Project Notes:

Project Title:
Name: Lee, Madison

Note Well: There are NO SHORT-cuts to reading journal articles and taking notes from them. Comprehension is paramount. You will most likely need to read it several times, so set aside enough time in your schedule.

Contents:

Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved, and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
Do invertebrates produce or use GnRH	They lack a hypothalamic pituitary Gondal axis and do not produce GnRH. They have equivalents.	Article 6	9/17
Are zebrafish a valid model for studying GnRH	Yes because zebrafish possess GnRh neurons	Article 7 and 8	9/22
What brain regions in zebrafish respond to GnRH-a exposure	The hypothalamus	Article 8	10/6
What drives the memory that habituation relies on?	Synaptic plasticity	Article 12	11/1
How does dopamine get regulated by AKH?	GABAergic Inhibition	Article 20	12/3
How can AKH be manipulated at a certain stages	GAL4 and Gal80 cross system	Article 22	12/3

Literature Search Parameters:

These searches were performed between (Start Date of reading) and XX/XX/2019.
List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
WPI Library	GnRH, Drosophila	I was able to determine whether drosophila was a good species for my project through similarities and difference drawn from the articles of drosophila gnrh equivalents and actual gnrh
WPI Library	Zebrafish, Habituation	From the articles that I derived from this search, I was able to look at the methods researchers used to assess factors like memory and learning behaviors. This was typically done through light and dark flashes
WPI Library	Drosophila, habituation, learning	This searches led me to olfactory and mechanosensory assays on how repeated stimuli altered behavioral responses
WPI Library	GAL 4, GAL 80	This search proivided methodological background on genetic tools for controlling gene expression timing

Tags:

Tag Name	

Article #1 Notes: Title

Article notes should be on separate sheets

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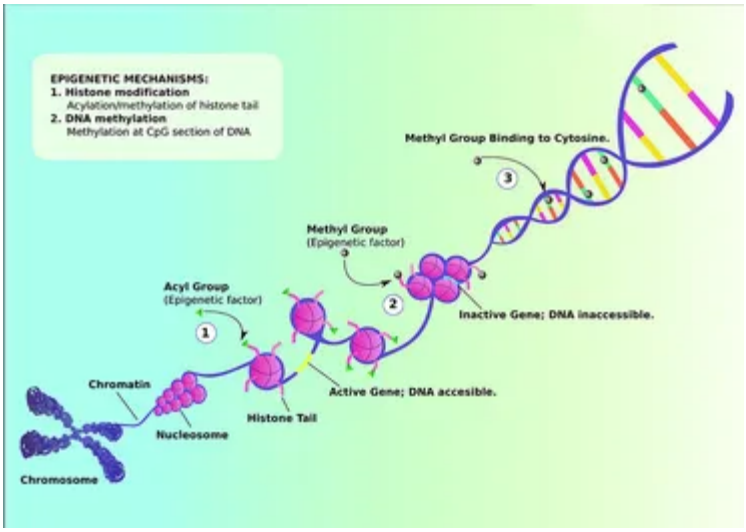
Source Title	
Source citation (APA Format)	
Original URL	
Source type	
Keywords	
#Tags	
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #1 Notes: Epigenetics and Autism Spectrum Disorder: Is There a Correlation?

Source Title	Epigenetics and Autism Spectrum Disorder: Is There a Correlation?
Source citation (APA Format)	Eshraghi, A. A., Liu, G., Kay, S.-I. S., Eshraghi, R. S., Mittal, J., Moshiree, B., & Mittal, R. (2018). Epigenetics and autism spectrum disorder: Is there a correlation? *Frontiers in Cellular Neuroscience*, *12*. https://doi.org/10.3389/fncel.2018.00078
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC5881102/
Source type	Journal Article
Keywords	DNAm, ASD(autism spectrum disorder), Epigenetics, neurologic
#Tags	#DNAm #ASD #epigenetixs #histone
Summary of key points + notes (include methodology)	Researchers suggest that autism spectrum disorder (ASD), a range of genetic and environmental disorders, is heavily influenced by epigenetics, DNA methylation (DNAm), and histone modifications. They further delve into this by analyzing factors that might predispose people to ASD—such as diet, maternal health, and environment—and their specific genetic effects. Although they believe in a strong correlation, their study of the brain and histone effects has conflicting results and potential flaws, including small sample sizes and non-controlled experiments. - Examine gene meth patterns, compare biomarkers, cell sorting - Measure epi enzymes - Rodent models - Maternal diet - Immune genes - Study with histone identifying acetylation changes
Research Question/Problem/ Need	How is ASD progressed by epigenetic modifications, and how should that influence medication?

Important Figures	 This figure shows how epigenetic modifications like histone acylation and methylation, canaffect these genes by alterig DNA accessinility. They show how the gene refulation may influence brain development.
VOCAB: (w/definition)	Pathogenetic- origin and development of disease Transcriptional Silencing-turning off gene so it doesn't produce RNA/protein Microglia- specialized immune cells in the brain and spinal cord that maintain neurons Lymphoblastoid cell lines- lab cells derived from whit blood cells
Cited references to follow up on	Loke, Y. J., Hannan, A. J., & Craig, J. M. (2015). The role of epigenetic change in autism spectrum disorders. *Frontiers in Neurology*, *6*. https://doi.org/10.3389/fneur.2015.00107 Zhubi, A., Chen, Y., Guidotti, A., & Grayson, D. R. (2017). Epigenetic regulation of *reln* and *gad1* in the frontal cortex (FC) of autism spectrum disorder (ASD) subjects. *International Journal of Developmental Neuroscience*, *62*(1), 63–72. https://doi.org/10.1016/j.ijdevneu.2017.02.003
Follow up Questions	How does genetics and the environment create lasting effects on epigenetics together? Can the modification of certain parts of drugs be used to help symptoms of ASD without the obstruction of development? How can you distinguish different forms of ASD without extensive testing and gather them in a generalized, unbiased group?

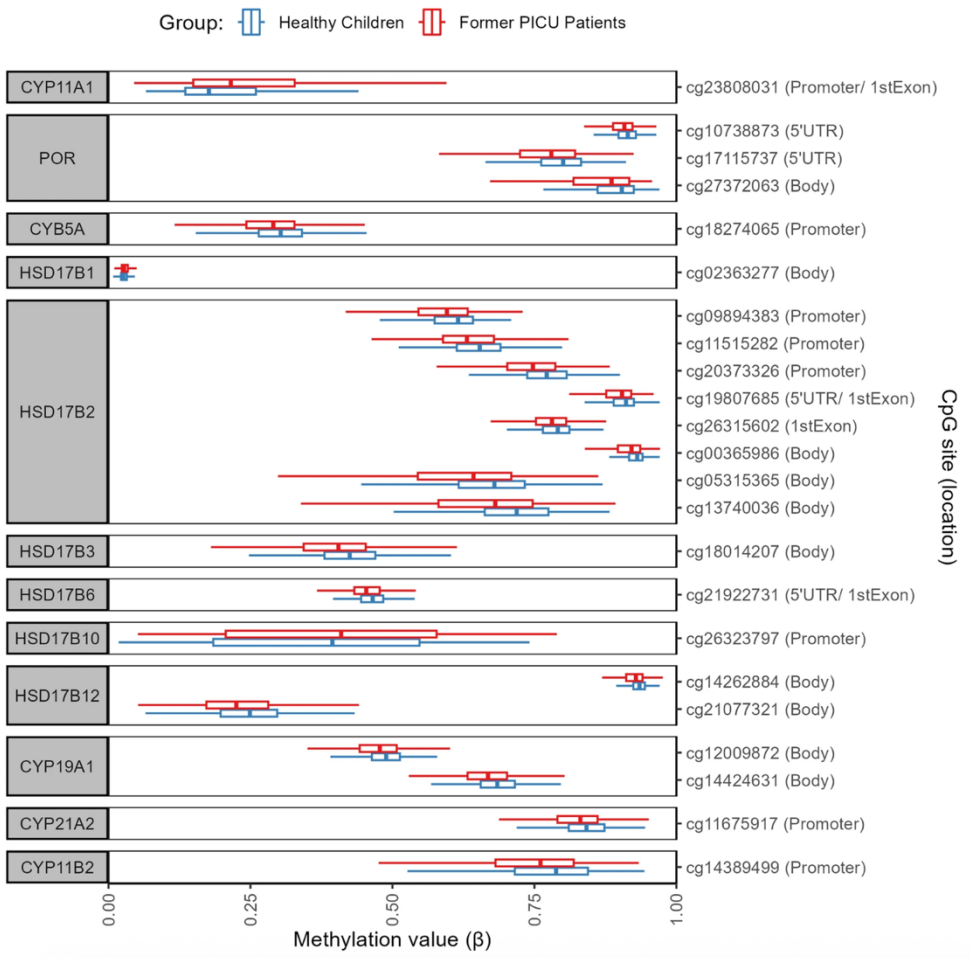
Article #2 Notes: Babies' Interactions with robots provide clues about infant development

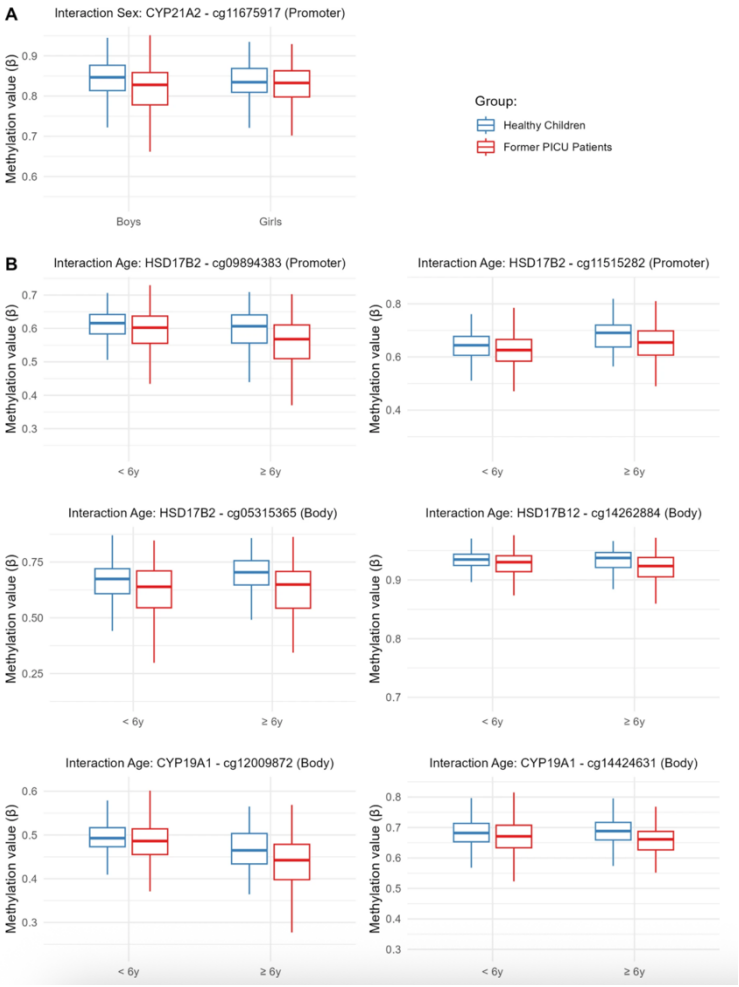
Source Title	Babies' Interactions with robots provide clues about infant development
Source citation (APA Format)	*Babies' interactions with robots provide clues about infant development - HSC News*. HSC News - News for USC's Health Sciences Campus community. (2020, February 18). https://hscnews.usc.edu/babies-interactions-with-robots-provide-clues-about-infant-development
Original URL	https://hscnews.usc.edu/babies-interactions-with-robots-provide-clues-about-infant-development
Source type	News Article/Research Summary
Keywords	Infant Development, Robotics, Early Detection Developmental Disabilities, Exploratory Movement, ADHD, Autism Spectrum Disorder, Robot
#Tags	#DevelopentalDiabilities#motionAnalysis#InfantDevelopment
Summary of key points + notes (include methodology)	• Lauren Klein and her team study how the use of robots can help detect developmental disorders early. • Use humanoid robot to encourage exploratory movement such as kicking grasping, etc, in infants which are important for healthy cognitive, motor and social development • Video recording and motion analysis to observe movements and risks • Infants who connect their movement with the robots show increased motor inactivity • Behavior can be monitored for early signs of developmental disorders
Research Question/Problem/ Need	How can robot interaction be used to detect developmental disorders in infant?
Important Figures	N/A
VOCAB: (w/definition)	N/A
Cited references to follow up on	https://www.researchgate.net/publication/332646782_Socially_Assistive_Infant Fitter, N. T., Funke, R., Pulido, J. C., Eisenman, L. E., Deng, W., Rosales, M. R., Bradley, N. S., Sargent, B., Smith, B. A., & Mataric, M. J. (2019). Socially

	assistive infant-robot interaction: Using robots to encourage infant leg-motion training. *IEEE Robotics & Automation Magazine*, *26*(2), 12–23. https://doi.org/10.1109/mra.2019.2905644 -Robot_Interaction_Using_Robots_to_Encourage_Infant_Leg-Motion_Training
Follow up Questions	1. How do exploratory motor movement patterns differ between developing infants and those at risk of developmental disorder? 2. What are the limitations of humanoid robots? 3. Can we integrate caregivers to improve early detection?

