The potential neuronal role of the activity-regulated gene Hr38 and dopamine-induced synaptic plasticity within long-term memory

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Abstract

Synaptic plasticity is a core mechanism responsible for neuronal functions such as long-term memory and learning, as when humans and animals learn, the synaptic plasticity within neuronal networks are responding to specific cues which elicit various changes in behavior[1]. Recent findings have suggested that activity-regulated genes (ARGs), such as *Hormone receptor-like in 38* (Hr38), and dopamine-driven synaptic plasticity are equally crucial for long-term olfactory memory consolidation, as well as the re-evaluation of learned information and the integration of various 'internal states'[1,2].
Surprisingly, ARGs are poorly studied in well-used model organisms like Drosophila, thus, in the hopes of further understanding the neuronal mechanisms behind the long-term memory (LTM), here we stimulated Drosophila neurons with appetitive conditioning to induce dopamine-driven plasticity, and then used the GAL4-UAS system, along with interference RNAs (RNAis), to knock-down and subsequently try to deduce the role of Hr38 within memory consolidation[2].

Introduction

- Synaptic plasticity, which is also termed activity-dependent synaptic plasticity, is the process by which perpetuated activity results in changes for the strength of connections between neurons[1].
- Theoretically, this leads to improved memory and learning capabilities by altering neuronal connections within the hippocampus, but interestingly, if the activity occurs concurrently with a positive or negative outcome/'valence' it is thought that the activity acts as a neutral stimulus which is associated with the subsequent outcome[1].
- This, is termed associative or olfactory association, and depending on what neutral stimuli is used, and when paired with a positive valence, dopaminergic neuros (DANs) induce dopamine secretion to modify neuronal connections and consolidate memory, something which is coined dopamine-driven synaptic plasticity[1].
- Activity-regulated genes (ARGs) also play important roles for neuronal functions including long-term memory (LTM), however, unlike synaptic plasticity, the basic understanding of ARGs is essentially incomplete, especially within model organisms such as Drosophila (figure 1)[3,4].
- Thus, to further understand the neuronal mechanisms behind LTM, the aim was to manipulate and try to deduce the role of one ARG of particular interest, *Hormone receptor-like in 38* (Hr38), which is believed to be involved with the LTM of drosophila via the process of appetitive conditioning (olfactory association with a positive valence)[3].

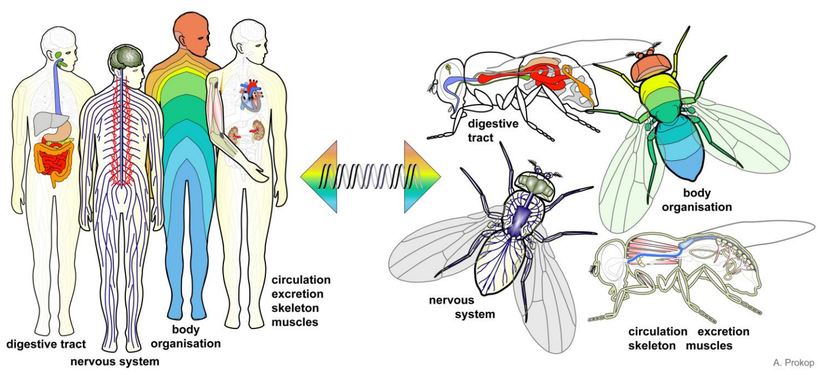


Figure 1[4]. Diagrammatic organization of Drosophila in comparison to humans
Figure 1 depicts the reason why Drosophila are used as model organisms. Specifically, they're used, as Drosophila have so many commonalities with human anatomy and various systems which is then applicable for further work based on higher vertebrates and humans.
For example, it is used here for investigations based on ARGs as Drosophila share commonalities via their nervous systems and corresponding nerve cells/neurons. Some of which include the facts that:

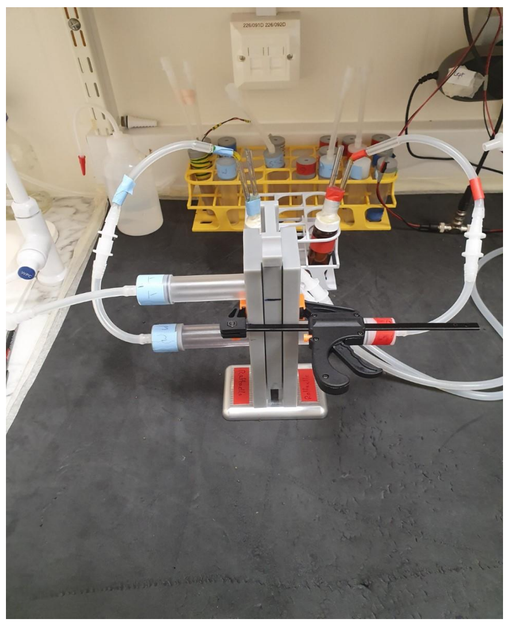
- both humans and Drosophila have sensory neurons that lie outside the central nervous system for assisting vision, smell, taste, hearing, mechanical resistance, etc.
- and many functional mechanisms centered on neurodegeneration and development were originally discovered in flies.

