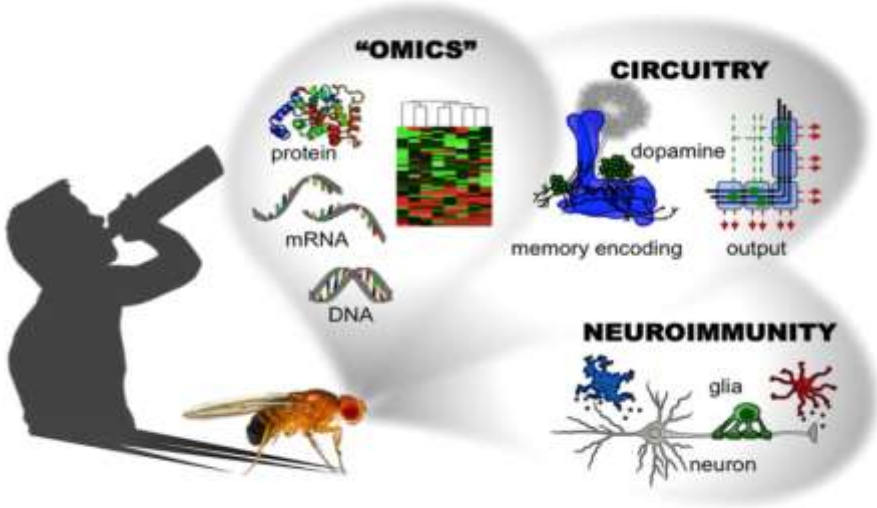
Alcohol and *Drosophila*

邢丽敏、纪小小、苏祥彬

2021/11/25

早C（coffee）晚A（alcohol）

Why alcohol is so addictive?



behaviors

1. Molecular mechanisms of alcohol addiction ——邢丽敏
2. Alcohol and dopamine——苏祥彬
3. Alcohol and social behaviors——纪小小
4. Discussion

