

The possible Neuronal  
roles of the activity-  
regulated gene Hr38 and  
dopamine-induced synaptic  
plasticity within long-term  
memory

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# 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. 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.
  - This, is termed associative or olfactory association, and depending on what neutral stimuli is used, and, when paired with a positive valence, dopaminergic neurons (DANs) induce dopamine secretion to again modify neuronal connections and consolidate memory, something which is coined dopamine-driven synaptic plasticity.
  - 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*3.
  - Thus, to further understand the neuronal mechanisms behind olfactory association leading to LTM, the aim was to manipulate and deduce the role of one ARG of particular interest, *Hormone receptor-like in 38* (Hr38), which is believed to be involved with LTM of *drosophila* via the process of appetitive conditioning (olfactory association with a positive valence).
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# 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 1)
- RNA extraction and cDNA synthesis kits
- QPCR machine

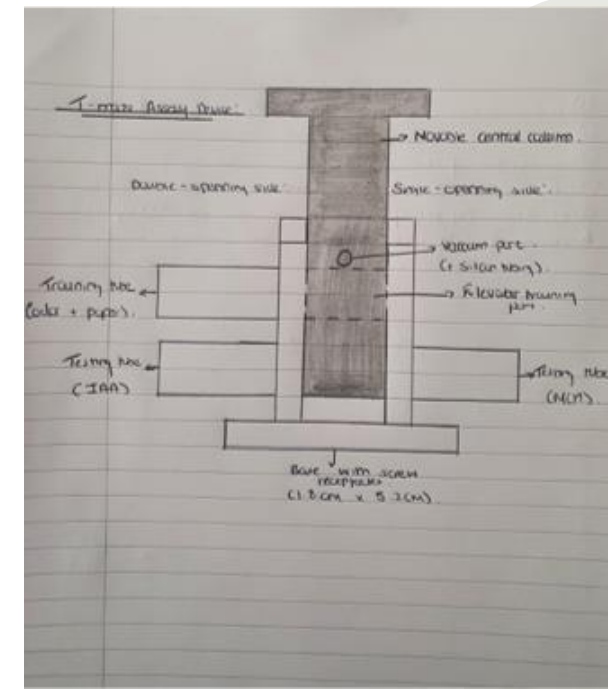


## Figure 1. T-maze memory assay device:

**1a.** Photograph of the physical T-maze used for testing is within the appendix of this report (top)

**1b.** Diagrammatic illustration of T-maze (bottom - labelled)

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 former is used for training (associating IAA or MCH with sugar paper) and the latter is used as the location for the sugar test tube which is connected to the IAA or MCH odor by silicone tubing. The latter 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 tubes where both MCH and IAA are being pumped in.