Article #3 Notes: Abnormal DNA methylation within genes of the steroidogenesis pathway two years after pediatric critical illness and association with stunted growth in height further in time

Source Title	Abnormal DNA methylation within genes of the steroidogenesis pathway two years after pediatric critical illness and association with stunted growth in height further in time
Source citation (APA Format)	Vanhorebeek, I., Coppens, G., Güiza, F., Derese, I., Wouters, P. J., Joosten, K. F., Verbruggen, S. C., & Van den Berghe, G. (2023). Abnormal DNA methylation within genes of the steroidogenesis pathway two years after paediatric critical illness and association with stunted growth in height further in time. *Clinical Epigenetics*, *15*(1). https://doi.org/10.1186/s13148-023-01530-9
Original URL	https://clinicalepigeneticsjournal.biomedcentral.com/articles/10.1186/s13148-023-01530-9
Source type	Journal Article
Keywords	Epigenetic Age deceleration, DNAm, PICU, steroidogenesis, buccal DNA, physical growth, developmental outcomes
#Tags	#Epigeneticageing #pediatriccriticalillness #DNAm#PICU#Deceleration
Summary of key points + notes (include methodology)	This article explores how pediatric critical illness impacts epigenetic aging in children, specifically by analyzing changes in DNA methylation and how those changes influence long term development. By studying former PICU patients aged six and older, the researchers were able to find and conclude that these children had significant epigenetic age and physical growth deceleration. To reinforce this, the study collected data on height, weight, and buccal DNA samples from 818 former PICU patients and compared them to healthy children. They then applied multiple statistical tests such as linear regression models, multivariable analyses, and confidence intervals to illustrate differences. As calculated p-values were less than .05, they had become statistically significant, and thus emanated that the differences between the 2 groups were not caused by variability or chance. - Buccal DNA samples were collected from 818 former PICU patients and compared to 392 healthy patients - Data was analyzed to detect biological aging differences

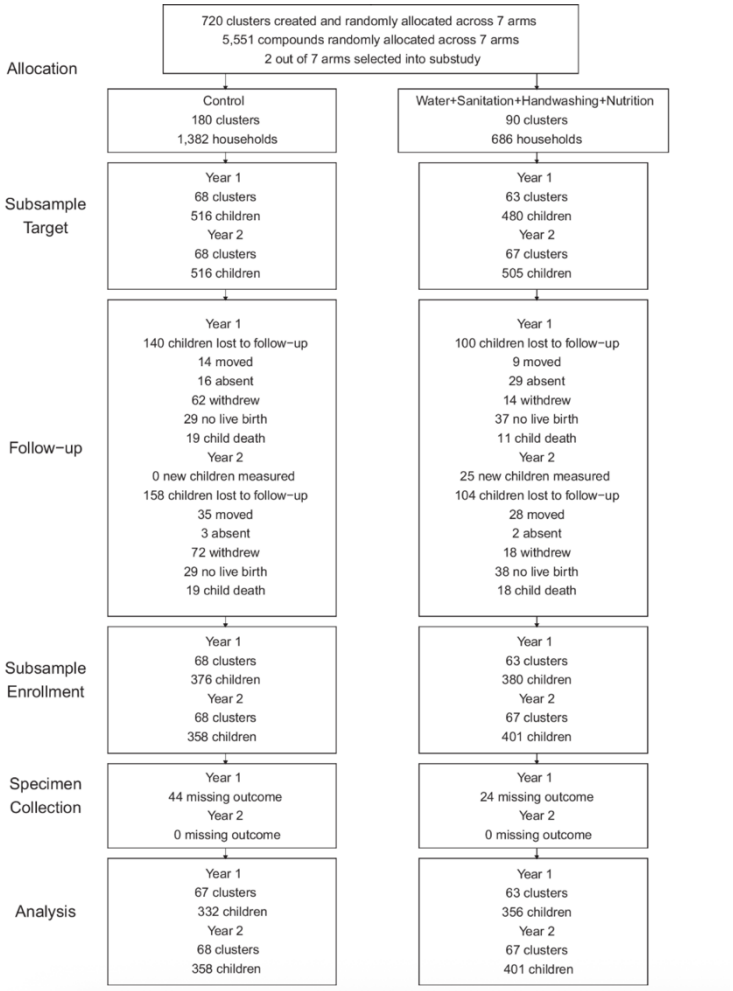
	- DNAm was used to measure epigenetic age - Linear regression models and multivariable analyses - Confidence intervals and statistical significance - Epigenetic Age deceleration and growth delays in former picus - Illness to growth impairments
Research Question/Problem/ Need	How does pediatric critical illness affect epigenetic aging and long-term physical development?
Important Figures	 Figure 3 shows the status of the methylation of CpG sites that differed between the former PICU patients and the healthy children. It shows the hypomethylation in patients.

	 It shows how the sex and age ay exposure affected the abnormal DNA, patterns in former PICU patients . Males showed greater hypomethylation and children older than 6showed signific methylation changes. So variability affects long term epigenetic effects
VOCAB: (w/definition)	Glucocorticoid treatment: Steroid hormone therapy often used in PICU setting to reduce inflammation Steroidogenesis pathway: biochemical process producing steroid hormones
Cited references to follow up on	Ko, M. S., Poh, P.-F., Heng, K. Y., Sultana, R., Murphy, B., Ng, R. W., & Lee, J. H. (2022). Assessment of long-term psychological outcomes after Pediatric Intensive Care Unit Admission. *JAMA Pediatrics*, *176*(3). https://doi.org/10.1001/jamapediatrics.2021.5767

	Mesotten, D., Gielen, M., Sterken, C., Claessens, K., Hermans, G., Vlasselaers, D., Lemiere, J., Lagae, L., Gewillig, M., Eyskens, B., Vanhorebeek, I., Wouters, P. J., & Van den Berghe, G. (2012). Neurocognitive development of children 4 years after critical illness and treatment with tight glucose control. *JAMA*, *308*(16), 1641. https://doi.org/10.1001/jama.2012.12424
Follow up Questions	1. What are the long-term effects of epigenetic age deceleration on cognitive and emotional development? 2. How to glucocorticoid treatments influence the epigenetic aging 3. Do interventions have an effect?

Article #4 Notes: **A cluster-randomized trial of water, sanitation, handwashing and nutritional interventions on stress and epigenetic programming**

Source Title	A cluster-randomized trial of water, sanitation, handwashing and nutritional interventions on stress and epigenetic programming
Source citation (APA Format)	Lin, A., Mertens, A. N., Rahman, Md. Z., Tan, S. T., Il'yasova, D., Spasojevic, I., Ali, S., Stewart, C. P., Fernald, L. C., Kim, L., Yan, L., Meyer, A., Karim, Md. R., Shahriar, S., Shuman, G., Arnold, B. F., Hubbard, A. E., Famida, S. L., Akther, S., … Granger, D. A. (2024). A cluster-randomized trial of water, sanitation, handwashing and nutritional interventions on stress and epigenetic programming. *Nature Communications*, *15*(1). https://doi.org/10.1038/s41467-024-47896-z
Original URL	https://www.nature.com/articles/s41467-024-47896-z
Source type	Journal Article
Keywords	WASH, epigenetic programming, stress physiology, oxidative stress, water, child development
#Tags	#Childdevelopment#DNAm#bangladesh#Epigenetics
Summary of key points + notes (include methodology)	This article delves into how early interventions in environmental factors such as nutrition, sanitation, and hygiene can impact child development and health on a genetic level, specifically through effects on stress physiology and epigenetic programming. By conducting the WASH Benefits trial in Bangladesh, a large integrated trial that examined over 5,500 pregnant women and their children and the influence of clean water, proper nutrition, and proper sanitation on their DNA methylation and stress. Through this, researchers were able to find that the children receiving the interventions showed reduced DNA methylation, and enhanced functioning of the stress-response system. It was also found that children also had reduced oxidative stress, where oxidative stress can contribute to the development of incurable illnesses. - They examined how nutrition, water, sanitation, and handwashing interventions affect oxidative stress, stress physiology, and epigenetic changes - Cluster trial with controls

	- Urinary oxidative stress markers, salivary cortisol, DNA m in the NR3C1 gene - Reduced oxidative stress in intervention group - Enhanced cortisol response - Lower DNAm—improved stress regulation
Research Question/Problem/ Need	How does improved nutrition, water, sanitation, and handwashing interventions impact oxidative stress, stress physiology, and epigenetic programming in children?
Important Figures	 This figure shows how the participants were assigned to the control or intervention group and followed up after one and 2 years. It tracks the the number of children in the stress response.
VOCAB: (w/definition)	Oxidative stress: Imbalances between free radicals and antioxidants which causes cellular damage Epigenetic Programming: environmentally induced changes in gene expression without altering DNA sequence NGFI-A transcription Factor: Protein regulating stress gene expression

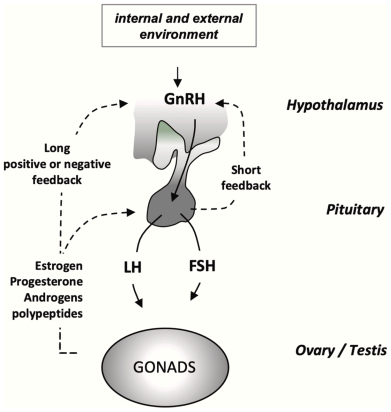
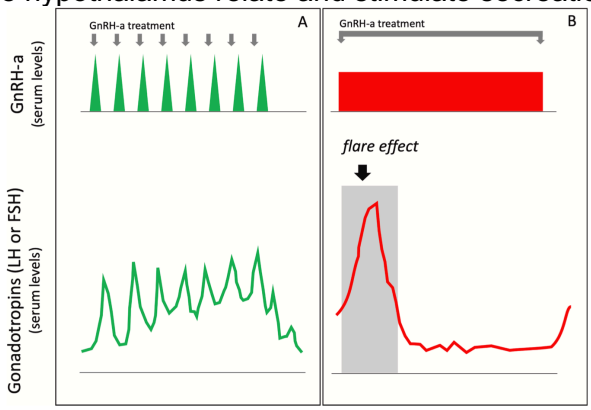
Cited references to follow up on	Ercumen, A., Benjamin-Chung, J., Arnold, B. F., Lin, A., Hubbard, A. E., Stewart, C., Rahman, Z., Parvez, S. M., Unicomb, L., Rahman, M., Haque, R., Colford, J. M., & Luby, S. P. (2019). Effects of water, sanitation, handwashing and nutritional interventions on soil-transmitted helminth infections in young children: A cluster-randomized controlled trial in rural Bangladesh. *PLOS Neglected Tropical Diseases*, *13*(5). https://doi.org/10.1371/journal.pntd.0007323 Slopen, N., McLaughlin, K. A., & Shonkoff, J. P. (2014). Interventions to improve cortisol regulation in children: A systematic review. *Pediatrics*, *133*(2), 312–326. https://doi.org/10.1542/peds.2013-1632
Follow up Questions	How do these interventions influence long term cognition Which intervention components have the greatest impact? Can similar methods be applied?

Article #5 Notes: Physiological and pharmacological overview of the gonadotropin releasing hormone

Source Title	Physiological and pharmacological overview of the gonadotropin releasing hormone
Source citation (APA Format)	Casati, L., Ciceri, S., Maggi, R., & Bottai, D. (2023). Physiological and pharmacological overview of the gonadotropin releasing hormone. *Biochemical Pharmacology*, *212*, 115553. https://doi.org/10.1016/j.bcp.2023.115553
Original URL	https://www.sciencedirect.com/science/article/pii/S0006295223001442#s0115
Source type	Literature Review Article
Keywords	GnRH, Pituitary gonadotropins, GnRH agonists, GnRH antagonists, Non-peptide GnRH antagonists
#Tags	#GnRH #Reproduction #PulsateSecreation #Agonists

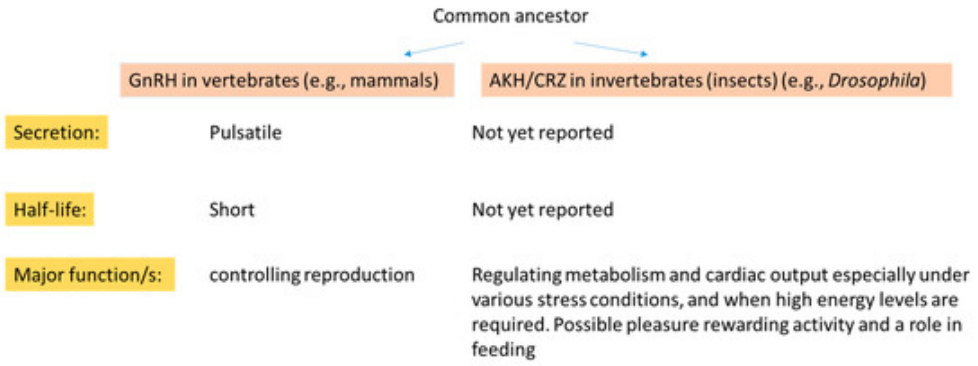
Summary of key points + notes (include methodology)	- GnRH regulates reproduction through the HPG axis and it stimulated the release of hormones like LH and FSH which control gonadal functions
	- GnrH releasing is pusatle and relucted by stim and inhibit signals
	- GnRH and receptors exist in reproductive and non-reproductive tissues which influences cell growth, the release of hormones, neurological genesis
	- Synthetic agonists and antagonists with longer held lives are used in fertility treatments
	  ○ Examines experimental evidence from animal models and human studies
	  ▪ Genetic
	  ▪ Molecular
	  ▪ Physiological
	  ○ Data sources cone from gene analysis, receptor cloning, peptide sequencing
	  ○ Clinical trials from infertility, reproduction disorders, and hormone related cancers

Research Question/Problem/ Need	How does GnRH regulate reproductive and non-reproductive functions, how are they applied in pharmaceutically?

Important Figures	 This figure shows the HPG axis, specifically where and how the GNRH neurons in the hypothalamus relate and stimulate secreation of other hormones.  This figure shows how different GnRH patterns affect gonadotropic levels where pulsatile cause LH and FSH release. GNRH superagonists produce a surge before long term suppression
VOCAB: (w/definition)	Pulsatile Secretion-Periodic release of GnRH, necessary for normal gonadotropin regulation. Superagonist- A synthetic GnRH analogue that produces a stronger receptor response than natural GnRH. HPG axis- The hypothalamo-pituitary–gonadal axis controlling reproductive hormone regulation GnRH Analogues- Synthetic peptides or non-peptides designed to mimic or block GnRH action for clinical use.
Cited references to follow up on	Fana, N. C., Eui-Bae, J., Chun, P., Olofssonb, J. I., Krisingera, J., & Leunga, P. C. K. (1994). The human gonadotropin-releasing hormone (gnrh) receptor gene:

	Cloning, genomic organization and chromosomal assignment. *Molecular and Cellular Endocrinology*, *103*(1–2). https://doi.org/10.1016/0303-7207(94)90087-6 Knobil, E. (1988). The hypothalamic gonadotrophic hormone releasing hormone (gnrh) pulse generator in The rhesus monkey and its neuroendocrine control. *Human Reproduction*, *3*(1), 29–31. https://doi.org/10.1093/oxfordjournals.humrep.a136647
Follow up Questions	1. How do different GNRH isoforms specifically affect reproductive and nonreproductive areas 2. What mutations stop or interrupt GnRH and how could they be treated

Article #6 Notes: GnRH-Related Neurohormone in the Fruit Fly Drosophila melanogaster

Source Title	GnRH-Related Neurohormone in the Fruit Fly Drosophila melanogaster
Source citation (APA Format)	Ben-Menahem, D. (2021). GnRH-related neurohormones in the fruit fly drosophila melanogaster. *International Journal of Molecular Sciences*, *22*(9), 5035. https://doi.org/10.3390/ijms22095035
Original URL	https://www.mdpi.com/1422-0067/22/9/5035
Source type	Peer Reviewed Scientific review article
Keywords	GnRH, corazonin, adipokineetic hormone, Drosophila,
#Tags	#GnRH #Corazonin #Drosophila #Neauropeptides #Evolution
Summary of key points + notes (include methodology)	The article reveals the similarities and differences between drosophila corazonin and vertebrate GnRH, which have similar structure, however very different signaling pathways and physiological roles. While GnRH primarily regulates reproduction through eh hypothalamic pituitary Gondal axis, CRZ functions are prevalent throughout metabolism, stress responses, and feeding behavior with minor roles in reproduction. Although they are predicted to share an ancestor, there is an evolutionary divergence between them. - Genomic analysis of peptides and receptors across the species - Behavioral assays under stress - Electrophysiological recordings of organ activity - Molecular analysis of structure and signaling pathways
Research Question/Problem/ Need	What are the roles of corazonin in Drosophila and how do they differ from vertebrate GnRH systems?
Important Figures	 This figure summarizes the main roles of GnRH, AKH, and CRZ in mammals and insects where GnRH controls reproduction and AKH and