Materials

To obtain accurate results that depict the activity of Hr38, and thus allude to a greater understanding of LTM, various materials are required, the majority of which include:

- Drosophila combined with the GAL4-UAS system (for knocking down Hr38)
- T-maze (for olfactory association training and testing – depicted by figure 2)
- RNA extraction and cDNA synthesis kits
- Both a PCR and qPCR machine

A



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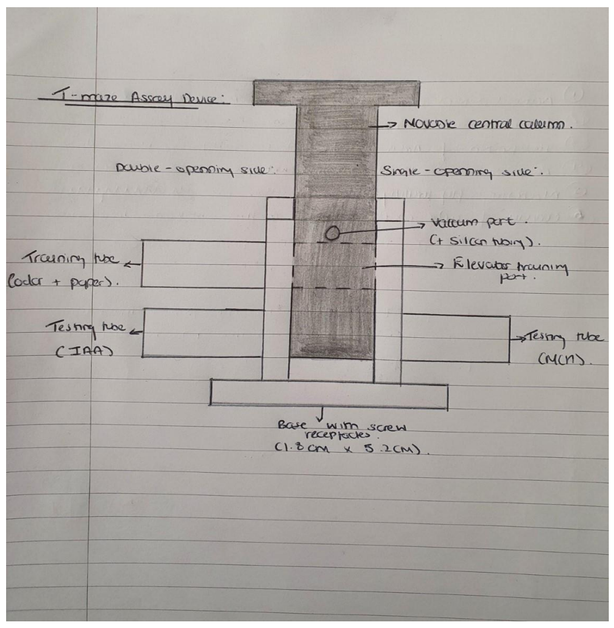


Figure 2. T-maze memory assay device:

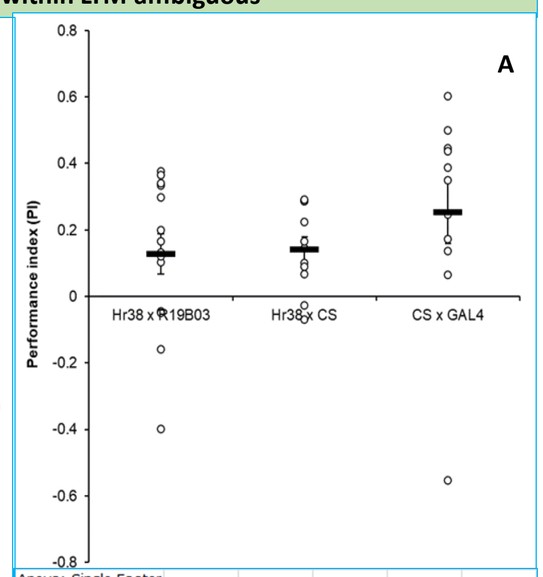
2a. Photograph of the physical T-maze used for testing.

2b. Diagrammatic illustration of T-maze.
A T-maze consists of three plastic columns with a lift centralized to the middle column. Additionally, the most left column contains 2 compartments each for test tubes that respectively allows training and testing, as the top compartment is used for training/associating IAA or MCH with sugar paper, and the bottom is used as the location for the sugar test tube which is connected to IAA or MCH by silicone tubing during testing. This is also the same for the further right column but for water instead of sugar. Finally, the central column also moves up and down to transport the Drosophila from the training test tube to the lower 'testing test tubes' where both MCH and IAA are being pumped in.

Results

Memory Assays keeps Hr38 role within LTM ambiguous

- From the memory assays, both parental controls, genotype 2 and 3, consistently preferred sugar over water, indicating that olfactory association/appetitive conditioning works, and the Drosophila progeny had their LTM functioning by associating IAA and MCH with both the nutritional reward of sugar over the more neutral water.
- However, as stated genotype 2, Hr38 UAS x CS, proved difficult to handle and there was consistently less progeny for genotype 2 than both genotype 1 and 3, Hr38UAS RNAi x R19B03 GAL4 and R19B03 GAL4 x CS. Potentially suggesting that the UAS system is 'leaky' and as a result there was impaired development and LTM during the development phase, something which occurred sooner than intended, but this does remain to be proven.
- Additionally, despite the positive result, the memory assay results were also not significant (p= 0.373851/p>0.05).
- Thus, it cannot be stated with confidence that Hr38 is involved in LTM periodically and instead remains ambiguous as samples of genotype 1 only performed an expected unbiased preference between IAA and MCH occasionally (figure 3b).



B

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	14	1.782977	0.127356	0.051056
Column 2	11	1.563257	0.142114	0.015481
Column 3	11	2.788973	0.253543	0.098442

ANOVA

ce of Varia	SS	df	MS	F	P-value	F crit
Between G	0.110781	2	0.05539	1.013824	0.373851	3.284918
Within Gro	1.802959	33	0.054635			
Total	1.91374	35				

Figure 3. Memory assay data (08/08/2022 - 09/09/2022)
Figure 3a. Scatterplot depicting memory assay data.
Figure 3b. One-way Anova of memory assay data.

