related protein kinase, ortholog of DYRK1 in humans, that regulates exo- and endocytosis of synaptic vesicles and reorganizes the cytoskeleton by directly and indirectly interacting with actin and microtubules, among other functions [44]. Mutant flies with reduced expression of Mnb present significant formation defects on the central nervous system, with 40–50% reduction in brain volume and drastic reduction in cell number [45], and also decreased learning in females [46]. In this way, the Mnb

downregulation found here in HSD flies could reflect not only memory deficits, but also neuroanatomical malformation. As its expression is modulated by the PKA/CrebA pathway, regulated by sNPF in Drosophila, [47], we propose that insulin signaling can be part of the signaling network orchestrated by Mnb expression in HSD flies.

In a similar way as Brp and Mnb, Sh was down regulated by HSD (Fig. 5d). It is also involved with synaptic plasticity, encoding a subunit of a voltage-gated potassium channel that regulates neurotransmitter release into the synapses [48]. There is evidence that Sh mutant flies have decreased STM performance, similar to the effect what we observed herein in HSD flies [49, 50], and take a longer habituation time compared to control as well [51]. Sh works along with a modulatory subunit, Hiperkinectic (Hk), that was also downregulated by HSD (Fig. 5e). Alternatively, changes in both Sh and Hk expression may indicate disruption in synapsis plasticity, that could contribute to STM deficits via decrease of potassium currents in MB neurons (Fig. 4i).

HSD also down regulated the gene Gish (Fig. 4c), a casein kinase preferentially expressed in the MB that regulates multiple cellular processes and signals in Wnt pathway [52, 53]. Decreased Gish in different Drosophila strains led to a decrease in memory performance tests, including a deficit in STM [54]. So, the down regulation observed in HSD flies might also be contributing to the deficits found in STM. In Gish mutants the calcium influx is disrupted in α'β' neurons, [54], which suggests that Gish is more related with the formation of memory traces in Drosophila, than the synaptic plasticity like brp, mnb and sh.

Other DEGs involved in LRN or STM emerged from this analysis in HSD flies, but do not appear to be potential candidates for further investigation. For example, HSD generated a decrease in expression in Shaggy (sgg) (Table 1), the ortholog of GSK-3β in humans, which is widely recognized as a potential molecular link between diabetes and Alzheimer's [55]. Usually, its over-expression is linked to deleterious effects in Drosophila [56][57, 58], which does not explain the phenotype found herein in HSD. Similarly, the reduced expression of Mushroom body miniature (Mbm) has been associated with phenotypes of malformation of MB [46, 59], causing deficits in several types of memory [60–62]. Unfortunately, there is no record of phenotypic changes when there is an increase in Mbm expression, which to some extent is similar to the findings obtained with flies raised on HSD (Table 1).

Given the importance of the dopaminergic signaling to the olfactory memory in *D. melanogaster*, we also analyzed dopaminergic genes to check possible changes at transcriptional level in HSD flies (Fig. 5). We found that the expression of genes related to dopamine synthesis had a significant decrease compared to the control (Th and Ddc) (Fig. 5a-b), while dopamine receptors and transporters were EEG (both DAT isoforms and both Dop1-R1 and Dop1-R2) (Fig. 5c-f). Interestingly, Dop1R1 and Ddc have already been described in our transcriptomic screening for memory genes (Supplemental Table 2), but were considered EEG or had a low FC. On the other hand, Th was not listed as a ‘memory’ associated gene in FlyBase, but it has been associated with memory impairment [63]. HSD induced a remarkable down-regulated in Th, with a FC>-26.105. This result indicates the possible participation of dopamine on the memory alterations in HSD flies, probably a dopamine deficiency, similar how occur in mutants without dopamine [63, 64].

Changes in dopaminergic system are especially relevant given that the action of dopaminergic neurons is critical for information processing in MB, and normal olfactory associative learning [65].

5. Conclusion

In this work, we demonstrated that HSD causes STM deficits in *D. melanogaster* and changes in the transcript levels of several memory related genes. Among the DEG, we brought attention to five genes that could be considered strong candidates to underlie STM deficits in HSD flies: Abl, Brp, Mnb, Sh and Gish. As their products are related to the synapses and memory trace formation, they and their regulators represent promising “biomarkers” to investigate the molecular networks shared between diabetes and Alzheimer disease. Besides, the down-regulation of Th gene suggests a probable dopamine deficiency in HSD flies, and the participation of dopaminergic system on the memory deficits induced by sugar diet.

Declarations

Ethics approval

The authors declare that the study was developed following all the ethical principles of the Committee on Publication Ethics.

Consent to participate

Not applicable.

Consent for publication

All authors have given final approval of this MS version and agreed with the publication of this study here.

Availability of data and materials

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was supported by grants from the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (process number: 88887.654237/2021-00) and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (process number: 306919/2020-6)

Authors' contributions

J.S.L and N.V.B designed the experimental procedures; J.S.L, P.A. and S.A.F performed the experiments; J.S.L, J.B.T.R and N.V.B analyzed the data and wrote the original paper.

Acknowledgments

The financial supports by Fundação de Amparo à Pesquisa do Estado de Rio Grande do Sul (FAPERGS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) are gratefully acknowledged. N.V.B and J.B.T.R are CNPq fellowship recipients.