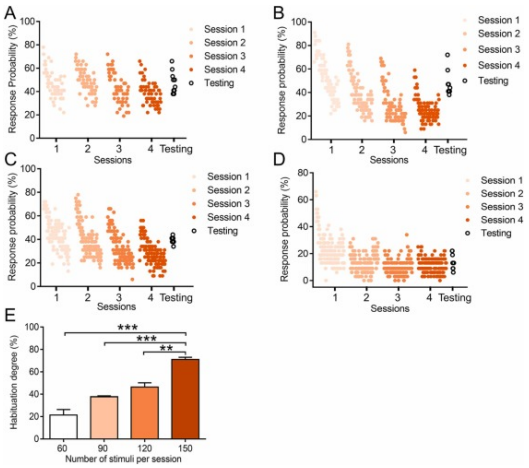
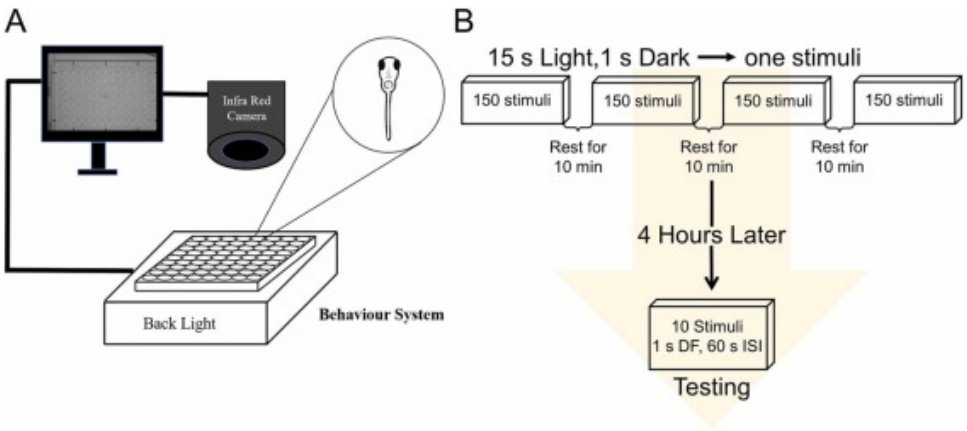
	CRZ regulate systems like metabolism Sequence comparison of the mature peptides: • Mammalian GnRH: pGlu -His -Trp -Ser -Tyr -Gly -Leu -Arg -Pro -Gly (1 2 3 4 5 6 7 8 9 10) • *Drosophila* AKH: pGlu -Leu -Thr -Phe -Ser -Pro -Asp -Trp (1 2 3 4 5 6 7 8) • *Drosophila* CRZ: pGlu -Thr -Phe -Gln -Tyr -Ser -Arg -Gly -Trp -Thr -Asn (1 2 3 4 5 6 7 8 9 10 11) This figure just compares the amino acid sequences of GnRH, AKH, and CRZ, where the similaries and differences are emulated, so while all of them belong to the same neurohormone, some small changes affect how the peptide functions
VOCAB: (w/definition)	Neuropeptide- Small protein like molecule used by neurons to communicate with each other (signaling molecule to regulate processes) Orthologs- Genes in different species that evolved from a common ancestral gene and retain the same function Neurohormone- a hormone produced and released by neurons into circulation
Cited references to follow up on	Knobil, E. (1988). The hypothalamic gonadotrophic hormone releasing hormone (gnrh) pulse generator in The rhesus monkey and its neuroendocrine control. *Human Reproduction*, *3*(1), 29–31. https://doi.org/10.1093/oxfordjournals.humrep.a136647
Follow up Questions	Could CRZ signaling in Drosophila share any similarities with GnRH beyond receptor structure? What experimental evidence would further research in the functional divergence hypothesis Are there any other invertebrates that have GnRH equivalents that regulate reproduction more directly

Article #7 Notes: Development and optimization of an effective method for evaluating habituation learning behavior in larval zebrafish

Source Title	Development and optimization of an effective method for evaluating habituation learning behavior in larval zebrafish
Source citation (APA Format)	Xu, H., Tang, X., Chen, J., Shi, Y., Liu, J., Han, C., Zhu, X., Zhang, T., Zhou, J., & Miao, W. (2023). Development and optimization of an effective method for evaluating habituation learning behavior in larval zebrafish. *Journal of Neuroscience Methods*, *386*, 109793. https://doi.org/10.1016/j.jneumeth.2023.109793
Original URL	https://www.sciencedirect.com/science/article/pii/S0165027023000122?via%3Dihub
Source type	Peer Reviewed Article
Keywords	Zebrafish Larvae, Habituation Learning, Behavioral Neuroscience. Protein Synthesis, Viewpoint system
#Tags	#LearningMemory #Zebrafish #Habituation #Behavioral Assay #Neuroscience

Summary of key points + notes (include methodology)	Summary
	- Habituation is a forma of learned where repeated stimuli cause decreased responses
	- There is not a standardized protocol for habituation testing in zebrafish, so they developed one through a standardized habituation assay using the Viewpoint system
	  ○ Uses controlled tanks, lighting systems, cameras, sensors, and behavioral analysis to detect the velocity function, sensitivity, and stimuli
	- Optimal Conditions:
	  ○ Velocity=13
	  ○ Luminous intensity=8%
	  ○ Sensitivity=32
	  ○ Stimuli number=150
	  ○ Age= 6 days post fertilization
	Methods
	- Used wild zebrafish larvae
	- Viewpoint system
	- Stimuli: 1 second dark flashes with 15 second break
	- Four training session with flashes that repeat
	- After 4 hours, they test with 60 second breaks to assess habituation

Research Question/Problem/	How can we develop a effective, reproducible and standard method for evaluating habituation learning in larval zebrafish?

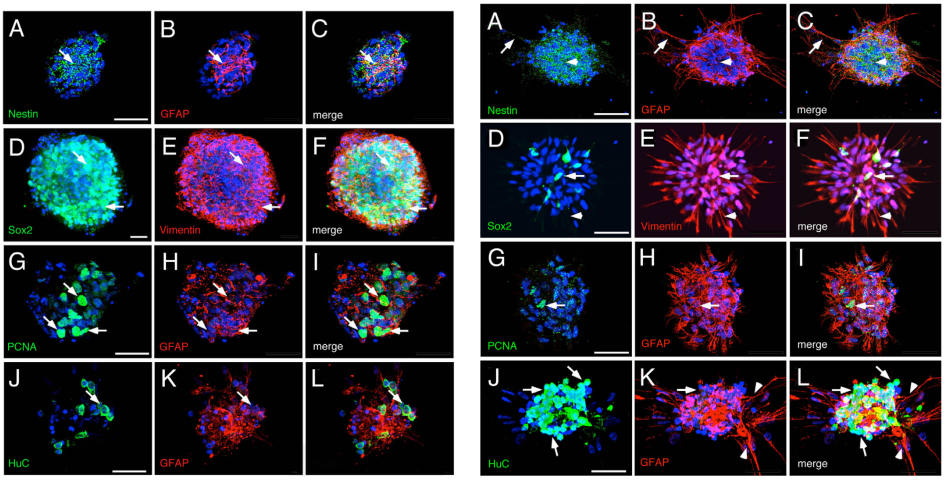
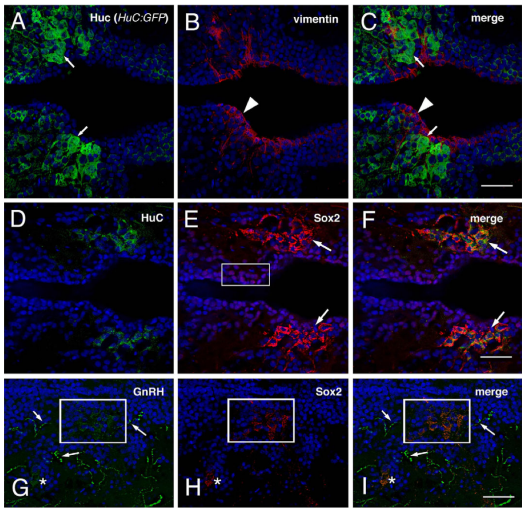
Need	
Important Figures	 Shows how the carrying the number of stimuli influences habituation degree, which is an essential part to our conclusion and findings  Give context on the viewpoint system, and how they decided t utilize it
VOCAB: (w/definition)	Habituation: a form of non-associative learning in which an organism decreases or stops its response to a repetitive, harmless stimulus Detection threshold of velocity: the minimum speed that a person or system can reliably perceive or measure Luminous intensity: measures the amount of visible light a source emits in a specific direction, and is expressed in units of candela (cd) Cycloheximide: a toxic antibiotic and protein synthesis inhibitor produced by the bacterium Streptomyces griseus that also has antifungal, plant growth regulator, and pesticide uses.

Cited references to follow up on	Basnet, R. M., Zizioli, D., Taweedet, S., Finazzi, D., & Memo, M. (2019). Zebrafish larvae as a behavioral model in neuropharmacology. *Biomedicines*, *7*(1), 23. https://doi.org/10.3390/biomedicines7010023 FUNATO, H. (2020). Forward genetic approach for behavioral neuroscience using animal models. *Proceedings of the Japan Academy, Series B*, *96*(1), 10–31. https://doi.org/10.2183/pjab.96.002
Follow up Questions	1. How does this work with different zebrafish strains 2. Can we use this for other factors of learning 3. How does the development of the brain between 4-7 days post fertilization influence habituation

Article #8 Notes: Zebrafish adult-derived hypothalamic neurospheres generate gonadotropin-releasing hormone neurons

Source Title	Zebrafish adult-derived hypothalamic neurospheres generate gonadotropin-releasing hormone neurons
Source citation (APA Format)	Cortés-Campos, C., Letelier, J., Ceriani, R., & Whitlock, K. E. (2015b). Zebrafish adult-derived hypothalamic neurospheres generate gonadotropin-releasing hormone (GnRH) neurons. *Biology Open*, *4*(9), 1077–1086. https://doi.org/10.1242/bio.010447
Original URL	https://journals.biologists.com/bio/article/4/9/1077/1544/Zebrafish-adult-derived-hypothalamic-neurospheres
Source type	Journal Article
Keywords	GnRH, Hypothalamus, Neurogenesis, Zebrafish, Hormone Treatment, Immunocytochemistry, Reproduction
#Tags	#Neurogenesis, #GnRH, #HypothalamicFunction, #CellDifferentiantion

Summary of key points + notes (include methodology)	- Adult zebrafish hypothalamus contains progenitor cells that can generate new GnRH neurons
	- These cells can differentiate into neurons, increasingly under hormone treatments
	- Both GNRH and testosterone stimulate neuron differentiation in the hypothalamus
	- GnRH progenitors, glial cells, and new neurons in the POA represent active neurogenesis
	- Neurogenesis in the hypothalamus that differentiated into GnRH was 'proven' which could provide as a biological explanation of the hormone cycles in HH
	  - Dissected hypothalamic tissue from adult male zebrafish
	  - Dissociated cells and cultured them to form neurospheres
	  - Grown in proliferation medium with growth factors
	  - Transferred to differentiation medium with treatments
	  - Used statistics to compare
	  - Used immunocytochemistry to detect markers of stem cells, neurons, glial cells, and GnRH
	  - Selected adult male zebrafish after a mating training protocol
	  - Fixed and sectioned brains
	  - Used antibody labeling for GnRH, Sox2, Huc, and vimentin
	  - Analyzed the parvocellular preoptic area for colocalization of the markers

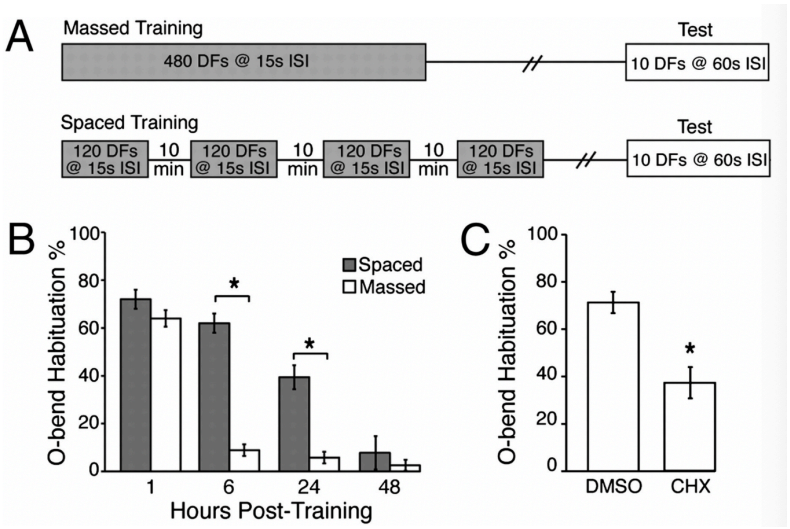
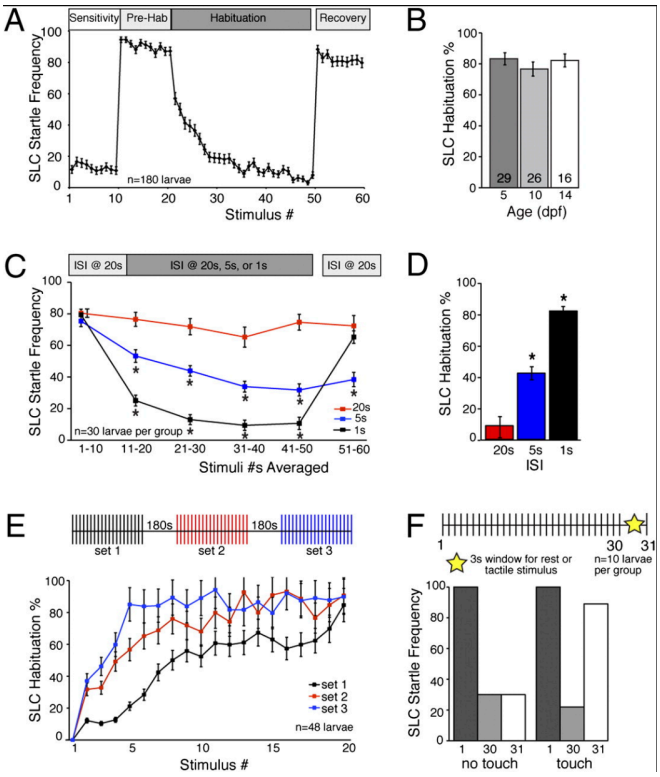
	○ Visualized with fluorescent microscopy and analyzed cell localization
Research Question/Problem/ Need	Can the adult zebrafish hypothalamus generate new GnRH neurons?
Important Figures	 These figures represent the quantity and prevalence of glial cells, progenitor cells, cell division, and newly formed neurons from a time of 0 to the 7 days into differentiation.  This figure visualizes the correlations between Sox2, Huc, and GnRH as differentiates. Vimentin and Huc don't overlap, the conclusion that they are separate can be drawn and that they do not have the same function.
VOCAB: (w/definition)	Gonadotropin-Releasing Hormone(GnRH)- a small peptide hormone produced in the hypothalamus that plays a key role in regulating reproduction Neurogenesis- the process by which new neurons are formed in the brain Hypothalamus- a small, vital region located at the base of the brain

	Immunocytochemistry- a technique used to visualize specific proteins or molecules inside cells Glial cells - a diverse group of cells in the nervous system that provide support, structure, and protection to neurons
Cited references to follow up on	Abraham, E., Palevitch, O., Gothilf, Y., & Zohar, Y. (2009). The zebrafish as a model system for forebrain gnrh neuronal development. *General and Comparative Endocrinology*, *164*(2–3), 151–160. https://doi.org/10.1016/j.ygcen.2009.01.012 Cheng, M.-F. (2013). Hypothalamic neurogenesis in the adult brain. *Frontiers in Neuroendocrinology*, *34*(3), 167–178. https://doi.org/10.1016/j.yfrne.2013.05.001
Follow up Questions	1. Would exposure to other hormone treatments like LH, FSH, or estrogen express the same effects of increased cell differentiation? 2. Does the prolonged exposure to GnRH or testosterone treatment alter this progenitor? 3. What are the applications of this model beyond HH and other reproductive disorders?