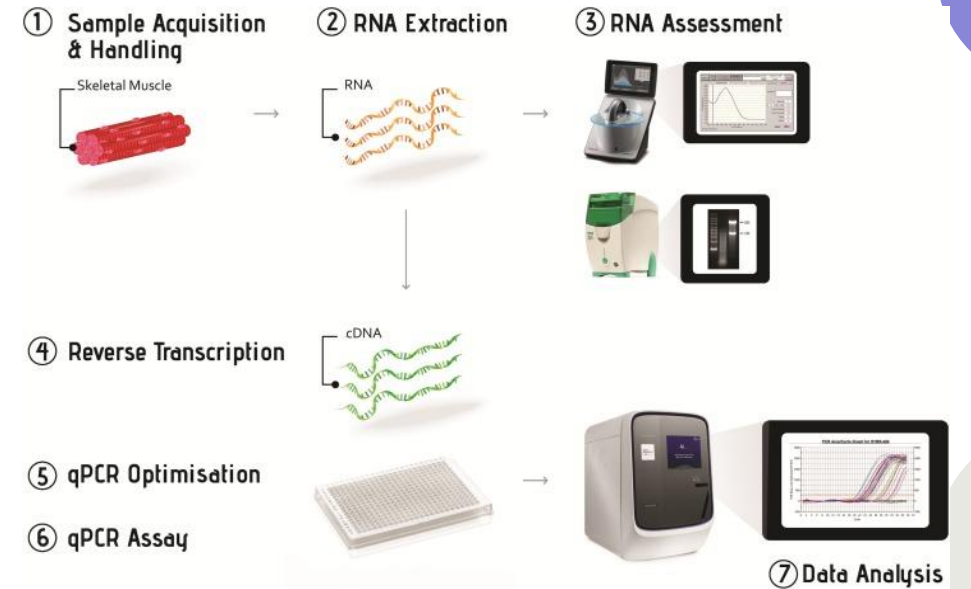


# Memory assay – protocol

- Flies are flipped into training test tube containing water paper in the top compartment for association with neutral stimulus/water = 2 minutes with MCH or IAA.
  - During this, filter paper within agar tubes is rewatered to make sure flies are hydrated (increases chance of survival during starvation).
  - After 2 minutes, flip flies into 2<sup>nd</sup> training test tube containing sugar paper and allow a 45 second break.
  - Place second tube into compartment for association with sugar/positive valence = 2 minutes with opposing odor.
  - Repeat a-f for all progeny and then flip back into agar tubes and starve/incubate for another 24 hours.
  - 24 hours later, testing begins by flipping flies back into a 'clean' training test tube.
  - Next lift up middle column of T-maze and then bash flies into lift of T-maze (same time push lift down half-way to prevent flies escaping back into training test tube).
  - Attach both sugar and water test tubes into lower compartments of T-maze (most left and right columns) with both MCH and IAA connected to one tube under 50 Pa of pressure, 23 degrees and 60% humidity.
  - Turn off lights (as flies are phototactic) and push lift fully down to the base of the T-maze for 2 minutes to allow flies to migrate to one tube (controls should migrate to sugar, Hr38 RNAi should be unbiased).
  - After 2 minutes, lift the lift back up to starting position (stops the flies from moving between the tubes) and turn the lights back on. Then place flies into collection tubes (flies in left tube = 'A' collection tubes, and right tube='B' collection tubes).
  - Repeat for all flies, and then freeze flies for counting.
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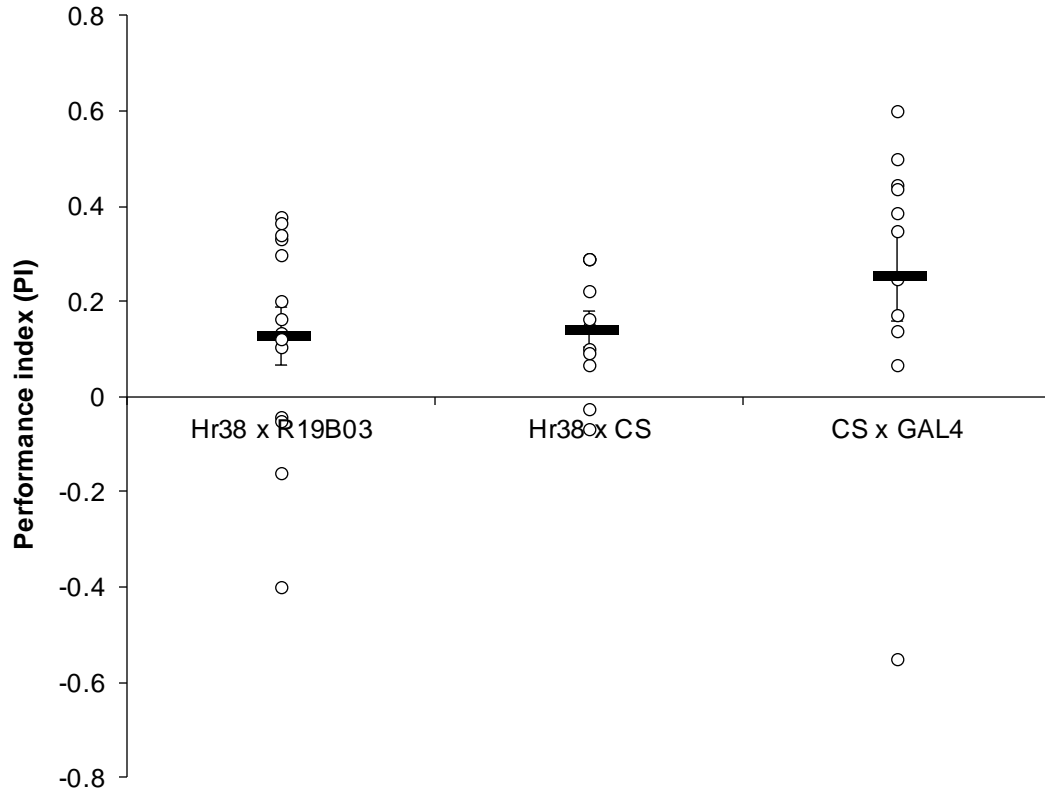
# RNA extraction & qPCR targets