References

1. Glovaci D, Fan W, Wong ND (2019) Epidemiology of Diabetes Mellitus and Cardiovascular Disease. Curr Cardiol Rep 21:21. https://doi.org/10.1007/s11886-019-1107-y
2. Koye DN, Magliano DJ, Nelson RG, Pavkov ME (2018) The Global Epidemiology of Diabetes and Kidney Disease. Adv Chronic Kidney Dis 25:121–132. https://doi.org/10.1053/j.ackd.2017.10.011
3. Sims-Robinson C, Kim B, Rosko A, Feldman EL (2010) How does diabetes accelerate Alzheimer disease pathology? Nat Rev Neurol 6:551–559. https://doi.org/10.1038/nrneurol.2010.130
4. Baglietto-Vargas D, Shi J, Yaeger DM, et al (2016) Diabetes and Alzheimer's disease crosstalk. Neurosci Biobehav Rev 64:272–287. https://doi.org/10.1016/j.neubiorev.2016.03.005
5. Yang Y, Song W (2013) Molecular links between Alzheimer's disease and diabetes mellitus. Neuroscience 250:140–150. https://doi.org/10.1016/j.neuroscience.2013.07.009
6. Chami B, Steel AJ, De La Monte SM, Sutherland GT (2016) The rise and fall of insulin signaling in Alzheimer's disease. Metab Brain Dis 31:497–515. https://doi.org/10.1007/s11011-016-9806-1
7. Salas IH, De Strooper B (2019) Diabetes and Alzheimer's Disease: A Link not as Simple as it Seems. Neurochem Res 44:1271–1278. https://doi.org/10.1007/s11064-018-2690-9
8. Akinola OB (2016) Sweet old memories: a review of the experimental models of the association between diabetes, senility and dementia. Metab Brain Dis 31:1003–1010. https://doi.org/10.1007/s11011-016-9876-0
9. Kandimalla R, Thirumala V, Reddy PH (2017) Is Alzheimer's disease a Type 3 Diabetes? A critical appraisal. Biochim Biophys Acta - Mol Basis Dis 1863:1078–1089. https://doi.org/10.1016/j.bbadis.2016.08.018
10. Han W, Li C (2010) Linking type 2 diabetes and Alzheimer's disease. Proc Natl Acad Sci 107:6557–6558. https://doi.org/10.1073/pnas.1002555107
11. Correia SC, Santos RX, Carvalho C, et al (2012) Insulin signaling, glucose metabolism and mitochondria: Major players in Alzheimer's disease and diabetes interrelation. Brain Res 1441:64–78. https://doi.org/10.1016/j.brainres.2011.12.063
12. Wang B, Moya N, Niessen S, et al (2011) A Hormone-Dependent Module Regulating Energy Balance. Cell 145:596–606. https://doi.org/10.1016/j.cell.2011.04.013

13. Zhang J, Little CJ, Tremmel DM, et al (2013) Notch-Inducible Hyperphosphorylated CREB and Its Ultradian Oscillation in Long-Term Memory Formation. J Neurosci 33:12825–12834. https://doi.org/10.1523/JNEUROSCI.0783-13.2013
14. Heier C, Kühnlein RP (2018) Triacylglycerol metabolism in drosophila melanogaster. Genetics 210:1163–1184. https://doi.org/10.1534/genetics.118.301583
15. Fitzsimons HL, Schwartz S, Given FM, Scott MJ (2013) The Histone Deacetylase HDAC4 Regulates Long-Term Memory in Drosophila. PLoS One 8:e83903. https://doi.org/10.1371/journal.pone.0083903
16. Moreira PI (2013) High-sugar diets, type 2 diabetes and Alzheimer's disease. Curr Opin Clin Nutr Metab Care 16:440–445. https://doi.org/10.1097/MCO.0b013e328361c7d1
17. Morris SNS, Coogan C, Chamseddin K, et al (2012) Development of diet-induced insulin resistance in adult Drosophila melanogaster. Biochim Biophys Acta - Mol Basis Dis 1822:1230–1237. https://doi.org/10.1016/j.bbadis.2012.04.012
18. Musselman LP, Fink JL, Baranski TJ (2019) Similar effects of high-fructose and high-glucose feeding in a Drosophila model of obesity and diabetes. PLoS One 14:1–13. https://doi.org/10.1371/journal.pone.0217096
19. Loreto JS, Ferreira SA, Ardisson-Araújo DM, Barbosa NV (2021) Human type 2 diabetes mellitus-associated transcriptional disturbances in a high-sugar diet long-term exposed Drosophila melanogaster. Comp Biochem Physiol Part D Genomics Proteomics 39:100866. https://doi.org/10.1016/j.cbd.2021.100866
20. Ecker A, Gonzaga TKS do N, Seeger RL, et al (2017) High-sucrose diet induces diabetic-like phenotypes and oxidative stress in Drosophila melanogaster: Protective role of Syzygium cumini and Bauhinia forficata. Biomed Pharmacother 89:605–616. https://doi.org/10.1016/j.biopha.2017.02.076
21. Rani L, Saini S, Shukla N, et al (2020) High sucrose diet induces morphological, structural and functional impairments in the renal tubules of Drosophila melanogaster: A model for studying type-2 diabetes mediated renal tubular dysfunction. Insect Biochem Mol Biol 125:103441. https://doi.org/10.1016/j.ibmb.2020.103441
22. Musselman LP, Fink JL, Narzinski K, et al (2011) A high-sugar diet produces obesity and insulin resistance in wild-type Drosophila. Dis Model Mech 4:842–849. https://doi.org/10.1242/dmm.007948
23. van Dam E, van Leeuwen LAG, dos Santos E, et al (2020) Sugar-Induced Obesity and Insulin Resistance Are Uncoupled from Shortened Survival in Drosophila. Cell Metab 31:710-725.e7. https://doi.org/10.1016/j.cmet.2020.02.016
24. Yu S, Zhang G, Jin LH (2018) A high-sugar diet affects cellular and humoral immune responses in Drosophila. Exp Cell Res 368:215–224. https://doi.org/10.1016/j.yexcr.2018.04.032
25. Eichler K, Li F, Litwin-Kumar A, et al (2017) The complete connectome of a learning and memory centre in an insect brain. Nature 548:175–182. https://doi.org/10.1038/nature23455