Article #9 Notes: Chemical Modulation of memory formation in larval zebrafish

Source Title	Chemical Modulation of memory formation in larval zebrafish
Source citation (APA Format)	Wolman, M. A., Jain, R. A., Liss, L., & Granato, M. (2011a). Chemical modulation of memory formation in larval zebrafish. *Proceedings of the National Academy of Sciences*, *108*(37), 15468–15473. https://doi.org/10.1073/pnas.1107156108
Original URL	https://pubmed.ncbi.nlm.nih.gov/21876167/
Source type	Journal Article
Keywords	Habituation, Nonassociative learning, Zebrafish, Behavioral Assays, Long term memory, Short term memory
#Tags	#BehavioralAssay, #CognitiveScreening #Zebrafish

Summary of key points + notes (include methodology)	- Zebrafish showed with longer term and short-term habituation which demonstrates memory formation and retention
	- Short term is frequency dependent and can be reversed by introducing tactile stimulus
	- Long term habituation requires protein synthesis to form
	- NMDA receptor antagonists negatively effected habituation, so zebrafish share learning pathways with mammals
	- The automated behavioral assay was reliable, scalable for testing potential cognitive drugs
	  ○ Used automated behavioral systems to record multiple larvae
	  ○ High speed cameras to be able to see and breakdown the rapid movement responses to stimulus
	  ○ Visual Learning assay they use was sudden light exposure with triggered o-bend responses
	  ○ Acoustic learning assay was sound pulses to induce a reflex
	  ▪ Repeated exposure allowed habituation measurements and decreased with repetition
	  ○ Short term Habituation was tested over minutes and were reversible
	  ○ Long term habituation was tested over hours and shower persistence up to 24 hours
	  ○ Exposed zebrafish to 1760 bioactive compounds with known molecular targets
	  ○ Statistical software identifies compounds that significantly changes habitation

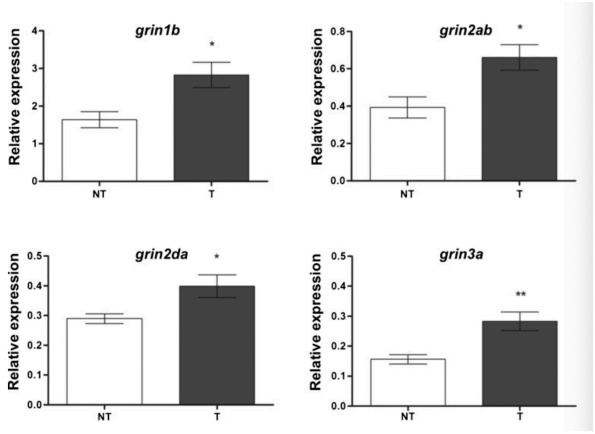
Research Question/Problem/ Need	How can a behavioral system using larval zebrafish be used to measure habituation learning?
Important Figures	 This figure just illustrates the setup in which the researchers created and used to study the habituation in the zebrafish. It shows that multiple larvae are placed individually, recorded, and then analyzed for the o-bend and c start responses.  This figure shows how evidence the zebrafish larvae get used to repeated sounds which their responses lessen with time. Despite this, their response returns after resting. Shorter breaks between the sounds help them get used to it, and a stimulus can break that.

VOCAB: (w/definition)	O-Bend Response- A characteristic bending movement of zebrafish larvae in response to sudden light-off stimuli. C-start Response- A rapid, C-shaped startle reflex triggered by acoustic or tactile stimuli. High Throughput Screening- A automated testing of many samples at once to identify active compounds or genetic effects quickly. Nonassociative learning – learnign that involves a change in response to a single type of stimulus, without pairing it with another stimulus or reward.
Cited references to follow up on	Burgess, H. A., & Granato, M. (2008). The neurogenetic frontier--lessons from misbehaving zebrafish. *Briefings in Functional Genomics and Proteomics*, *7*(6), 474–482. https://doi.org/10.1093/bfgp/eln039 Muto, A., Orger, M. B., Wehman, A. M., Smear, M. C., Kay, J. N., Page-McCaw, P. S., Gahtan, E., Xiao, T., Nevin, L. M., Gosse, N. J., Staub, W., Finger-Baier, K., & Baier, H. (2005). Forward genetic analysis of visual behavior in zebrafish. *PLoS Genetics*, *1*(5). https://doi.org/10.1371/journal.pgen.0010066
Follow up Questions	1. Could the same habituation-based assay be adapted to study associative learng in zebrafish 2. How does the environmental controls influence the habituation learning

Article #10 Notes: Growth hormone (GH) increases cognition and expression of ionotropic glutamate receptors (AMPA and NMDA) in transgenic zebrafish (*Danio rerio*)

Source Title	Growth hormone (GH) increases cognition and expression of ionotropic glutamate receptors (AMPA and NMDA) in transgenic zebrafish (*Danio rerio*)
Source citation (APA Format)	Studzinski, A. L., Barros, D. M., & Marins, L. F. (2015). Growth hormone (GH) increases cognition and expression of ionotropic glutamate receptors (AMPA and NMDA) in transgenic zebrafish (danio rerio). *Behavioural Brain Research*, *294*, 36–42. https://doi.org/10.1016/j.bbr.2015.07.054
Original URL	https://www.sciencedirect.com/science/article/pii/S016643281530125X
Source type	Journal Article
Keywords	Long Term Memory, AMPA receptors, NMDA receptors, Zebrafish, Insulin-like growth factor I
#Tags	#GH, #Cognition #Neurobiolofu #Memory

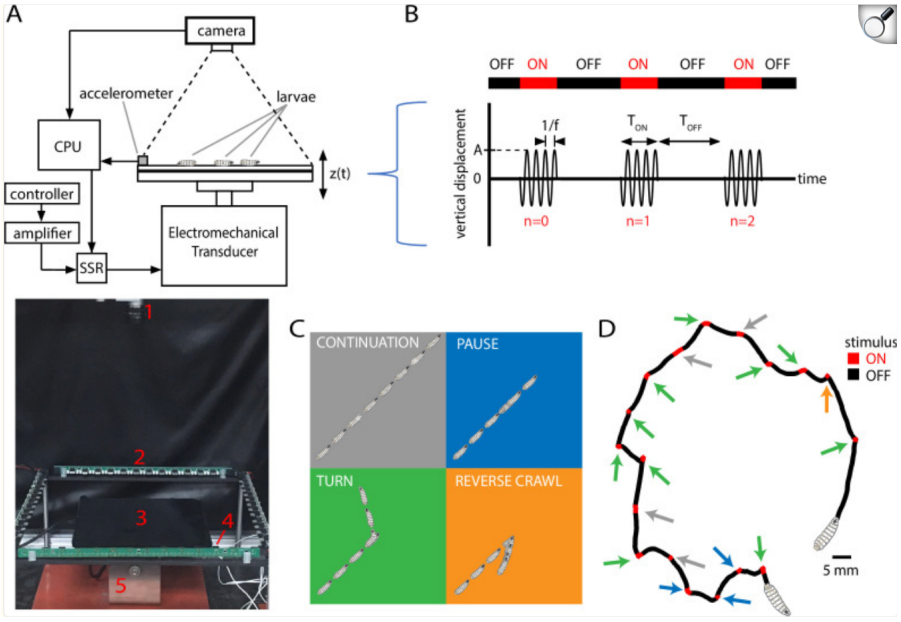
Summary of key points + notes (include methodology)	- GH overexpression improves long term memory which was found through the observation that transgenic zebrafish with extra growth hormone remembered stimulus better than a normal fish because it hesitated
	- AMPA and NMDA units were more active in transgenic fish which means that the GH overexpression improves learning related processes by increasing the receptors that function for memory
	- Transgenic fish had higher levels of insulin growth in the brain because GH increases those which supports neurogenesis
	- Connected these to where GH stimulates insulin growth factors, those effect receptor activities, better synaptic, and better memory
	  - Zebra fish were engineered to overexpress GH
	  - 2 genes were injected into embryos with one of them being GH and the other for a marker
	  - Breeded them to produce the control fish
	  - Ethical approval was obtained and kept under controlled environments
	  - Used tube to create mild shock from the difference between dark and light

	▪ Entering the dark zone, they had 2 electrical shocks ▪ Tested 48 hours later without shock ○ Brains were frozen then RNA was extracted and compared transgenic and non-transgenic levels
Research Question/Problem/ Need	Does the overexpression of growth hormone influence the long-term memory is Zebrafish?
Important Figures	 This figure shows that the transgenic zebrafish have a higher expression of AMPA receptor genes when compared to non-transgenic fish. So, it essentially increases gene activity.
VOCAB: (w/definition)	AMPA Receptors- ionotropic glutamate receptors involved in fast excitatory synaptic transmission; important for learning and memory NMDA Receptors- Ionotropic glutamate receptors important for synaptic plasticity, long-term potentiation (LTP), and memory formation. Transgenic organism- An organism genetically modified to contain and express genes from another species. Synaptic Plasticity- he ability of synapses to strengthen or weaken over time, critical for learning and memory.
Cited references to follow up on	Sonntag, W. E., Ramsey, M., & Carter, C. S. (2005). Growth hormone and insulin-like growth factor-1 (IGF-1) and their influence on cognitive aging. *Ageing Research Reviews*, *4*(2), 195–212. https://doi.org/10.1016/j.arr.2005.02.001 Schneider-Rivas, S., Rivas-Arancibia, S., Vazquez-Pereyra, F., Vázquez-Sandoval, R., & Borgonio-Pérez, G. (1995). Modulation of long-term memory and extinction responses induced by growth hormone (GH) and growth hormone releasing hormone (GHRH) in rats. *Life Sciences*, *56*(22). https://doi.org/10.1016/0024-3205(95)00171-2

Follow up Questions	1. Would GH over expression also improve short term memory in zebrafish? 2. How would the results differ if the shock intensities were higher?

Article #11 Notes: Mechanical Vibration Patterns Elicit Behavioral Transitions and Habituation in crawling Drosophila Larvae

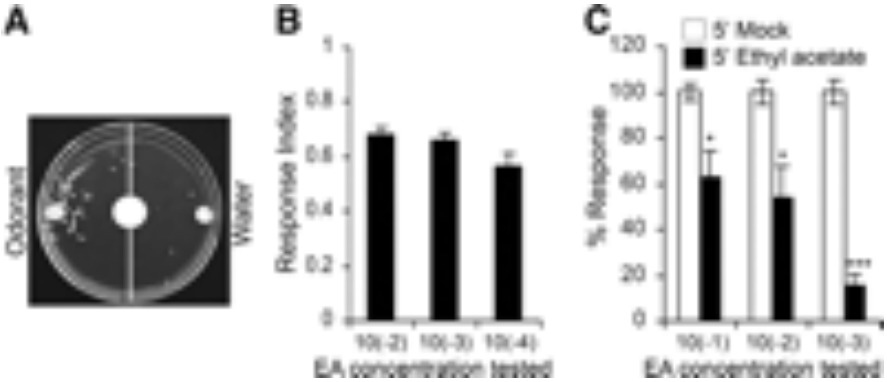
Source Title	Mechanical Vibration Patterns elicit Behavioral Transitions and Habituation in Crawling Drosophila Larvae
Source citation (APA Format)	Berne, A., Zhang, T., Shomar, J., Ferrer, A. J., Valdes, A., Ohyama, T., & Klein, M. (2023). Mechanical vibration patterns elicit behavioral transitions and habituation in crawling drosophila larvae. *eLife*, *12*. https://doi.org/10.7554/elife.69205
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC10586805/
Source type	Journal Article
Keywords	Drosophila, Short-Term Memory, Cognition, Habituation, Stimuli,
#Tags	
Summary of key points + notes (include methodology)	Summary - The larvae respond o vibration with small sets of behaviors which are ranked by avoidance strength - Habituation is robust where avoidance behaviors decrease quickly and return to normal behavior within seconds - Habituation is mostly one directional where their reversal rate almost never returns to the strong stimuli response - Mechanosensory habituation shared principles with olfactory and associative learning Methodology Animal Controls/General Info - Drosophila larvae crawling on agar gel - 20 larvae per experiment - 300-600s per experiment Vibrations - Used an electromechanical transducer (EMT) to induce the vertical sinusoidal vibrations - Crawled on metal platmore for uniform vibration - EMT shakes up and down - CCD camera - Z(t)=Asin(2(pi)ft) o F is stimulus frequency and how fast it vibreates o Displacement up and down o 4 measurements of vibration

	▪ TON: how long vibration is ▪ TOFF: Time between vibrations ▪ T=TON+TOFF ○ Formula for how strong vibration acceleration is Larvae Behavior ○ 4 responses ○ Continuation in crawling forward- no avoidance ○ Pause and keep going- weak avoidance ○ Turn and change direction- weak avoidance ○ Reverse and crawl backwards- strong avoidance
Research Question/Problem/ Need	The need for this project stems from the few studies on the different stimuli you can expose Drosophila to habituation.
Important Figures	 Figure 1 shows the built vibration assay used to deliver the precise stimulations to the Drosophila larvae. It shows a platform connected to an electromechanical transducer which creates vertical sinusoidal vibrations with a camera above to record larval movement. B shows how the vibration Is applied. C shoes the direction in which they defined as continuation, pause, turn. And reverse crawling.
VOCAB: (w/definition)	Linear Time Invariant- A modeling framework assuming responses are linear and do not change over time; shown here to be insufficient. Circuit analogy- A conceptual model using electrical components to represent biological processes underlying behavior
Cited references to follow up on	Brea, J., Urbanczik, R., & Senn, W. (2014). A normative theory of forgetting: lessons from the fruit fly. *PLoS computational biology*, *10*(6), e1003640. https://doi.org/10.1371/journal.pcbi.1003640
Follow up Questions	1. Does habituation reflect changes in sensory neurons or motor neuron

	excitability? 2. How would habituation change if vibration was combined with another sensory modality? 3. Why does habituation appear to be largely irreversible?