PART1

Molecular mechanisms of alcohol addiction

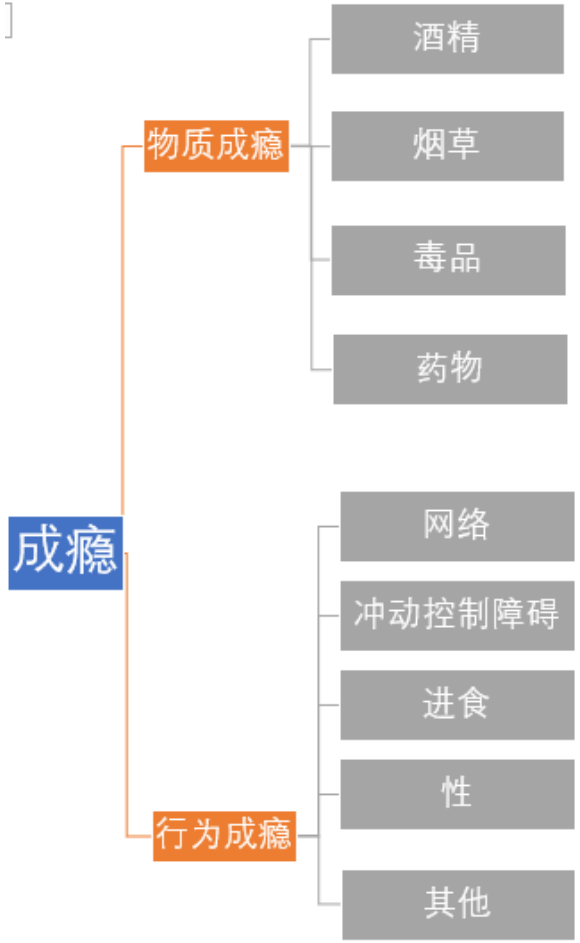
——XLM

ICD-10中依赖诊断标准里，依赖（成瘾）的核心要素为：

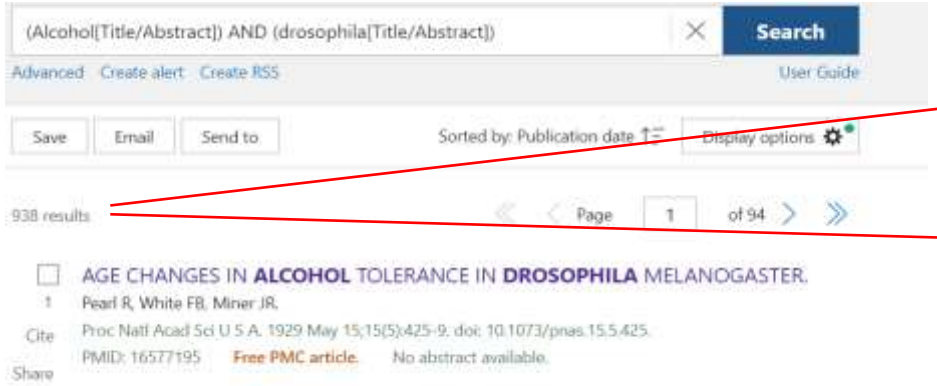
(1) **失控：** 持久强烈使用精神活性物质的欲望，尽管明白有害，但仍然继续使用。

(2) **渴求：** 强烈的欲望或强迫性觅药与用药行为。

(3) **耐受性与戒断状态：** 耐受性指明显增加物质的使用量才能达到中毒量或预期效果；戒断状态为当物质使用减少或终止时出现的特殊症状群。



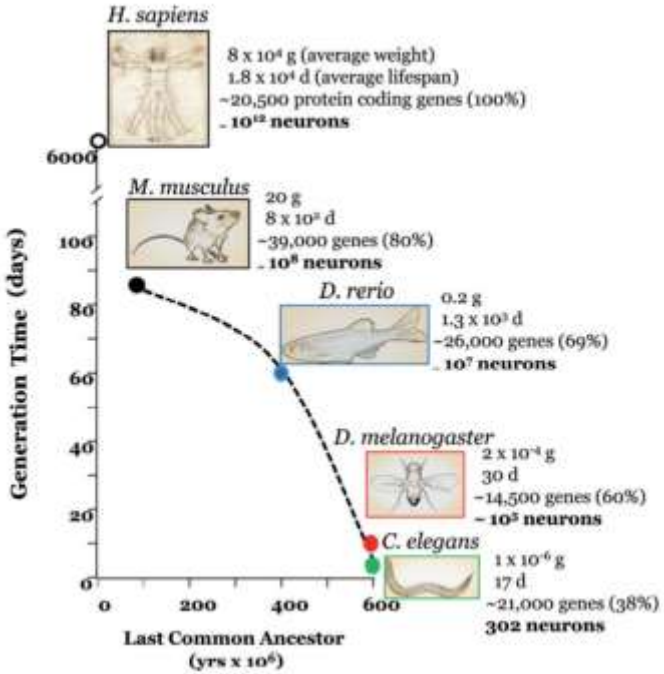
Why use fruit flies as model organisms to study alcohol addiction ?



Proc Natl Acad Sci U S A. 1929

1990

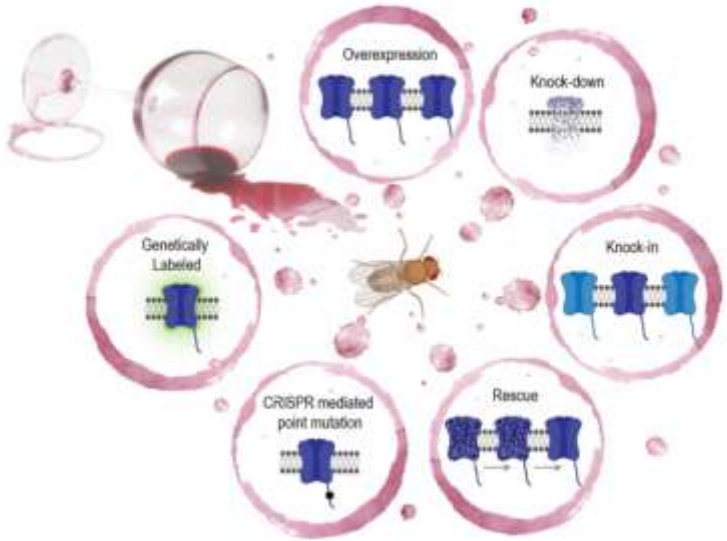
938 results



White BH. J Neurogenet. 2016.