26. Davis RL (2011) Traces of Drosophila Memory. Neuron 70:8–19. https://doi.org/10.1016/j.neuron.2011.03.012
27. Winding M, Pedigo BD, Barnes CL, et al (2023) The connectome of an insect brain. Science (80-) 379:. https://doi.org/10.1126/science.add9330
28. Sokolowski Marla B. (2001) Drosophila: Genetics meets behaviour. Nat Rev Genet 2:879–890
29. Eddison M, Belay AT, Sokolowski MB, Heberlein U (2012) A Genetic Screen for Olfactory Habituation Mutations in Drosophila: Analysis of Novel Foraging Alleles and an Underlying Neural Circuit. PLoS One 7:e51684. https://doi.org/10.1371/journal.pone.0051684
30. Kacsoh BZ, Barton S, Jiang Y, et al (2019) New Drosophila Long-Term Memory Genes Revealed by Assessing Computational Function Prediction Methods. G3 Genes|Genomes|Genetics 9:251–267. https://doi.org/10.1534/g3.118.200867
31. Préat T (1998) Decreased Odor Avoidance after Electric Shock in Drosophila Mutants Biases Learning and Memory Tests. J Neurosci 18:8534–8538. https://doi.org/10.1523/JNEUROSCI.18-20-08534.1998
32. Blum AL, Li W, Cressy M, Dubnau J (2009) Short- and Long-Term Memory in Drosophila Require cAMP Signaling in Distinct Neuron Types. Curr Biol 19:1341–1350. https://doi.org/10.1016/j.cub.2009.07.016
33. Lee PT, Lin G, Lin WW, et al (2018) A kinase-dependent feedforward loop affects CREBB stability and long term memory formation. Elife 7:1–16. https://doi.org/10.7554/eLife.33007
34. Rivera O, McHan L, Konadu B, et al (2019) A high-fat diet impacts memory and gene expression of the head in mated female Drosophila melanogaster. J Comp Physiol B 189:179–198. https://doi.org/10.1007/s00360-019-01209-9
35. Cordner ZA, Tamashiro KLK (2015) Effects of high-fat diet exposure on learning & memory. Physiol Behav 152:363–371. https://doi.org/10.1016/j.physbeh.2015.06.008
36. McLean FH, Grant C, Morris AC, et al (2018) Rapid and reversible impairment of episodic memory by a high-fat diet in mice. Sci Rep 8:11976. https://doi.org/10.1038/s41598-018-30265-4
37. Watson LS, Stone TD, Williams D, et al (2020) High-Fat Diet Impairs Tactile Discrimination Memory in the Mouse. Behav Brain Res 382:112454. https://doi.org/10.1016/j.bbr.2019.112454
38. Lietzau G, Nyström T, Wang Z, et al (2020) Western Diet Accelerates the Impairment of Odor-Related Learning and Olfactory Memory in the Mouse. ACS Chem Neurosci 11:3590–3602. https://doi.org/10.1021/acschemneuro.0c00466
39. Bothwell M, Giniger E (2000) Alzheimer's Disease: Neurodevelopment Converges with Neurodegeneration. Cell 102:271–273. https://doi.org/10.1016/S0092-8674(00)00032-5
40. Lin H, Lin T-Y, Juang J-L (2007) Abl deregulates Cdk5 kinase activity and subcellular localization in Drosophila neurodegeneration. Cell Death Differ 14:607–615. https://doi.org/10.1038/sj.cdd.4402033