Article #12 Notes: Central Synaptic Mechanisms Underlie Short Term olfactory Habituation in Drosophila Larvae

Source Title	Central Synaptic Mechanisms underlie Short term olfactory Habituation in Drosophila Larvae
Source citation (APA Format)	Larkin, A., Karak, S., Priya, R., Das, A., Ayyub, C., Ito, K., Rodrigues, V., & Ramaswami, M. (2010b). Central synaptic mechanisms underlie short-term olfactory habituation in *drosophila* larvae. *Learning & Memory*, *17*(12), 645–653. https://doi.org/10.1101/lm.1839010
Original URL	https://pubmed.ncbi.nlm.nih.gov/21106688/
Source type	Journal Article
Keywords	Drosophila, Short-Term Memory, Drosophila Larvae, Habituation, Stimuli, Synapse, Olfactory, Odor
#Tags	#Shorttermmemory #Shorttermhabituation #DrosophilaLarvae #Olfactory
Summary of key points + notes (include methodology)	Key Points ○ Drosophila move towards certain smells (chemotaxis), like ethyl acetate. After short exposure to EA, which was 5 mins, attraction drops and reestablishes in 40 minutes. This is short-term habituation ○ Response is specific to odor and is reversible and can be erased by a strong stimulus ○ Short term habituation is not just induced by odor, meaning that that the circuit of the drosophila is being altered not receptors ○ Local inhibitor interneurons are needed for habituation ○ Projection Neurons need to be able to receive GABA or else no habituation ○ NMDA receptors on PNs are needed because the allow calcium influx which triggers synaptic plasticity ○ Fly larvae nmda receptor activity is parallel to that of mammals and can we apply to disorders like schizophrenia and autism Notes ○ Short term habituation is decreased behavioral response to EA lasting for hour with good recovery ○ Other tests with direct depolarization, blue light and heat, induces similar behavior Methodology ○ Had 4 types of fly, 3 gene reductions with gal 4 ○ 9cm petri dish, 10 ml 2% agar, 2 small caps on opposite sides -one with odor -one with water

	- Filter paper disks with diluted odor, decreasing powers of 10 - Larvae were 3rd instar 20-55 per dish - 5 min to do, them count on odor vs control - RI= #odor-#control/#total where 1 is full odor attraction and -1 I repulsion - Dis habituation was measure with 1 min cold shock
Research Question/Problem/ Need	The need for this project stems from the lack of knowledge there was on how habituation actually altered the organism--previous studies did not separate peripheral and central mechanisms.
Important Figures	 Figure 1 illustrates the components of the testing assay, and the results of the statistical test the performed on raw data. 1A shows the attraction to the odorant vs. water, and the testing set up where the larvae were put on a petri dish. 1B illustrates the mean avoidance indexes when exposed to difference concentrations of the odorant where they showed slight decreases. 1Cillusgtrated the control variable vs the odorant treated group
VOCAB: (w/definition)	Chemotaxis: directed movement of flies toward or away from odor Olfactory Sensory Neurons: neurons in the antennae that detect odors and send signals to antennal lobe GABA/GABA-Receptor: inhibitory signaling that reduces neural activity and helps drive olfactory habituation Rutabaga: gene encoding adenyl cyclase required for learning and long term habitation Adenylyl Cyclase: an enzyme that converts ATP into cAMP during neural signaling Cyclic AMP: molecule that controls synaptic plasticity and memory formation
Cited references to follow up on	Bahk, S., & Jones, W. D. (2016). Insect odorant receptor trafficking requires calmodulin. *BMC Biology*, *14*(1). https://doi.org/10.1186/s12915-016-0306-x Eddison, M., Belay, A. T., Sokolowski, M. B., & Heberlein, U. (2012a). A genetic screen for olfactory habituation mutations in drosophila: Analysis of novel foraging alleles and an underlying neural circuit. *PLoS ONE*, *7*(12). https://doi.org/10.1371/journal.pone.0051684

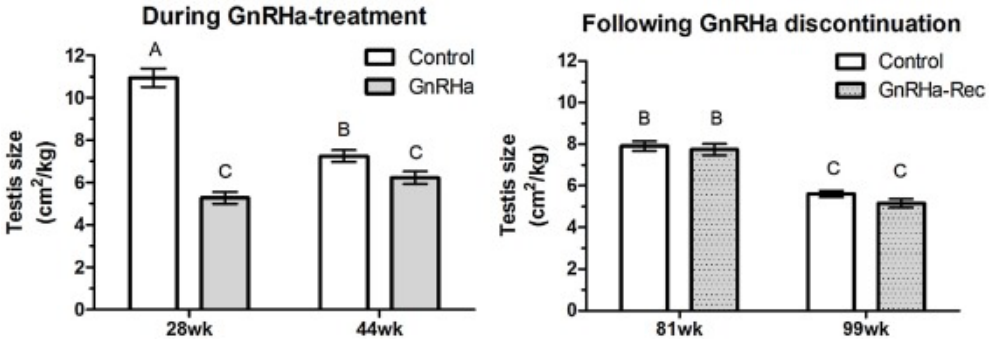
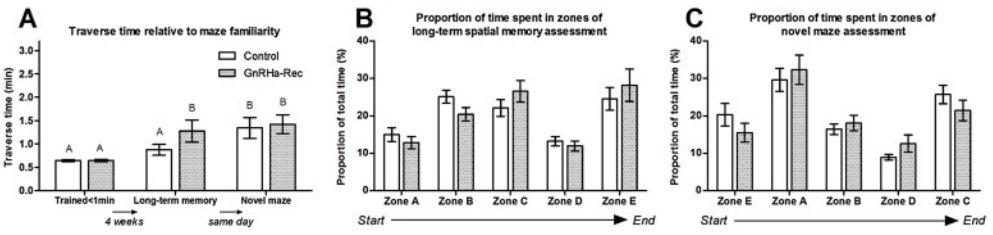
	Sudhakaran, I. P., Holohan, E. E., Osman, S., Rodrigues, V., VijayRaghavan, K., & Ramaswami, M. (2012). Plasticity of recurrent inhibition in the *drosophila* antennal lobe. *The Journal of Neuroscience*, *32*(21), 7225–7231. https://doi.org/10.1523/jneurosci.1099-12.2012
Follow up Questions	1. Why is ethyl Acetate used as an odorant? 2. What was used to dilute the odorant and how did they decide the odor concentration? 3. Are/How are the memory processes different from larvae to adults?

Article #13 Notes: A Reduction in Long-Term spatial Memory Persists After Discontinuation of Peripubertal GnRH agonist Treatment in Sheep

Source Title	A reduction in long term spatial memory persists after discontinuation of peripubertal gnrh agonist treatment in sheep
Source citation (APA Format)	Hough, D., Bellingham, M., Haraldsen, I. R. H., McLaughlin, M., Rennie, M., Robinson, J. E., Solbakk, A. K., & Evans, N. P. (2017a). Spatial memory is impaired by peripubertal GnRH agonist treatment and testosterone replacement in sheep. *Psychoneuroendocrinology*, *75*, 173–182. https://doi.org/10.1016/j.psyneuen.2016.11.029
Original URL	https://pubmed.ncbi.nlm.nih.gov/27987429/
Source type	Journal Article
Keywords	Gonadotropin-releasing Hormone, Gnrh agonist, Puberty, Peripubertal Development, Spatial memory, Hippocampus, Cognition, Ovine
#Tags	Gnrha, Puberty Blockers, Cognition, Hippocampus, Spatial Memory, Development, Memmory, behavioral testing

Summary of key points + notes (include methodology)	Key Points
	- Gnrh-a suppressed the reproductive axis during treatment and testes sizes recovered after discontinuation
	- Spatial orientation within the control group and the GnRH-a groups were not significantly different after withdrawal from treatment
	  o Same-day learning and orientation
	  o Short term plasticity not permanent
	  o 1 trial
	  o baseline
	- Spatial Learning lest
	  o 3 trials
	  o Improvement was in the same day
	  o Non treaded
	  o Recovery rams showed differing pattern movements as they went through certain maze zones
	  ▪ With higher testosterone in breeding periods, they went faster through zones
	  ▪ During non-breeding season, they were slower than controls
	- Ability to learn maze was the same and the speed was same as control group

	- Long term spatial referent memory stayed impaired as the recovery rams were about 1.5x slower than the controls group which were tested 4 weeks after training in same maze
	  o Same from when they were still on GnRH-a
	  o Permanent effect on spatial reference memory
	- Problem solving had no difference
	Methods
	- Control had no treatment
	- Gnrh-a recovery group receive goserelin acetate every 4 week to delay puberty
	- Tested testes size every 4 weeks by scrotal length and circumference
	- Spatial behavior testing at 41 week, 83, and 95
	- Orientation testing at 41, 83, 95
	- Long term memory testing at 95 weeks
	  o Rams were ready/educated when they completed mat in les than 1 min 2 times in a row
	  o 4 weeks after training, the ram got 1 try to complete maze
	- Used significance levels

Research Question/Problem/ Need	There is a requirement to test whether studied effect such as physiological changes in the limbic system, reduced spatial reference memory, etc. are reversible when GnRH-a treatment is discontinued.
Important Figures	 This figure showing the difference between the sizes of the control and tested testes in relation to body size and weight of the ram through different time periods. The first graph shows that the GnRH-a treatment indeed had an effect on the rams, suppressing their reproduction and puberty. The second graph shows that after discontinuation, the hormones returned to normal.  This graph showed that that during same day trial tests, the traverse times of the 2

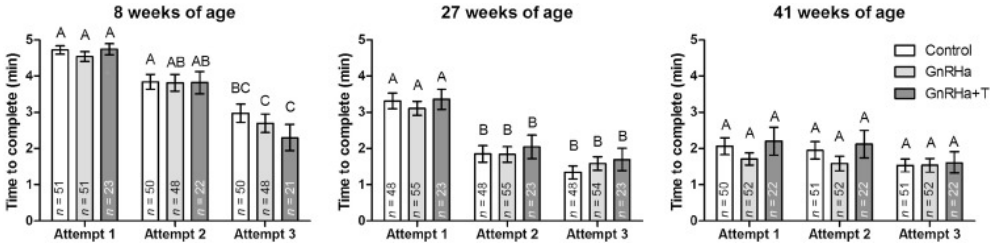
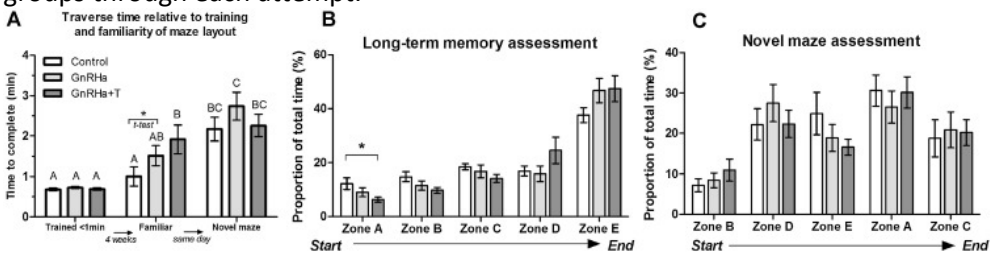
	groups remained significantly indifferent, however when tested for long term memory, there was a significant difference.
VOCAB: (w/definition)	Traverse Time: time it takes for the animal to complete the maze Working Memory: Short term and flexible problem-solving ability Emotional Reactivity Behavioral responses to stress like escape attempts influenced by amygdala
Cited references to follow up on	Carel, J.-C., Eugster, E. A., Rogol, A., Ghizzoni, L., & Palmert, M. R. (2009). Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics*, *123*(4). https://doi.org/10.1542/peds.2008-1783 Nuruddin, S., Bruchhage, M., Ropstad, E., Krogenæs, A., Evans, N. P., Robinson, J. E., Endestad, T., Westlye, L. T., Madison, C., & Haraldsen, I. R. (2013). Effects of peripubertal gonadotropin-releasing hormone agonist on brain development in sheep—a magnetic resonance imaging study. *Psychoneuroendocrinology*, *38*(10), 1994–2002. https://doi.org/10.1016/j.psyneuen.2013.03.009
Follow up Questions	1. Why does peripubertal GnRH-a treatment permanently impar long-term spatial reference memory but not short term learning? 2. Could earlier stops to treatment prevent the long-term effects? 3. Does it matter the dose and duration of treatment?

Article #14 Notes: Spatial memory is impaired by peripubertal GnRH agonist Treatment and Testosterone Replacement in Sheep

Source Title	Spatial memory is impaired by peripubertal GnRH agonist Treatment and testosterone replacement in sheep
Source citation (APA Format)	Hough, D., Bellingham, M., Haraldsen, I. R. H., McLaughlin, M., Rennie, M., Robinson, J. E., Solbakk, A. K., & Evans, N. P. (2017b). Spatial memory is impaired by peripubertal GnRH agonist treatment and testosterone replacement in sheep. *Psychoneuroendocrinology*, *75*, 173–182. https://doi.org/10.1016/j.psyneuen.2016.10.016 /
Original URL	https://www.sciencedirect.com/science/article/pii/S0306453016305595?via%3Dihub
Source type	Journal Article
Keywords	Spatial orientation Spatial memory Hippocampus GnRH Puberty, Gender dysphoria
#Tags	

Summary of key points + notes (include methodology)	Key Points
	- Long term spatial memory was depleted by GnRH-a treatments where rams hate had GnRH signaling blocked took longer to complete the same maze 4 weeks after training/learning
	- The additive of testosterone replacement with GnRH-a treatment still showed weaker spatial memory
	- The spatial orientation and same day learning speed(the time it takes for the sheep to perform the maze) were unchanged by the treatment across the various ages and time periods
	  o Maze assay can be shaped by motivation and anxiety
	  o 4-week retention test tests memory
	- Emotional effects were significant and depended on hormones, which GnRH secretes
	  o Low testosterone animals, the ones with the GnRH-a treatment were more vocal and emotional
	  o Testosterone replacement reduced emotions with fewer escape attempted and vocalizations. It could've reduced motivation to finish the maze due to flocking instinct reliance
	  o Testosterone replacement improves behavior and not memory in this study

	- Spatial memory was reliant on GnRH signaling and Spatial working memory was influenced by testosterone
	Methods
	- They had 3 control groups consisting of untreated rams, GnRH-a treated rams, and GnRH-a treated with injected testosterone
	  o The GnRH-a treatments were implanted every 4 weeks from the time period of 8-44 weeks of age
	  o Testosterone replacement happened every 2 weeks with adjusted doses based on variables
	- They tested spatial orientation with mazes that included traps and measure motivation through ages 8,27,44,41 weeks
	  o TRAVERSE TIMES WERE MEASURED, vocalizations, escape attempts, and progress through different zones
	- The learning was done through 3 attempts per day and 2 hours apart
	- Long term memory was tested by training the rams to get under 1 min of completing the maze, and testing it at 45 weeks (4 weeks later) in the maze layout
	- They also had a test where they rearranged the trap order to measure familiarity
	THEY USED ANOVA TESTING TO measure significant differences.