- Despite the straightforward protocol listed above, there is one caveat that must be discussed prior to moving onto to the next protocol focused on RNA extraction. Within this project, both training and testing act as memory assays that indicate whether the Hr38 crosses/progeny of *Drosophila* had their Hr38 downregulated and whether this in turn influenced memory consolidation and LTM, which shall be discussed later.
- However, to support this indication, both RNA extraction and qPCR are needed. Thus, during training there is allocated vials of *Drosophila* that are then frozen in liquid nitrogen at specific time points post-training. These time points include 0 hours after training, 2 hours after and 4 hours after and this subdivision were frozen so quickly to allow us to quantify the regulation of Hr38 and physically see whether Hr38 was downregulated in comparison to more 'stable housekeeping' (HK) genes, including GADPH and SdhA, that rarely fluctuate in the majority of cells, as well as various target genes of interest also believed to be involved in memory or interact with Hr38.
- In addition to freezing this subdivision, the remaining flies post-testing are then frozen at -4 degrees Celsius for approximately an hour and are counted for the aforementioned indication centered on Hr38.
- For clarity, The target genes of interest for qPCR include:: Hr38 , Nckx30c , Hsc703, Fntb, Aqz, SF2, Dikar, Tau, Wdb, Mrp33, NACHRap1ha5, GluRII, GADPH, Mpcp2, SdhA.

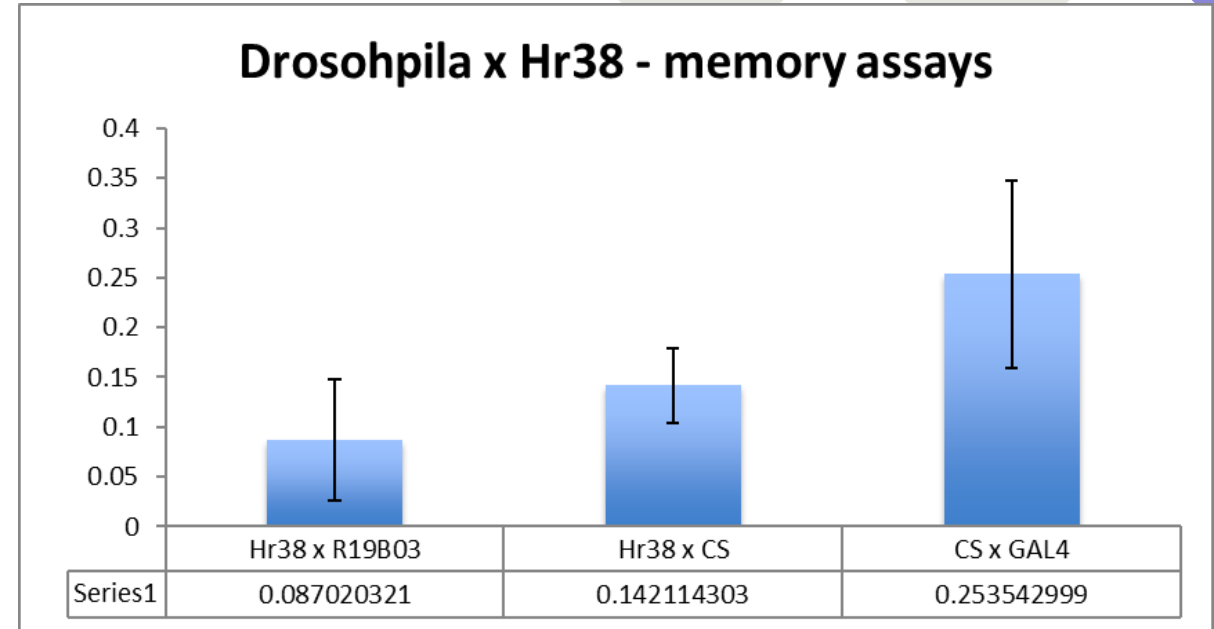


**Figure 2. Summary of RNA extraction and qPCR workflow.** Primarily shows the stages involved in the 'post-testing stages' of the project. Steps 1-4 =RNA extraction, 5-6 =qPCR.

# Memory assay – results



- Memory assay data was collected from 08/08/2022 - 09/09/2022.
- Focused on 3 genotypes of Drosophila:
  1. Hr38 RNAi x R19B03 GAL4 (Hr38 knockdown –expected 50/50 split between odors/sugar and water)
  2. Hr38 RNAi x CS (parental control 1 – no knockdown, expected preference for sugar, however **proved difficult to handle**)
  3. R19B03 GAL4 x CS (parental control 2 – no knockdown, expected preference for sugar)