41. Marquilly C, Busto GU, Leger BS, et al (2021) Htt is a repressor of Abl activity required for APP-induced axonal growth. PLOS Genet 17:e1009287. https://doi.org/10.1371/journal.pgen.1009287
42. Miskiewicz K, Jose LE, Yeshaw WM, et al (2014) HDAC6 Is a Bruchpilot Deacetylase that Facilitates Neurotransmitter Release. Cell Rep 8:94–102. https://doi.org/10.1016/j.celrep.2014.05.051
43. Knapek S, Sigrist S, Tanimoto H (2011) Bruchpilot, A Synaptic Active Zone Protein for Anesthesia-Resistant Memory. J Neurosci 31:3453–3458. https://doi.org/10.1523/JNEUROSCI.2585-10.2011
44. Arbones ML, Thomazeau A, Nakano-Kobayashi A, et al (2019) DYRK1A and cognition: A lifelong relationship. Pharmacol Ther 194:199–221. https://doi.org/10.1016/j.pharmthera.2018.09.010
45. Fischbach KF, Heisenberg M (1984) Neurogenetics and Behaviour in Insects. J Exp Biol 112:65–93. https://doi.org/10.1242/jeb.112.1.65
46. Heisenberg M, Borst A, Wagner S, Byers D (1985) Drosophila Mushroom Body Mutants are Deficient in Olfactory Learning. J Neurogenet 2:1–30. https://doi.org/10.3109/01677068509100140
47. Hong S-H, Lee K-S, Kwak S-J, et al (2012) Minibrain/Dyrk1a Regulates Food Intake through the Sir2-FOXO-sNPF/NPY Pathway in Drosophila and Mammals. PLoS Genet 8:e1002857. https://doi.org/10.1371/journal.pgen.1002857
48. Gasque G, Labarca P, Reynaud E, Darszon A (2005) Shal and Shaker Differential Contribution to the K + Currents in the Drosophila Mushroom Body Neurons. J Neurosci 25:2348–2358. https://doi.org/10.1523/JNEUROSCI.4384-04.2005
49. Cowan TM, Siegel RW (1986) Drosophila Mutations that Alter Ionic Conduction Disrupt Acquisition and Retention of a Conditioned Odor Avoidance Response. J Neurogenet 3:187–201. https://doi.org/10.3109/01677068609106849
50. Bushey D, Huber R, Tononi G, Cirelli C (2007) Drosophila Hyperkinetic Mutants Have Reduced Sleep and Impaired Memory. J Neurosci 27:5384–5393. https://doi.org/10.1523/JNEUROSCI.0108-07.2007
51. Joiner MA, Asztalos Z, Jones CJ, et al (2007) EFFECTS OF MUTANT DROSOPHILA K + CHANNEL SUBUNITS ON HABITUATION OF THE OLFACTORY JUMP RESPONSE. J Neurogenet 21:45–58. https://doi.org/10.1080/01677060701247375
52. Davidson G, Shen J, Huang Y-L, et al (2009) Cell Cycle Control of Wnt Receptor Activation. Dev Cell 17:788–799. https://doi.org/10.1016/j.devcel.2009.11.006
53. Han N, Chen C, Shi Z, Cheng D (2014) Structure of the kinase domain of Gilgamesh from Drosophila melanogaster. Acta Crystallogr Sect F Struct Biol Commun 70:438–443. https://doi.org/10.1107/S2053230X14004774
54. Tan Y, Yu D, Pletting J, Davis RL (2010) Gilgamesh Is Required for rutabaga-Independent Olfactory Learning in Drosophila. Neuron 67:810–820. https://doi.org/10.1016/j.neuron.2010.08.020
55. Zhang Y, Huang N, Yan F, et al (2018) Diabetes mellitus and Alzheimer's disease: GSK-3β as a potential link. Behav Brain Res 339:57–65. https://doi.org/10.1016/j.bbr.2017.11.015

56. Sofola O, Kerr F, Rogers I, et al (2010) Inhibition of GSK-3 Ameliorates Aβ Pathology in an Adult-Onset Drosophila Model of Alzheimer's Disease. PLoS Genet 6:e1001087. https://doi.org/10.1371/journal.pgen.1001087
57. Jia D-D, Zhang L, Chen Z, et al (2013) Lithium Chloride Alleviates Neurodegeneration Partly by Inhibiting Activity of GSK3β in a SCA3 Drosophila Model. The Cerebellum 12:892–901. https://doi.org/10.1007/s12311-013-0498-3
58. Aqsa, Sarkar S (2021) Age dependent trans-cellular propagation of human tau aggregates in Drosophila disease models. Brain Res 1751:147207. https://doi.org/10.1016/j.brainres.2020.147207
59. Hovhanyan A, Herter EK, Pfannstiel J, et al (2014) Drosophila Mbm Is a Nucleolar Myc and Casein Kinase 2 Target Required for Ribosome Biogenesis and Cell Growth of Central Brain Neuroblasts. Mol Cell Biol 34:1878–1891. https://doi.org/10.1128/MCB.00658-13
60. de Belle JS, Heisenberg M (1996) Expression of Drosophila mushroom body mutations in alternative genetic backgrounds: a case study of the mushroom body miniature gene (mbm). Proc Natl Acad Sci 93:9875–9880. https://doi.org/10.1073/pnas.93.18.9875
61. Wolf R, Wittig T, Liu L, et al (1998) Drosophila Mushroom Bodies Are Dispensable for Visual, Tactile, and Motor Learning. Learn Mem 5:166–178. https://doi.org/10.1101/lm.5.1.166
62. Liu L, Wolf R, Ernst R, Heisenberg M (1999) Context generalization in Drosophila visual learning requires the mushroom bodies. Nature 400:753–756. https://doi.org/10.1038/23456
63. Riemensperger T, Isabel G, Coulom H, et al (2011) Behavioral consequences of dopamine deficiency in the Drosophila central nervous system. Proc Natl Acad Sci 108:834–839. https://doi.org/10.1073/pnas.1010930108
64. Berry JA, Cervantes-Sandoval I, Nicholas EP, Davis RL (2012) Dopamine Is Required for Learning and Forgetting in Drosophila. Neuron 74:530–542. https://doi.org/10.1016/j.neuron.2012.04.007
65. Cervantes-Sandoval I, Phan A, Chakraborty M, Davis RL (2017) Reciprocal synapses between mushroom body and dopamine neurons form a positive feedback loop required for learning. Elife 6:1–16. https://doi.org/10.7554/eLife.23789

Tables

Table 1 is available in the Supplementary Files section.