Research Question/Problem/ Need	The long-term impact of GnRH-a treatment on cognition is not widely studied despite its wide use during puberty and effect on hippocampal functions and
Important Figures	 Figure 2 essential shows the spatial orientation results inform of a bar graph. We can observe that as the rams get older and the number of attempts increases, the response times decrease. There is an inverse relationship. We can also observe that there was not a significant difference between the control groups and treated groups through each attempt.  Figure 5 shows that all of the grouped learned at the same rate during training with the simar times and bar graph, but during the long term memory test 4 weeks later, the GnRH-a treated groups took significantly longer than the control group. This show the there is impaired spatial memory.

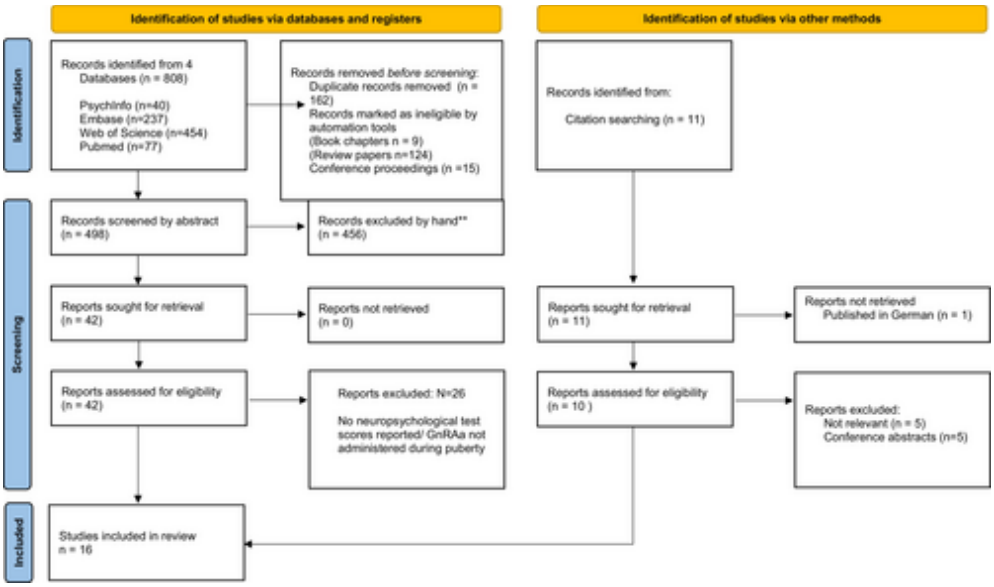
VOCAB: (w/definition)	Traverse time the time it takes for the model organism to complete the maze from start to finish Spatial memory: The ability to remember the layout of an environment and where to go within it Long-term spatial memory memory from stable features off a space Spatial working memory: short term memory used to handle changes or sequences Peripubertal: the development period around puberty
Cited references to follow up on	Berenbaum, S. A., & Beltz, A. M. (2011). Sexual differentiation of human behavior: Effects of prenatal and pubertal organizational hormones. *Frontiers in Neuroendocrinology*, *32*(2), 183–200. https://doi.org/10.1016/j.yfrne.2011.03.001 / Skinner, D. C., Albertson, A. J., Navratil, A., Smith, A., Mignot, M., Talbott, H., & Scanlan-Blake, N. (2009a). Effects of gonadotrophin-releasing hormone outside the hypothalamic-pituitary-reproductive axis. *Journal of Neuroendocrinology*, *21*(4), 282–292. https://doi.org/10.1111/j.1365-2826.2009.01842.x /
Follow up Questions	1. Does peripubertal GnRH-a treatment cause permanent changes, are they reversible after treatment stops? 2. How would female ovine models react under the same treatment? 3. How much of the differences between memory and learning is caused by motivation?

Article #15 Notes: The Impact of Suppressing Puberty on Neuropsychological Function: A review

Source Title	The impact of suppressing puberty on neuropsychological function: a review
Source citation (APA Format)	Baxendale, S. (2024). The impact of suppressing puberty on neuropsychological function: A Review. *Acta Paediatrica*, *113*(6), 1156–1167. https://doi.org/10.1111/apa.17150/
Original URL	https://onlinelibrary.wiley.com/doi/10.1111/apa.17150
Source type	Journal Article
Keywords	Pubertal suppression, neurodevelopment, neuropsychological, animals, humans, central precious puberty, transgender, gender affirming care
#Tags	Brain, Gnrh-a, Hippocampus, Cognition, Animals, Reversibility, Development

Summary of key points + notes (include methodology)	Key Points (No methods because it is a review)
	- Puberty is a time for important neurodevelopment
	  o Executive Functions like impulse control, decision making, etc
	  o Social cognition like emotional responses and intelligence
	
	- Puberty plays a part in systems like Synaptic Pruning, Myelination and Hormone changes in the amygdala, hippocampus which are involved in learning, memory, and emotional processing (brain functions)
	
	- GnRH-a affects brain and behavior in experiments on animals where they are often specific to sex, impact: learning, social behavior, responses, emotional reactions
	
	- The data on humans are very lacking because there are only 5 studies covered and all of them lack a repeated follow-up or cannot fully test the effect of it. There was a study that said that it resulted in IQ declines.
	
	- There is no evidence the cognitive effect are fully reversable because there is not enough data. There are some clinical claims that say the effects of puberty blockers are fully reversible but other scientific articles say that you can adapt but rarely return to the original state.
	
	  o It states that if the effect associated with puberty blockers were 100% reversible, then puberty would have to be different than other developmental stages in life, which is not realistic

Research Question/Problem/	The neuropsychological consequences of puberty suppression through GnRH

Need	agonists are lack extensive research that is needed to fully inform users on its potentially permanent effects.
Important Figures	 Figure 1 essential just showing how the authors condensed and picked out specific article to write their review on. Although there were several hundred studies relating to puberty suppression and the neurological effects of them, they reduced their paper to the ones that specifically tested GnRH-a in humans/animal, used standardized test, and measured cognitive data.
VOCAB: (w/definition)	Synaptic Pruning: developmental process where the brain eliminates weak, unused, redundant synaptic connects so that neural circuits become more efficient - Enhanced during puberty to shape decision making and regulation Neuroplasticity: the brains ability to adapt structurally and functionally in response to experience or hormonal signals Myelination: process where axons are wrapped in myelin to speed up electrical signaling in the brain. - Enhanced during puberty because of involvement in attention, learning, memory, executive function Central Precocious Puberty: early activation of puberty which needs clinical hormone suppression
Cited references to follow up on	Benes, F. M. (1994). Myelination of a key relay zone in the hippocampal formation occurs in the human brain during childhood, adolescence, and adulthood. *Archives of General Psychiatry*, *51*(6), 477. https://doi.org/10.1001/archpsyc.1994.03950060041004/ Ismail, F. Y., Fatemi, A., & Johnston, M. V. (2017). Cerebral plasticity: Windows of opportunity in the developing brain. *European Journal of*

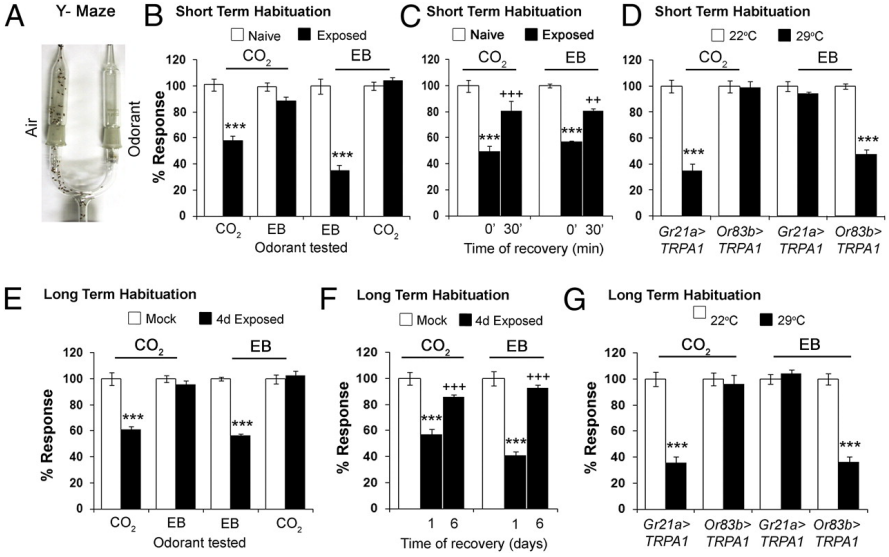
	Paediatric Neurology, *21*(1), 23–48. https://doi.org/10.1016/j.ejpn.2016.07.007/ Skinner, D. C., Albertson, A. J., Navratil, A., Smith, A., Mignot, M., Talbott, H., & Scanlan-Blake, N. (2009b). Effects of gonadotrophin-releasing hormone outside the hypothalamic-pituitary-reproductive axis. *Journal of Neuroendocrinology*, *21*(4), 282–292. https://doi.org/10.1111/j.1365-2826.2009.01842.x/
Follow up Questions	1. What specific neural circuits are sensitive to GnRH suppression 2. Is there any part of cognition that is more susceptible to alteration 3. How does sex alter the effect of GnRH-a treatment

Article #16 Notes: Plasticity of local GABAergic interneurons drives olfactory habituation

Source Title	Plasticity of local GABAergic interneurons drives olfactory habituation
Source citation (APA Format)	Das, S., Sadanandappa, M. K., Dervan, A., Larkin, A., Lee, J. A., Sudhakaran, I. P., Priya, R., Heidari, R., Holohan, E. E., Pimentel, A., Gandhi, A., Ito, K., Sanyal, S., Wang, J. W., Rodrigues, V., & Ramaswami, M. (2011a). Plasticity of local GABAergic interneurons drives olfactory habituation. *Proceedings of the National Academy of Sciences*, *108*(36). https://doi.org/10.1073/pnas.1106411108/
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC3169145/
Source type	Journal Article
Keywords	learning, memory, homeostasis, feedback
#Tags	Olfactory, GABA, inhibition, synaptic plasticity, habituation, learning, Drosophila, Y-maze

Summary of key points + notes (include methodology)	Key Points
	- Short Term Habituation (STH) happens after 30 minutes of odor exposure where their avoidance reduces, and is odor specifics
	- Habituation in drosophila is learning is not just sensory adaptation because the reduction in avoidance happens even when the odor receptors are bypassed and OSNs are depolarized with TrPA1. Habituation and learning is from the central olfactory circuits
	  o Because they mimicked the effects of odor reduction without actually exposing to the odor, it changes brain circuits.
	  o If it was just sensory adaptation then that would mean that the receptors just get tired and the olfactory sensory neurons get temporality less responsive because of exposure.
	- Long Term Habituation happens after 4 days of odor exposure and is reversed after several days
	- The rutabaga is needed for habituation because with the Rut mutant, the flies were not able to habituate
	  o Rut is needed in local interneurons and GABAergic interneurons
	  o It produces cAMP which is important because it is the chemical signal that communicates to the interneuron
	  o Inhibitory neurons inside the antennal lobe
	- Projector Neuron knockdown of GABAergic receptors depletes habituation to odors that activates projector neurons.
	- CREB2b induction prevents LTH but not STH. Thus, LTH depends on CREB communication in GABAergic local neurons. It changes gene transcription

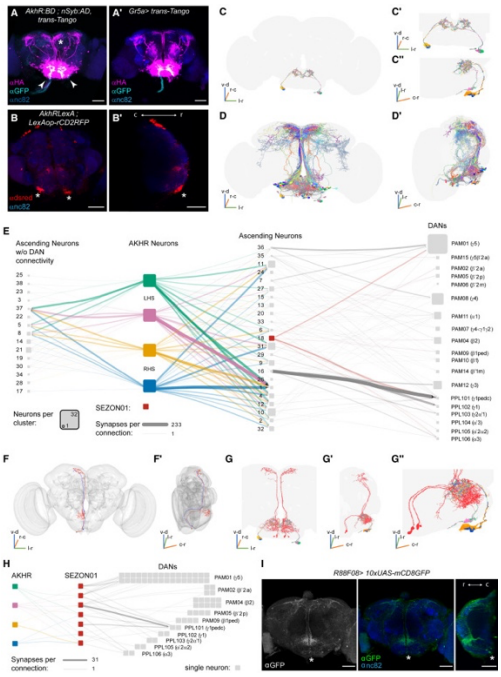
	Methodology
	- They did a Y-maze assay where they tested avoidance between the flies and if they choose between the odorant arm and the clean air air. They then calculated a response index. They did batches bigger than 8
	  o (# in odor- # in clean air)/ total # of flies
	- In STH they starved the flies and measures their baseline avoidance to CO2 and ethyl burate and then they exposed them to odorant for 30 minutes and tested their avoidance.
	- For long term habituation, they exposed new adult plies to continuous 5% CO2 or Eb in paraffin oil for 4 days. Right after the 4 days, they were tested for avoidance and were continuously tested across multiple days
	- Phantom habituation used TrpA1 to express in OSNs
	- Adult manipulations were completed using Tub- Gal 80
	- Statistical analysis
	  o T-test

Research Question/Problem/ Need	How does the antennal love circuit produce olfactory habituation and what molecules are needed for short term and long-term habituation?
Important Figures	 Figure 1 shows that Y-Maze assay they uses to measure the responses of the flies and get them to react. The proceeding graphs of B and E show the reduction of avoidance as the flies are exposed, through the comparison between tested naïve and exposed odorant flies. Graphs C and F show the differences in recovery time. D and G show the habituation responses when the drosophila had manipulated genes.
VOCAB: (w/definition)	Rutabaga- a gene that encodes adenylate cyclase Adenylate Cyclase- an enzyme that produces cAMP which helps neurons undergo synaptic plasticity Cyclic AMP- a signaling molecule that facilitates synaptic changes involved in

	learning TrpA1- heat activated ion channel used to artificially activate neurons in experiments GABA- an inhibitory neurotransmitter that reduces neuronal activity. It is released by LN1 neurons to suppress PN responses GABAA receptor- a receptor on projector neurons that binds GABA and causes inhibition Antennal Lobe- first olfactory processing center in flies. It is similar to the mammalian olfactory bulb
Cited references to follow up on	Devaud, J.-M., Acebes, A., & Ferrús, A. (2001). Odor exposure causes central adaptation and morphological changes in selected olfactory glomeruli in drosophila. *The Journal of Neuroscience*, *21*(16), 6274–6282. https://doi.org/10.1523/jneurosci.21-16-06274.2001
Follow up Questions	1. What differentiates short-term habituation from long-term habituation on mechanistically? 2. Why does long-term habituation need CREB for transcription despite short-term not needing it? 3. Why is odor specificity an important thing to measure in olfactory habituation?