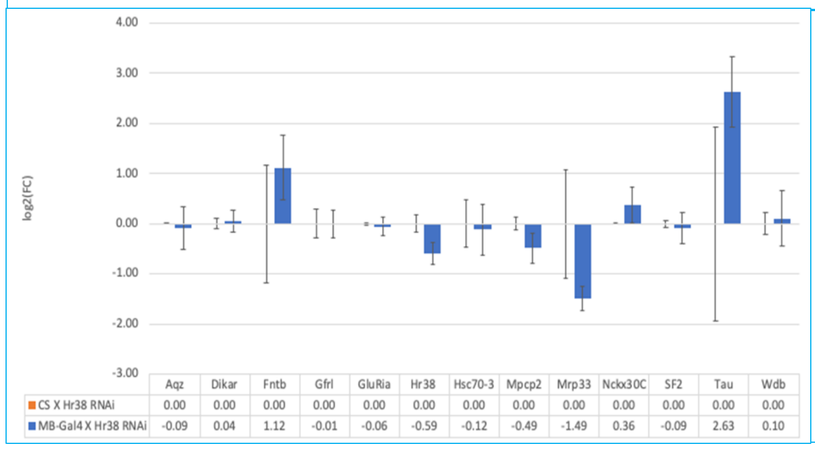
Both figure 4a and 4b focused on the 3 genotypes of Drosophila used during the memory assay protocols:

- Hr38 RNAi x R19B03 GAL4 (Hr38 knockdown –expected 50/50 split between odors/sugar and water)
- Hr38 RNAi x CS (parental control 1 – no knockdown, expected preference for sugar, however **proved difficult to handle**)
- R19B03 GAL4 x CS (parental control 2 – no knockdown, expected preference for sugar)

Unfortunately, as displayed by figure 3b, none of the results proved to be significantly different (p>0.05), and thus difficult to interpret whether Hr38 is involved in memory or not.

qPCR expression tests indicate Hr38's downstream effects

- It is demonstrated that Hr38 is decreased in genotype 1 samples, showing that the RNAi line used in the project worked successfully by downregulating Hr38 as expected.
- Additionally, it was seen that by downregulating Hr38, the RNAi in turn caused downstream fluctuations in the levels of expression of the other target genes, including a decrease in Mrp33 and an increase in Tau, implying a respective decrease in mitochondrial RNA processing and an accumulation of tau, possibly relating to an increased chance of developing tauopathies, such as Alzheimer's[5].
- However, some of the qPCR samples obtained were quite poor in quality and had to be removed from calculations as a result, thus, in the future, I would wish to re-attempt the qPCR protocol and expression tests to obtain more accurate results on the expression of Hr38 and its downstream effects on tau and the other target genes involved.



	Aqz	Dikar	Fntb	Gfrl	GluRia	Hr38	Hsc70-3	Mpcp2	Mrp33	Nclox30C	SF2	Tau	Wdb
CS X Hr38 RNAi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MB-Gal4 X Hr38 RNAi	-0.09	0.04	1.12	-0.01	-0.06	-0.59	-0.12	-0.49	-1.49	0.36	-0.09	2.63	0.10

Figure 4. Gene expression of Hr38 and other target genes.
Figure 4 represents the data obtained from the qPCR utilized to quantify the expression of Hr38. Interestingly, Hr38 is not so strongly downregulated as expected, while for the other target genes, I had to remove both biological replicates #3 as the values were completely off and expression values were extraordinarily high, thus there were only 2 replicates for each genotype. Also, only SdhA was used as a HK as some values were missing for GAPDH, and unfortunately there was no good data for nAChRalpha5.

Future work & Conclusions

- In relation to future work, despite the lack of significance, I would also like to use another GAL4 line to investigate whether Hr38 is involved in other parts of the Drosophila mushroom body, the center of learning and memory for Drosophila, and other functions relating to memory. This could also be done in co-ordination with using another Hr38 line to disprove or prove the results displayed here in figure 4 and in turn disprove or prove Hr38's involvement with LTM specifically. Finally, I would also wish to perform more memory assays based on female Drosophila and male Drosophila separately to investigate the sex-specific differences with Hr38 and LTM, as Hr38 is believed to be upregulated in males when compared to females.
- Overall, despite the room for expansion, it can be said that greater knowledge on neuronal mechanisms and networks was achieved as I managed to show that appetitive conditioning does indeed induce olfactory association and LTM in wild-type Drosophila, and while it was not possible to deduce the role of Hr38, it can be suggested that Hr38 is involved with memory as dysregulation of Hr38 fluctuated the expression of other genes downstream, possibly leading to impaired functions that would be normally be performed at least in conjuncture with LTM. However, further work will be required to clarify this suggestion.

Acknowledgements

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