Schemes

Scheme 1 is available in the Supplementary Files section

Figures

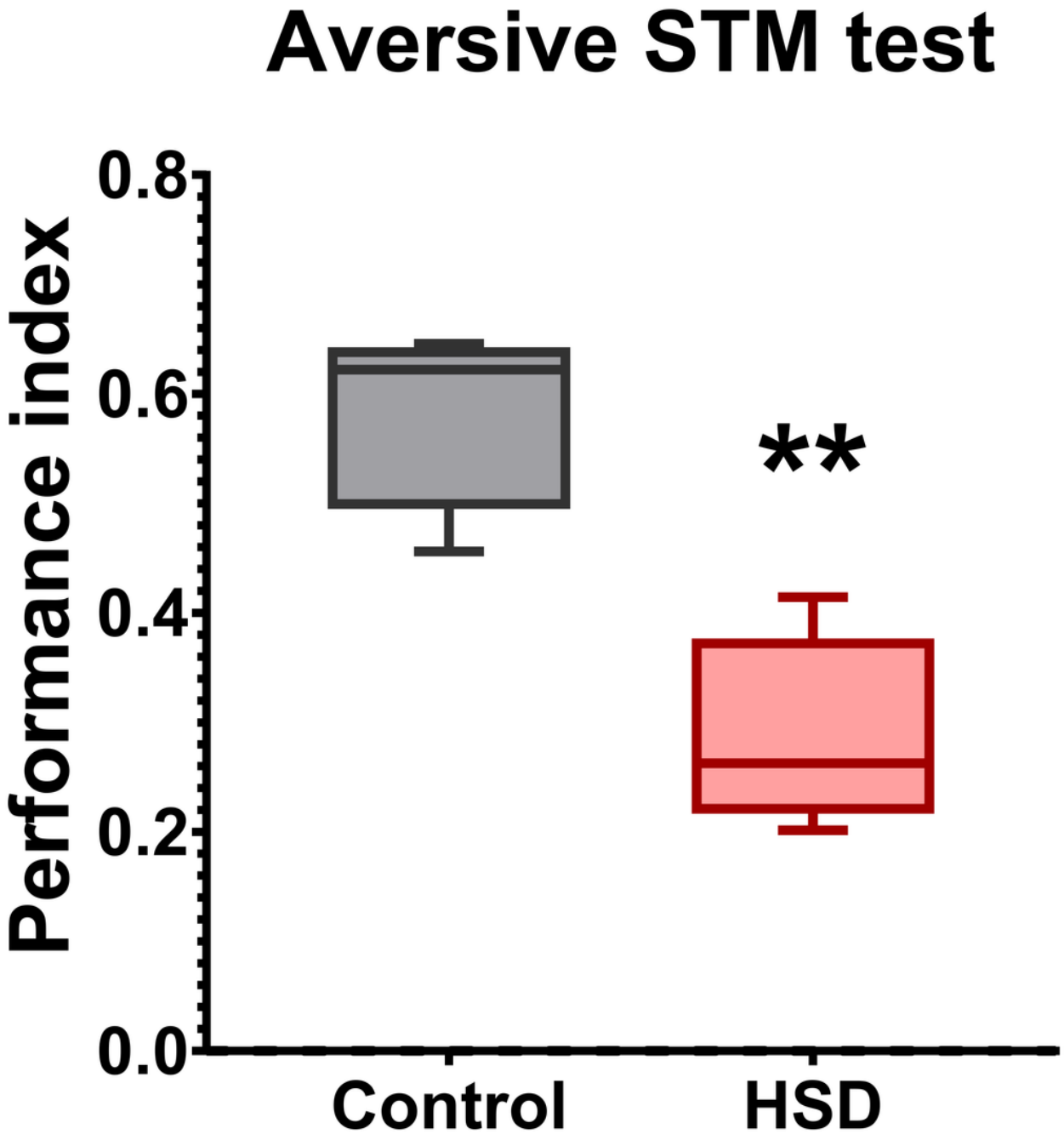

Figure 1

Effects of HSD diet on fly's aversive short-term memory by association. The test was accessed in flies hatched from and reared on control and/or HDS diet for 7 days. Statistical analyses were done by Unpaired t test with **p<0.005