Article #17 Notes: Compensatory Enhancement of Input maintains Aversive Dopaminergic Reinforcement in hungry Drosophila

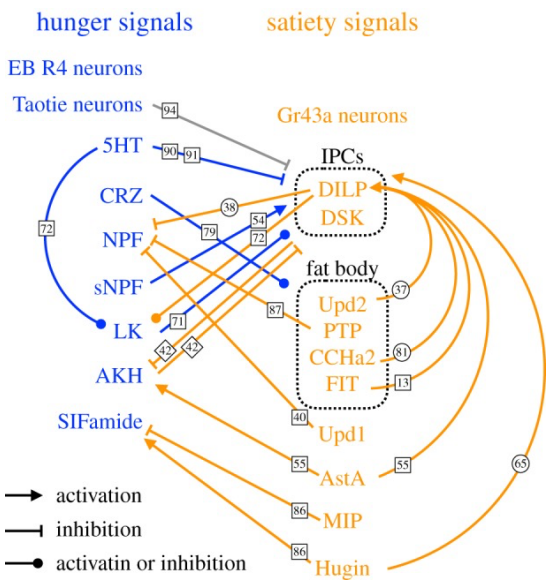
Source Title	Compensatory Enhancement of Input Maintains Aversive Dopaminergic Reinforcement in Hungry Drosophila
Source citation (APA Format)	Meschi, E., Duquenoy, L., Otto, N., Dempsey, G., & Waddell, S. (2024). Compensatory enhancement of input maintains aversive dopaminergic reinforcement in hungry drosophila. *Neuron*, *112*(14). https://doi.org/10.1016/j.neuron.2024.04.035
Original URL	https://pubmed.ncbi.nlm.nih.gov/38795709/
Source type	Journal Article
Keywords	Neural circuits, homeostasis, modulation, hunger, aversive learning, adipokine tic hormone, drosophila, dopaminergic system, reinforcement
#Tags	Dopaminergic, starvation, GABAergic, neural development, habituation, synaptic, neural plasticity
Summary of key points + notes (include methodology)	Key Points - The hungry flies showed better aversive olfactory learning than fed flies. This is not the same with water deprivation - Aversive learning and the increased learning by hunger need the adipokinetic hormone and the receptor which is shown by the mutants with both suppressed AKH and/or AKHR show impaired impaired shock responses - AKH is released by the corpora cardiaca, where aversive learning is depleted when the corpora cardiaca celled are removed - The akh receptors are found in 4 neurons in the suboesophageal zone that are directly activated but AKH and are required for aversive learning Methodology - Used odor shock test (stress and fear), and used a standard T-maze where the flies were exposed to an odor and 12 90 voltage shocks over a span of 1 minutes, and then an odor without shock o They tested memory right after - They also used an odor with a bitter diet - Hunger state was induced by 20-24 food deprivation period on 1% agar without refeeding and water was still provided during this period. Shibire was used to block out synaptic responses from certain neurons and tub Gal 80 was used to knock down AKH Notes

	- Habituation is NOTTTTT aversive learning. But they share similar learning circuits - Endocrine hormones can directly differ the strength of the learning circuit - AKH is released during hinder, enhances shock induced dopaminergic signals which increases the magnitude of pathways that help enhance aversive learning. - Memory formation also relies on internal systems where hormones adjust the inhibition of plasticity. - Habituation uses GABA, NMDA, and synapses
Research Question/Problem/ Need	It is not widely researched how internal states of neurons under different environmental conditions affect the behavioral state that those circuits and neurons regulate.
Important Figures	 Figure 5 shows that AKH signaling connects to dopaminergic neurons through SEZ output neurons.
VOCAB: (w/definition)	Hemolymph: insect equivalent of blood Octopamine: neuromodulator that increases neuronal excitability
Cited references to follow up on	Chatterjee, N., & Perrimon, N. (2021). What fuels the fly: Energy metabolism in *Drosophila* and its application to the study of obesity and diabetes. *Science advances*, *7*(24), eabg4336. https://doi.org/10.1126/sciadv.abg4336
Follow up Questions	1. Is this transferable to memory tests such has habituation? 2. Is there a reason for the 24 hours starvation period, how did they determine the time? 3. How does aversive learning differ from habituation in terms of neural

	circuiting?

Article #18 Notes: Neural Basis of Hunger-Driven Behavior in Drosophila

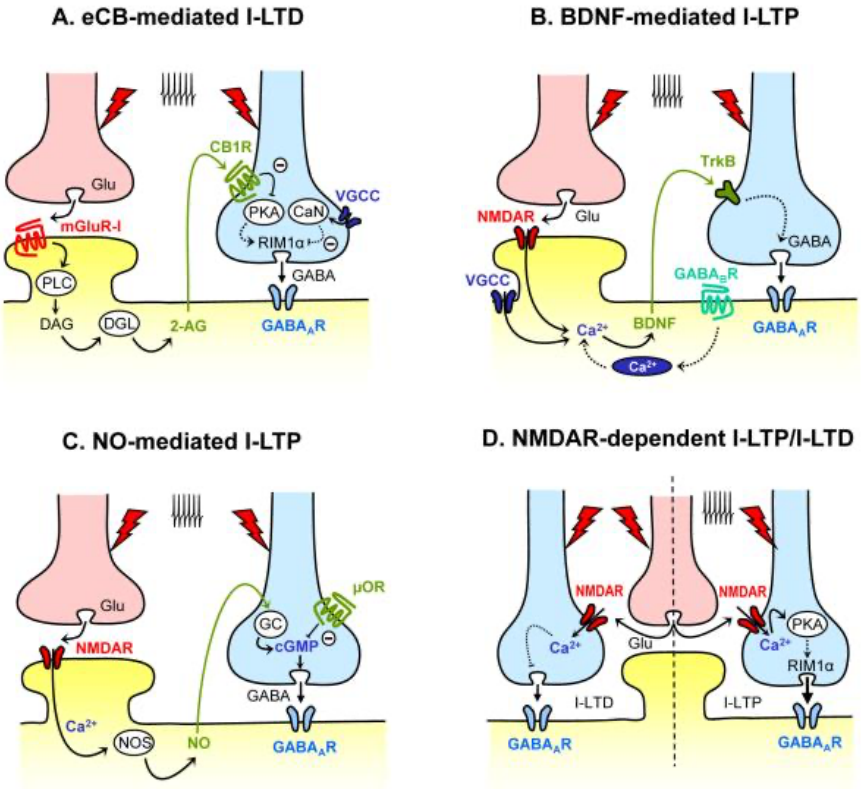
Source Title	Neural Basis of Hunger-Driven Behavior in Drosophila
Source citation (APA Format)	Lin, S., Senapati, B., & Tsao, C.-H. (2019). Neural basis of hunger-driven behaviour in *drosophila*. *Open Biology*, *9*(3). https://doi.org/10.1098/rsob.180259
Original URL	https://pubmed.ncbi.nlm.nih.gov/30914005/
Source type	Journal Article
Keywords	*Drosophila*, neural circuits, hunger, feeding behavior, food-seeking behavior
#Tags	
Summary of key points + notes (include methodology)	- AKH is the main endocrine hunger signal in Drosophila, and it is similar to human glucagon. It is released from the corpora cardiaca during starvation and signals low energy state to peripheral tissues and the brain - Starvation increases AKH release - AKH stores energy for survival and food seeking ability - AKH and insulin inhibit each other - AKH is needed for starvation hyperactivity which is essential just their ability to store/forage for food. Adult flies that lack AKH or AKHR do not show increased locomotion seen during starvation - AKH influences feeding circuits through SEZ neurons where AKH activate AKHR that influence the hunger state - Flies can evaluate food nutritional value when taste is removed, They used 2 choice assays to see if flies lacking taste receptor could still prefer sucrose. They can learn nutrition through odor pairs with nutritious sugar
Research Question/Problem/ Need	How does the internal state of hunger of hunger get transferred to the nervous system for behaviors in Drosophila?

Important Figures	 Figure 1 shows how each neuromodulator interact, I shows whether the neuromodulator influences inhibition and/or activation. Hunger is a distributed, multifaceted state.
VOCAB: (w/definition)	Fat body: fly organ that is analogous to the liver, where it senses nutrients and releases endocrine signals Gr43a neurons: brain fructose's sensors that monitor carbohydrate intake
Cited references to follow up on	Lee, G., & Park, J. H. (2004). Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in Drosophila melanogaster. *Genetics*, *167*(1), 311–323. https://doi.org/10.1534/genetics.167.1.311
Follow up Questions	1. How do hunger signals interact with each other? 2. How does brain prioritize competing needs? 3. Does long term hunger reshape neurons?

Article #19 Notes: Long Term Synaptic Plasticity at Inhibitory Synapses

Source Title	Long term Synaptic Plasticity at Inhibitory Synapses
Source citation (APA Format)	Castillo, P. E., Chiu, C. Q., & Carroll, R. C. (2011). Long-term plasticity at inhibitory synapses. *Current Opinion in Neurobiology*, *21*(2), 328–338. https://doi.org/10.1016/j.conb.2011.01.006
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC3092861/
Source type	Journal Article
Keywords	GABA, GABA receptor, LTP, LTD, metaplasticity, heterosynaptic plasticity, homeostatic plasticity, epilepsy, autism, schizophrenia
#Tags	

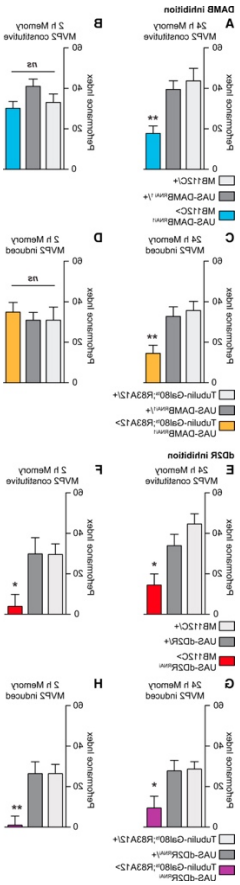
Summary of key points + notes (include methodology)	- Learning, memory and brain development depend on excitatory plasticity and long-term plasticity at inhibitory synapses that use GABA
	  o Researchers mainly focused on excitatory synapses as the learning developer. Inhibitory plasticity helps control the balance in the brain (making it stable), which affects memory and learning
	- Excitatory Synapses essentially controls inhibitory plasticity because when the excitatory strength changes, then the inhibitory strength knows how to adjust and maintain balance
	- The retrograde messengers travel from the postsynaptic cell back to the GAB-releasing terminal and change how much GABA is released
	  o Retrograde messengers are the chemical signals that the neuron releases when the excitatory synapse is very active and they move backward from the post to the presynaptic terminal
	  o Travel until they reach GABA and tell it to release less or more
	- Excitatory neurons make and release glutamate
	  o Glutamate is the main excitatory neurotransmitter in the brain. When a neuron releases glutamate onto another neuron, it makes the neuron more likely to fire
	- Inhibitory synapses can get stronger over long periods of time called LTP or weaker over long periods of time called LTD.
	  o Occur in many regions
	- Because inhibitory pathways dictate how much is the neurons are exited, they decide whether excitatory synapses can undergo LTP or LTD
	- Glutamate can activate NMDA receptors and change GABA release
	- Inhibitory plasticity can also happen on the postsynaptic side where the

	change involves the GABA receptors. Neurons can add more receptors to strengthen inhibition, remove them to weaken - Disruptions of inhibitory plasticity is a factor in disorders and conditions like epilepsy, autism, etc.
Research Question/Problem/ Need	The article is needed because research has often entirely focused on excitatory plasticity as the drive odor memory, which left inhibitor plasticity out.
Important Figures	 Figure 1 shows how different molecules can change the amount of GABA released from the inhibitory synapse.
VOCAB: (w/definition)	Inhibitory Synapses- connection between 2 neurons where the message sent from the presynaptic neuron makes the post synaptic neuron react accordingly GABA- Gamma-Aminobutyric Acid is the main inhibitory neurotransmitter in the brain
Cited references to follow up on	Gaiarsa, J.-L., Caillard, O., & Ben-Ari, Y. (2002). Long-term plasticity at GABAergic and glycinergic synapses: Mechanisms and functional significance. *Trends in Neurosciences*, *25*(11), 564–570. https://doi.org/10.1016/s0166-2236(02)02269-5 McBain, C. J., & Kauer, J. A. (2009). Presynaptic plasticity: Targeted control of

	Inhibitory Networks. *Current Opinion in Neurobiology*, *19*(3), 254–262. https://doi.org/10.1016/j.conb.2009.05.008
Follow up Questions	1. How does the brain determine whether inhibition should be stronger or weaker? 2. How does inhibition develop with time?