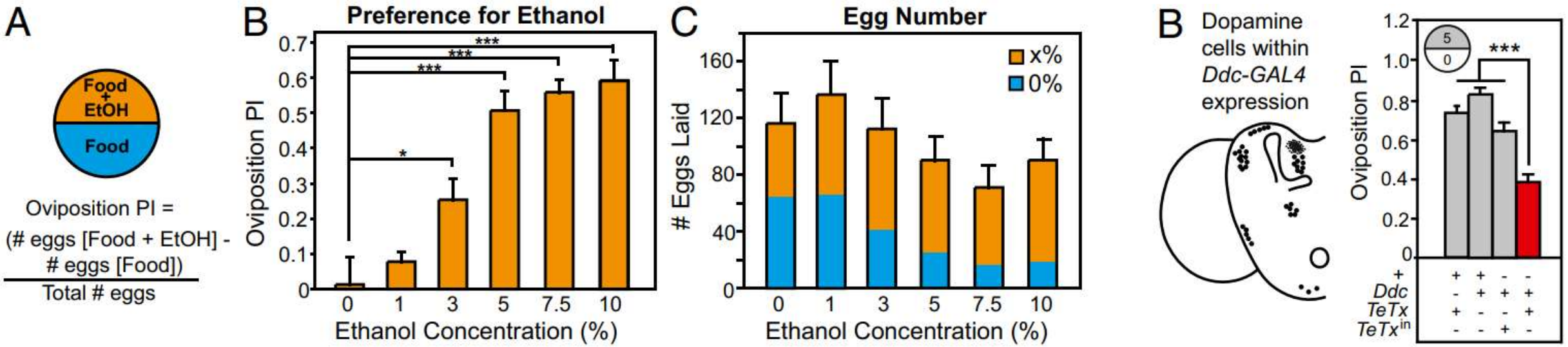
65%-75%

Fortini ME, et al., J Cell Biol. 2000.



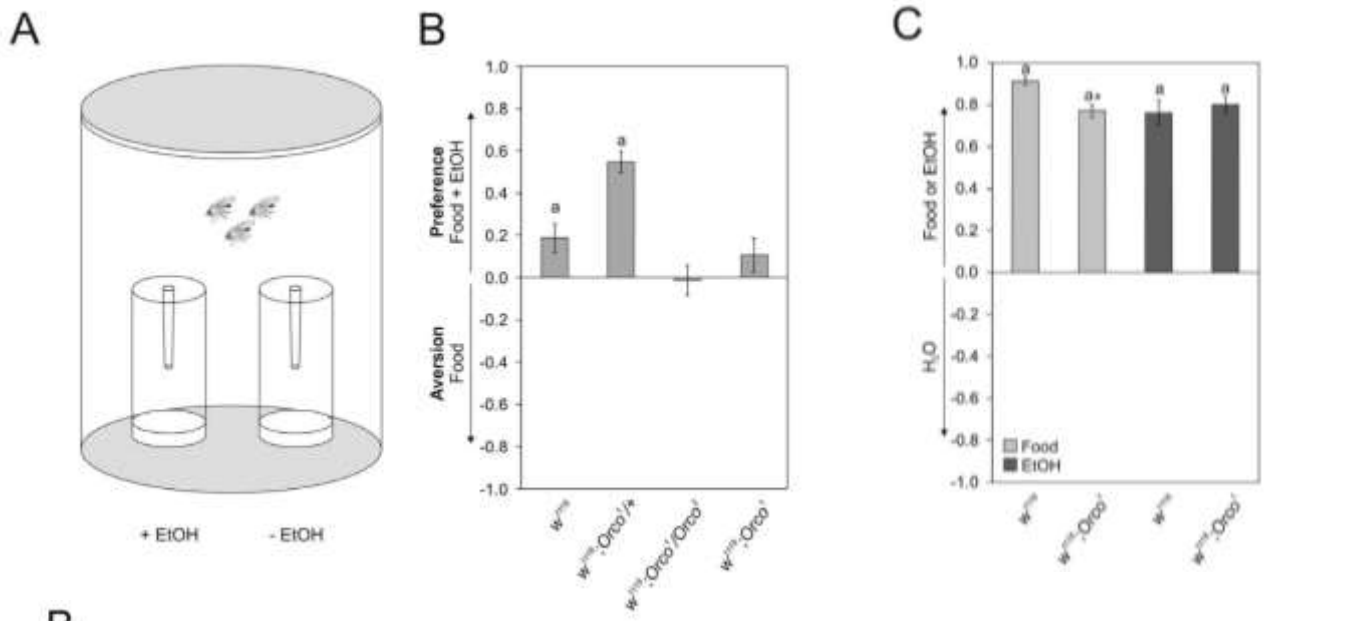
Scaplen and Petruccelli., Neuroscience Insights ., 2021