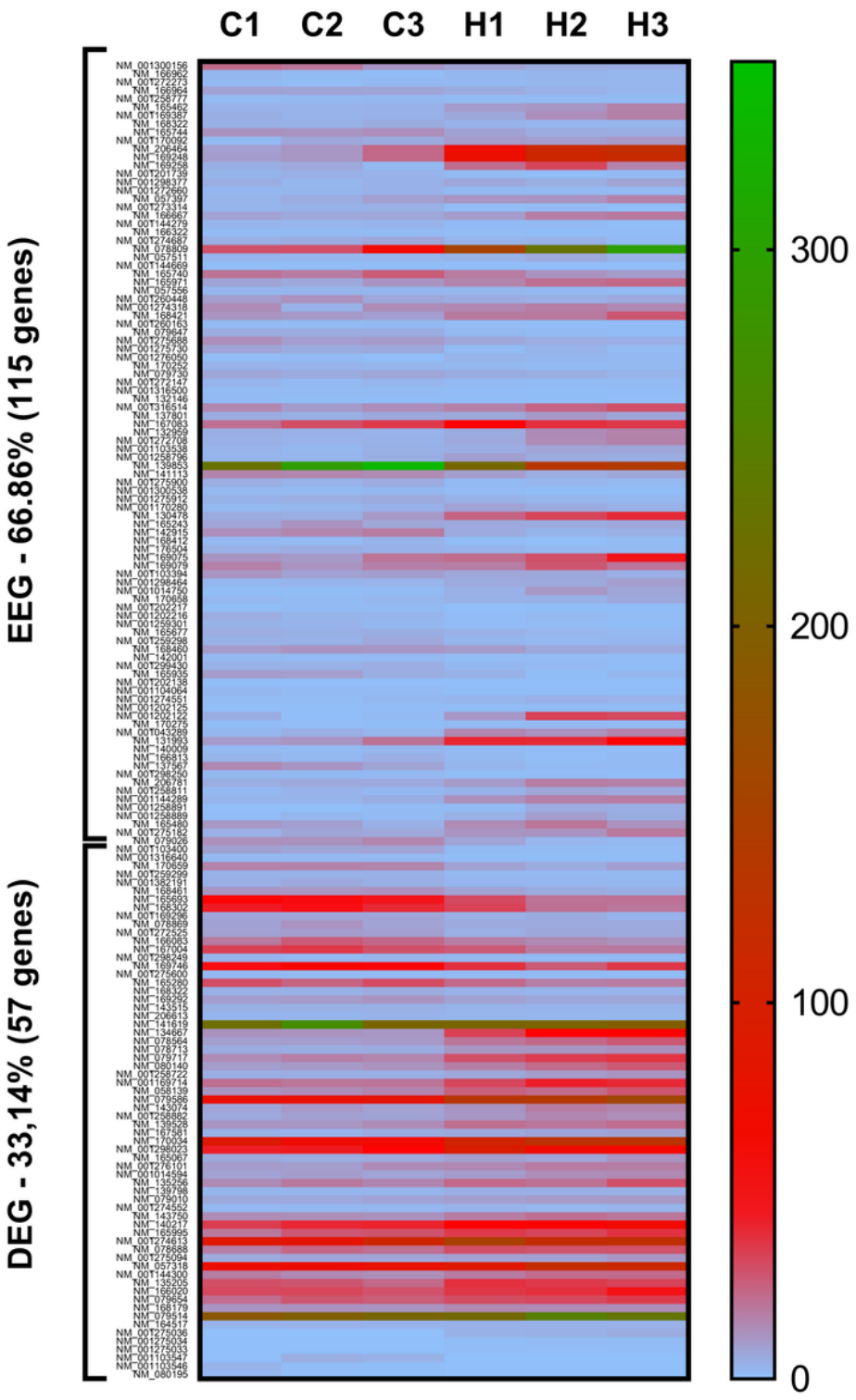

Figure 2

Heat-Map containing the expression values in rpkm of all genes marked as ‘memory or learning’ in FlyBase dataset in Control (C1, C2 and C3) and HSD libraries (H1, H2 and H3). Proportions of DEG are also indicated