Article #20 Notes: A GABAergic Feedback Shapes Dopaminergic Input on the Drosophila Mushroom Body to Promote Appetitive Long-term Memory

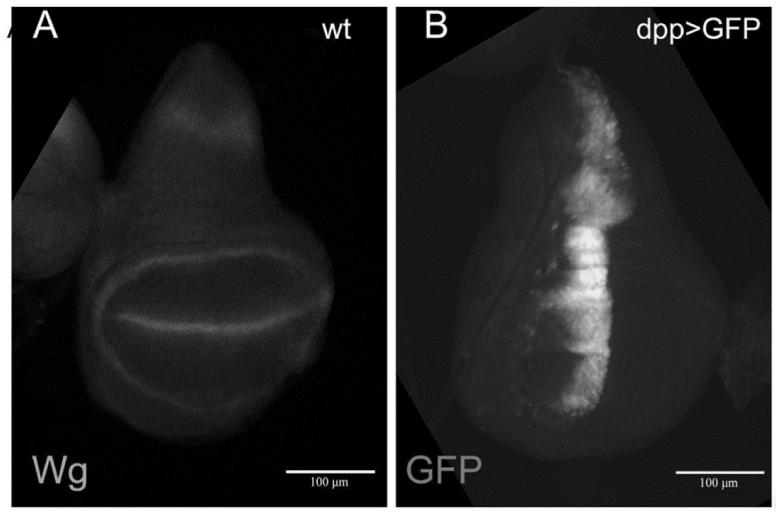
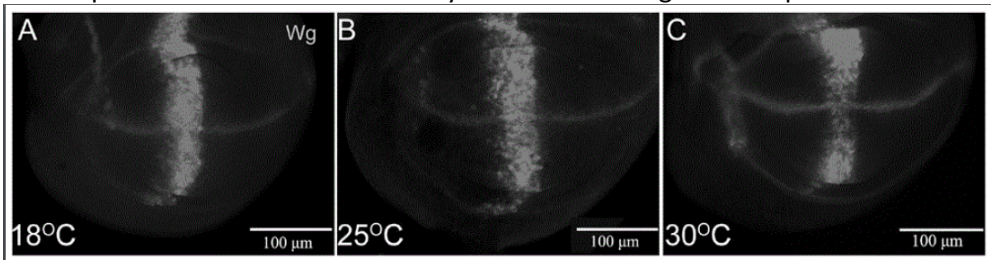
Source Title	A GABAergic Feedback Shapes Dopaminergic Input on the Drosophila Mushroom Body to Promote Appetitive Long-term Memory
Source citation (APA Format)	Pavlowsky, A., Schor, J., Plaçais, P.-Y., & Preat, T. (2018). A GABAergic feedback shapes dopaminergic input on the drosophila mushroom body to promote appetitive long-term memory. *Current Biology*, *28*(11). https://doi.org/10.1016/j.cub.2018.04.040
Original URL	https://pubmed.ncbi.nlm.nih.gov/29779874/
Source type	Journal article
Keywords	appetitive long-term memory, *Drosophila*, memory consolidation, dopamine, DAMB, D2, oscillations, GABAergic inhibition, negative feedback
#Tags	
Summary of key points + notes (include methodology)	Key Points - MP1 neuron and MVP2 neuron which regulates appetitive long-term memory are in a loop. MP1 is related to dopamine and MVP2 is involved with inhibition - MP1 activity is needed right after to initial long-term memory - MVP2 is not activated during this time because activating too early blocks long term memory - After 30 minutes, the inhibitor receptor of dD2R allows MP1 to suppress MVP2 so MP1 oscillations can rise - The DAMB which is the activating dopamine receptor switches on MVP2 which shuts down the MP1 oscillations through inhibition - GABAergic feedback needs D-GABABR1 in MP1 - MP1 oscillation frequency increases with nutrient odor - This is similar to mammals' dopamine GABA loops, where dopamine and inhibition is needed for learning and memory Methodology - MP1 and MVP2 dopaminergic neurons were targeted using GAL4 flies so they could manipulate each neuron to see its effect - They did not target developmental chances; they only targeted adults using tubulin GAL 80ts. Kept at low temps to keep GAL4 off then shifted to higher temps for 2 days during adulthood - They starved the fires, trained with one odor paired with 1.5 M sucrose

	and another without sucrose - Memory was tested in a T-maze choice test for 1 minutes - 2 hour and 24-hour long-term memory - They performed in vivo imaging after appetitive training
Research Question/Problem/ Need	Long term memory is influenced by memory consolidation, but there is a lack of research and transparency of how that memory consolidation is process at the neural level.
Important Figures	 Figure 1 shows that MP1 provides input to the mushroom body during appetitive long term memory formation and that it is shaped by GABAergic loops from the mushroom body output neuron, MVP2. MP1 is high after learning while MVP2 is inhibited. After some time, MVP2 becomes active and sends GABAergic inhibitory feedback onto MP1 which reduces MP1 activity.
VOCAB: (w/definition)	Mushroom Body: a brain structure in drosophila essential for learning and memory Appetitive Long term Memory: a durable memory formed when an odor is associated with a rewarding stimulus Dopaminergic Neurons, DANs: neurons that release dopamine MP1 neuron: specific dopaminergic neuron that provides prolonged signaling required for LTM MVP2: A GABAergic output neuron that provides inhibitory feedback to MP1

Cited references to follow up on	Krashes, M. J., DasGupta, S., Vreede, A., White, B., Armstrong, J. D., & Waddell, S. (2009). A neural circuit mechanism integrating motivational state with memory expression in drosophila. *Cell*, *139*(2), 416–427. https://doi.org/10.1016/j.cell.2009.08.035
Follow up Questions	1. What happened to appetitive long term memory if MVP2 is silenced? 2. Is the dopamine and GABAergic loop specific to appetitive memory? 3. What does the MP1 activation time period do to strength of memory?

Article #21 Notes **A method for regulating the expression level of Drosophila transgenes**

Source Title	A method for regulating the expression level of Drosophila Transgenes
Source citation (APA Format)	Zhang, X., Li, K., Dong, W., & Zhang, J. (2020). *A method for regulating the expression level of Drosophila transgenes*(China Patent No. CN107201379B). China National Intellectual Property Administration. https://patents.google.com/patent/CN107201379B/en
Original URL	https://patents.google.com/patent/CN107201379B/en
Source type	Patent
Keywords	N/A
#Tags	
Summary of key points + notes (include methodology)	Notes - The Issue with the normal GAL4-UAS crossed system is that the uncontrolled and/or excessive expression that they cause can lead to cell death or developmental lethality - The genes typically are dose dependent in which different levels of expressions activate different pathways - Temperature doesn't only turn GAL 4 on or off because the method they invented a way to also incorporate fine tune expression levels by modulation RNAi strength - They combined UAS overexpression and UAS RNAi for the same gene so that the system has a self-balancing process that could be controlled by culture temperature - RNAi efficiency increases with temperature - At low temperatures around 16-18 degrees Celsius, the RNAi knock down is weak which mean the gene will be activated by GAL4 and overexpressed - At high temperatures around 30 degrees Celsius, the RNAi is dominant which means that the gene is suppressed - Enables stage specific gene manipulation which is needed for studying genes that have carrying roles during development and adulthood - They used Wing patterning genes to support that the method works for developmentally sensitive pathway - Approach is generalizable to other GAL4/UAS systems models which means that it could be applicable to neural circuits, hormone signaling, etc. Methodology - Designed in order to precisely regulate the strength and timing of a gene expression in drosophila using GAL4/UAS systems with RNAi and

	temperature - Flies are crossing in order to target the same gene. 2 UAS contructs with one driving over expression and the second one driving RNAi suppression - This drosophila strain is then crossed with a GAL 4 driver with help determine where the gene will be present and activated in the Drosophila - The low temperatures reduce RNAi efficiency, so the gene is overexpressed - The high temper increases RNAi efficiency, so the gene is suppressed - Gene expression levels were measured at third instar larvae and gene expression was visualized using immunostaining
Research Question/Problem/ Need	The normal GAL4/UAS system can lead to uncontrolled and excessive expression that can lead to cell death or developmental lethality, making it unideal for studies and experiments with developmental pathways. It can disrupt and lead to changes in the genes function.
Important Figures	 Figure 1 compares the wing expression with GAL4/UAS system expression in third-instar larvae which establishes that the GAL4 driver has a defined spatial pattern and is specific to certain tissue. This system activates a gene in a specified area.  Figure 2 shows that GAL4/UAS system that influences overexpression along is not temperature sensitive by showing wing expression at different temperatures.

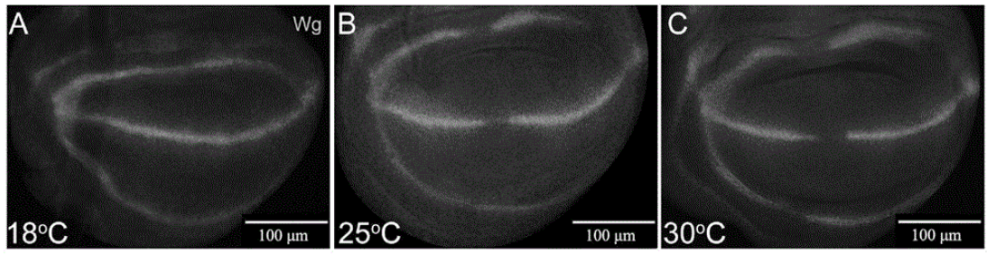


Figure 3 shows the wg expression differences as GAL4/UAS RNAi third instar larvae were used at different temperatures. RNAi suppression increases with temperature

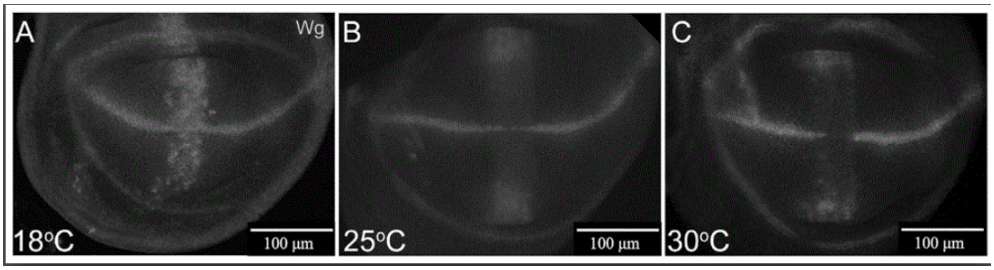


Figure 4 shows the wg changes in expression after a GAL4-UAS and UASRNAi crossing at different temperatures. Over expression is seen are low temperature while normal expression was seen at 25 Celsius and low expression was seen at high temperature

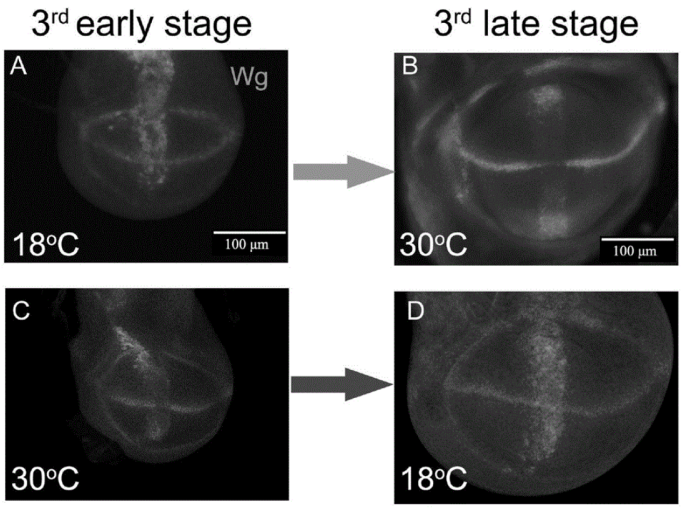


Figure 5 shows temporal regulation using temperature shifts where it shows that early overexpression and late overexpression using temperature.

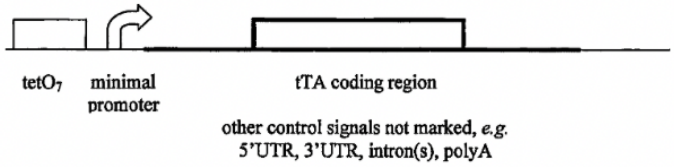
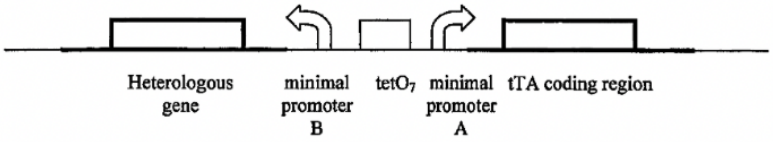
VOCAB: (w/definition)

Recombinant Strain- A genetically engineered fly line containing **both** UAS-X and UAS-X-RNAi, allowing simultaneous activation and inhibition of the same gene.
RNA interference- A biological mechanism that reduces gene expression by degrading target mRNA, preventing protein production
Morphogen gradient- A concentration gradient of a signaling molecule (e.g., Wingless) that produces different biological outcomes at different levels.

Cited references to follow up on	Fortier, E., & Belote, J. M. (2000). Temperature-dependent gene silencing by an expressed inverted repeat in Drosophila. *Genesis (New York, N.Y. : 2000)*, *26*(4), 240–244. https://doi.org/10.1002/(sici)1526-968x(200004)26:4<240::aid-gene40>3.0.co;2-p
Follow up Questions	1. How can gene expression be quantified at each temperature point 2. Is the relationship between temperature and gene expression a trend other than downward? Is it linear, exponential, etc.? 3. Why did they choose third instar larvae to test on?

Article #22 Notes: Expression System for Insect Pest Control

Source Title	Expression System for Insect Pest Control
Source citation (APA Format)	Alphey, L. (2015). *Expression system for insect pest control* (U.S. Patent No. US 9121036 B2). U.S. Patent and Trademark Office. https://patents.google.com/patent/US9121036B2/en
Original URL	https://patents.google.com/patent/US20070056051A1/en
Source type	Patent
Keywords	N/A
#Tags	
Summary of key points + notes (include methodology)	Notes - Describes an insect gene expression system that uses positive transcriptional feedback to overcome the limitations of normal promoter with them showing g weak, variable expressions once integrated - They use positive transcriptional feedback which means that the ene being expressed produces a protein that enhances its own transcription - Expression is controllable which allows them to turn the expression on and off - It is most clearly implemented using the tetracycline repressible transcriptional activator which binds to the tetO sequences near the promoter - When tetracycline is absent, the gene is expressed - When tetracycline is present, it suppresses transcription - Embryos and larvae are more sensitive to high transcriptional activity than adults which makes it so that stage specific effects can happen - The patent mentions other transcriptional activators including GAL4 which can be incorporated into the same process - In GAL4 versions regulation can happen with temperature sensitive GAL80 - It incorporates sex-specific splicing, tissue-specific promoter, etc. - The invention is designed to function across a wide range of insect species Methods - Built genetic constructs which a transcriptional activator gene is placed under the control of promoter that the activator can turn on - Added DNA specific binding sites near the promoter so the activator protein could bind onto and increate transcription - More activator leads to stronger gene expression - Expression is controlled by adding or removing food with tetracycline - When tetracycline is absent, expression is activated and vice versa - Used reporter genes and developmental survival analysis
Research Question/Problem/ Need	Existing insect promoters show weak, inconsistent, varying expression which mean their usefulness is limited.

Important Figures	 Fig.1  Fig.2 Figure one shows how the tTa and tetO positive feedback loop starts. It shows how the transcriptional activator enhances its own expression by binding upstream. Figure 2 shows how construct size can be reduced while maintaining a strong feedback loop.
VOCAB: (w/definition)	Positive Feedback-A regulatory mechanism in which a gene product increases transcription of its own gene, amplifying expression. Minimal Promoter- A stripped-down promoter with low basal activity that requires enhancers or activators for strong transcription.
Cited references to follow up on	Alphey, N., Bonsall, M. B., & Alphey, L. (2011). Modeling resistance to genetic control of insects. *Journal of theoretical biology*, *270*(1), 42–55. https://doi.org/10.1016/j.jtbi.2010.11.016
Follow up Questions	1. How stable is positive feedback expression across different insertion places? 2. What is the line for high expression and developmental lethality? 3. How do GAL4/GAL80 differ in terms of effectiveness and reversibility?