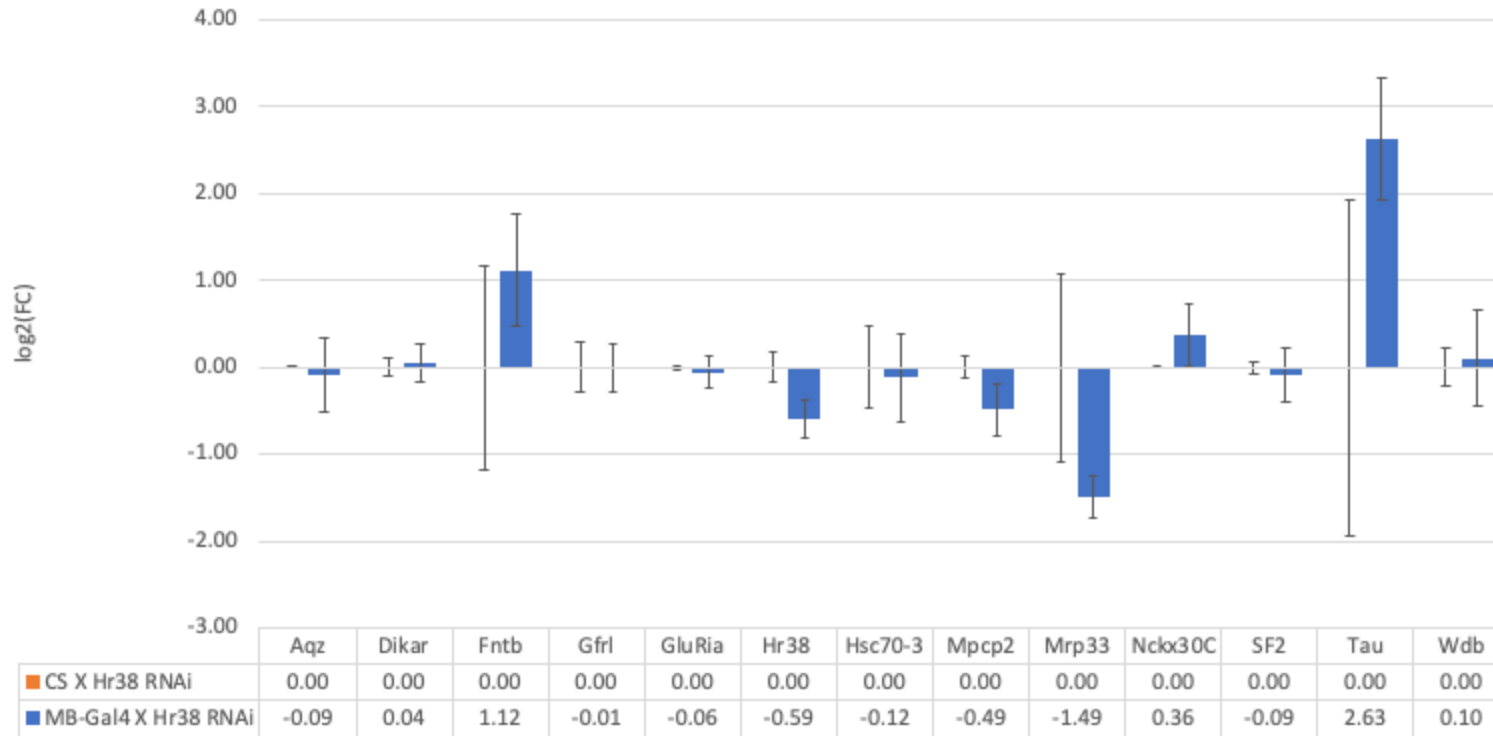


Anova: Single Factor				
SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	14	1.782977	0.127356	0.051056
Column 2	11	1.563257	0.142114	0.015481
Column 3	11	2.788973	0.253543	0.098442

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.110781	2	0.05539	1.013824	0.373851	3.284918
Within Groups	1.802959	33	0.054635			
Total	1.91374	35				

# QPCR – results Hr38



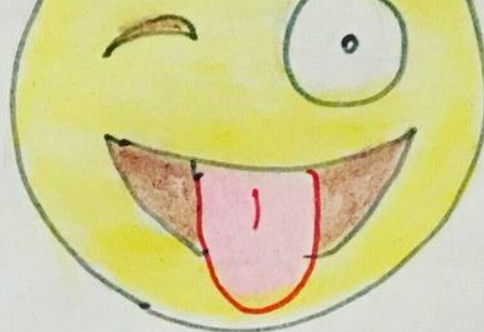
# Further work

1. Memory assays – despite lack of significance wish to still see if Hr38 is involved in memory thus use another GAL4 line to see if Hr38 is involved in other parts of the Drosophila's mushroom body for consolidation of memory/LTM.

- Also, this goal could be done via another Hr38 RNAi line to confirm or disprove result from current RNAi line used.

2. Finally, as Hr38 is upregulated in males, could investigate sex-specific differences (differences between female and male LTM and their 'concentration' of Hr38).

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Thank you