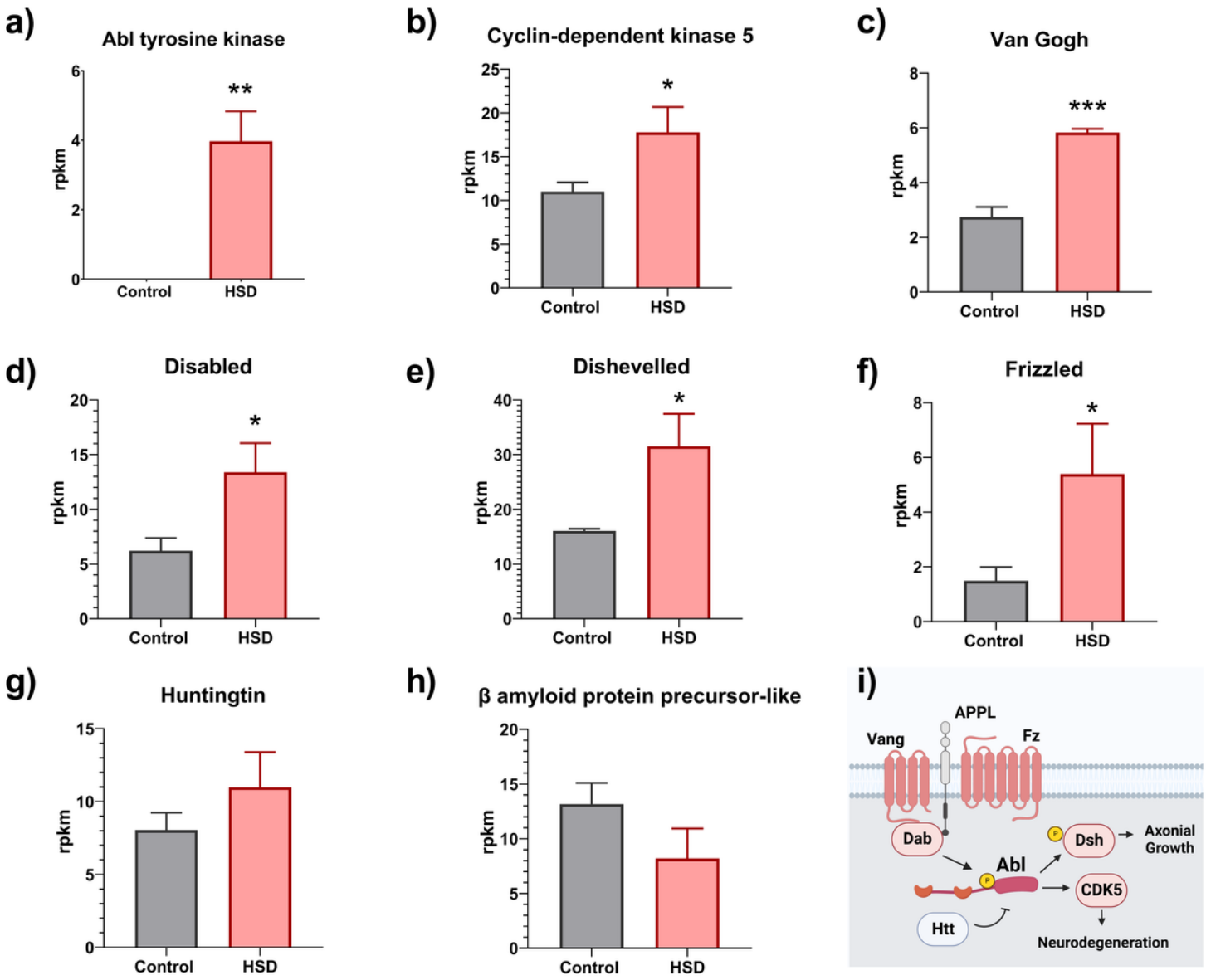

Figure 3

Expression values of genes involved with the Abl and Wnt signaling in control and HSD flies. a) Abl tyrosine kinase (Abl), isoform I b) Cyclin-dependent kinase 5 (CDK5) Frizzled c) Van Gogh (Vang), isoform B d) Disabled (Dab) e) Dishevelled (Dsh) f) (Fz), isoform A g) Huntingtin (Htt), isoform A h) β amyloid protein precursor-like (APPL), isoform F i) Abl signaling on memory through alteration on axonal growth in MB and neurodegeneration. DEG are represented in red and EEG in grey colors. The figure was based on the proposed models of Bothwell and Giniger, 2000 and Marquilly et al, 2021.Statistical analyses were done by Unpaired t test (* indicates $p<0.05$, ** indicates $p<0.002$ and *** indicates $p<0.0002$)

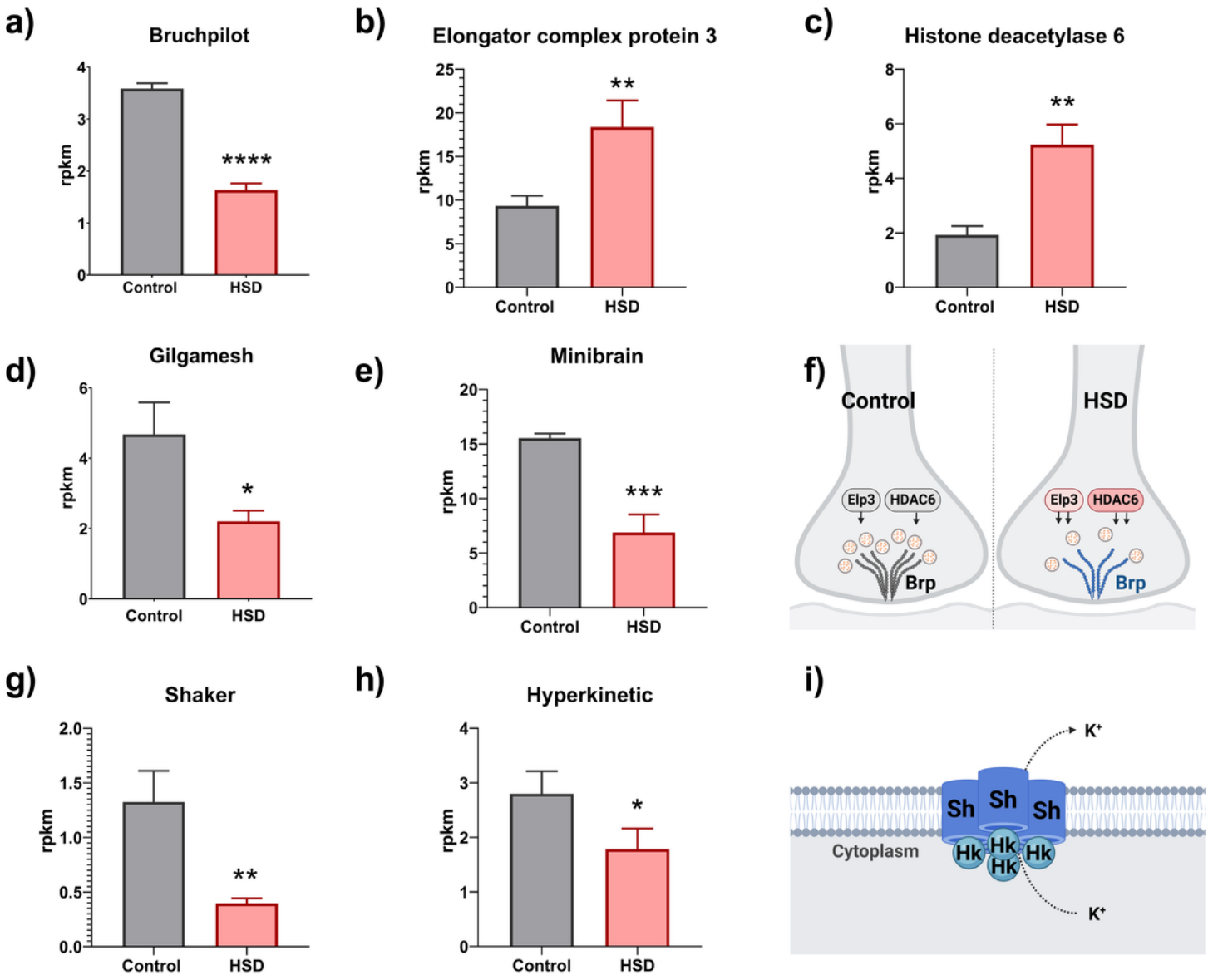

Figure 4

Expression values of genes involved with the STM in control and HSD flies. a) Bruchpilot (Brp), isoform J. b) Elongater complex protein 3 (Elp3). c) Histone deacetylase 6 (HDAC6), isoform A. d) Gilgamesh (Gish), isoform P. e) Minibrain (Mnb), isoform H. f) Proposed model of Brp action in control and HSD flies. g) Shaker (Sh), isoform T. h) Hyperkinectic (Hk), isoform K. i) Shaker and Hiperkinectic signaling was based on proposed model of Bushey et al, 2007. Statistical analyses were done by Unpaired t test (* indicates $p<0.05$, ** indicates $p<0.002$, *** indicates $p<0.0002$ and **** indicates $p<0.0001$)

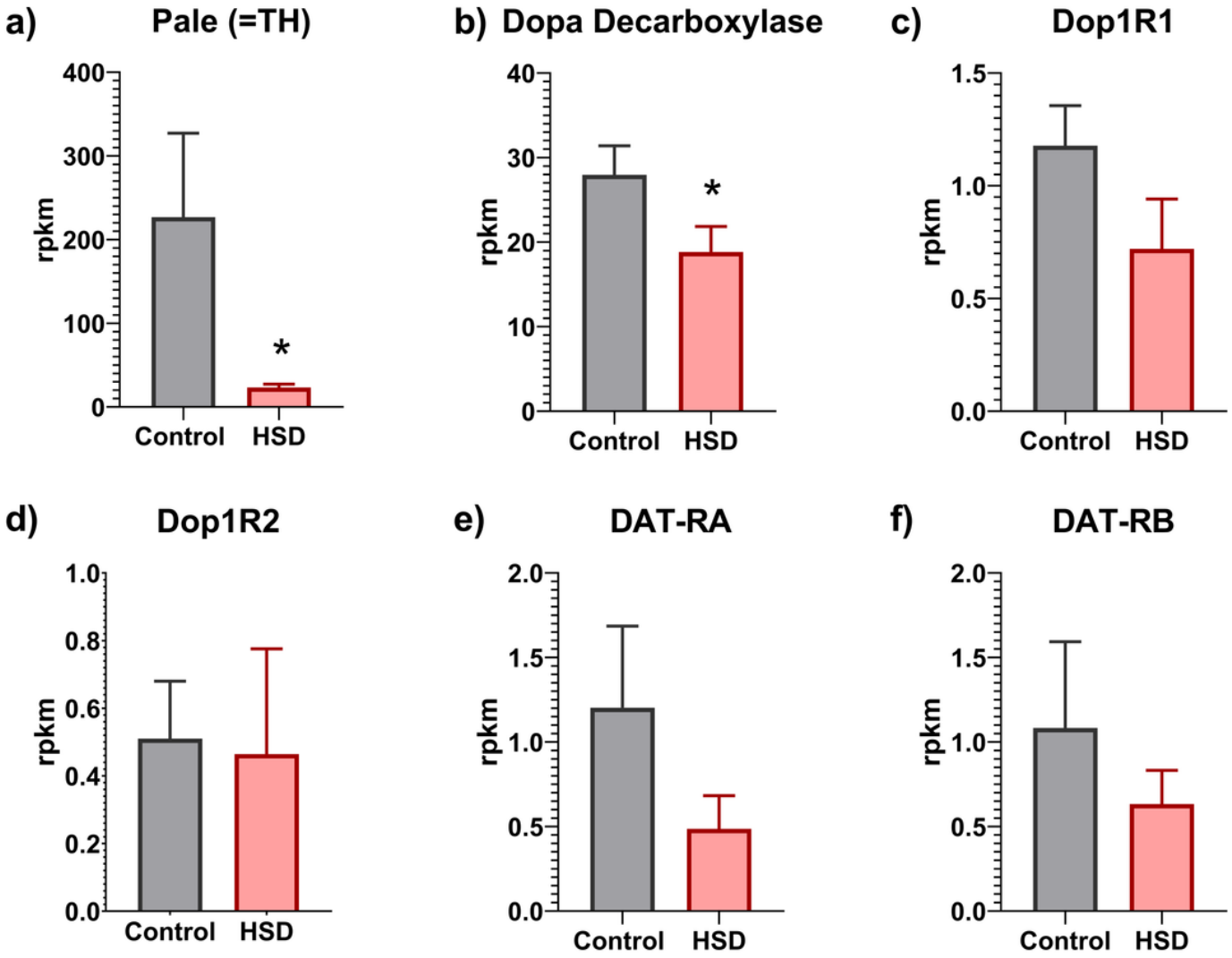

Figure 5

Expression values of genes involved with the dopamine regulation in control and HSD flies. a) Pale (Ple, also known as Tyrosine Hydroxylase [TH]), isoform B b) Dopa Decarboxylase (Ddc), isoform B c) Dopamine 1-like Receptor-1 (Dop1R1, also known as dumb), isoform D d) Dopamine 1-like Receptor-2 (Dop1R2, also known as damb), isoform A e) Dopamine Transporter (DAT), isoform A and f) Dopamine Transporter (DAT), isoform B. Statistical analyses were done by Unpaired t test for parametric data and Mann-Whitney test for nonparametric data (* indicates $p<0.05$)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.docx
- SupplementalTable2.docx

- Table1.tif
- GraphicalAbstract.tif
- Scheme1.png