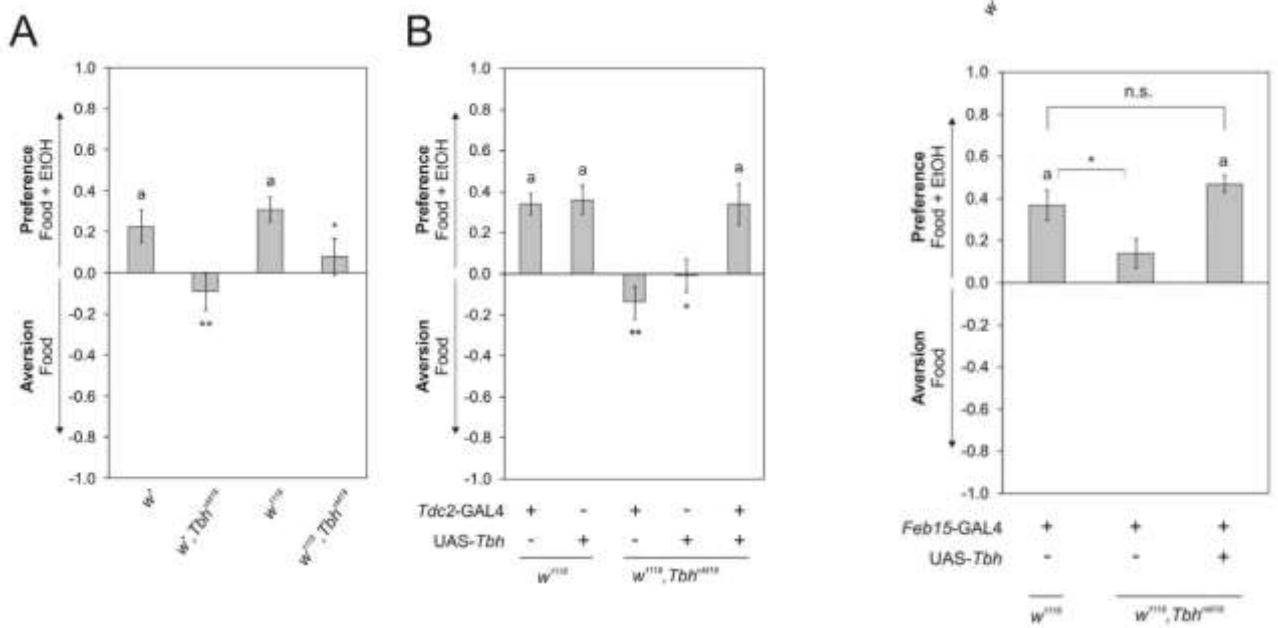
Fruit flies have a natural attraction to ethanol

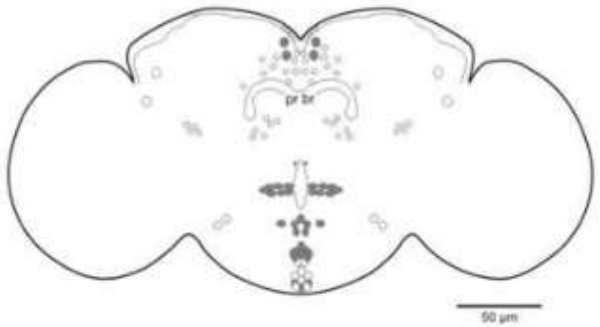


Azanchi., *et al*., *PNAS*., 2013

orco regulates ethanol perception, *tβh* is required for olfactory ethanol preference



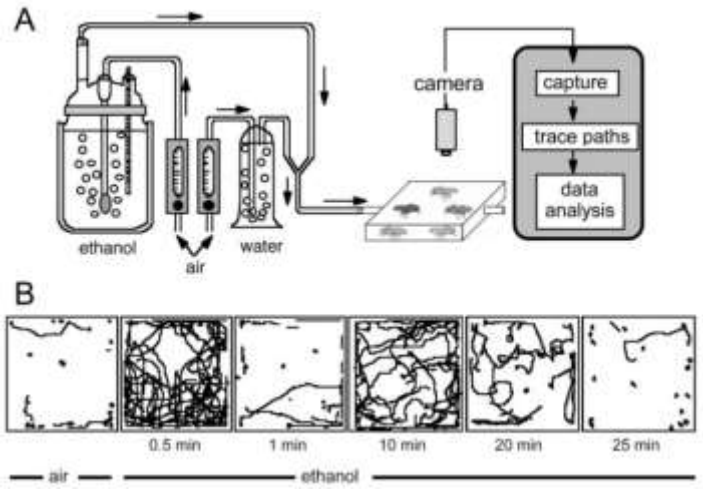


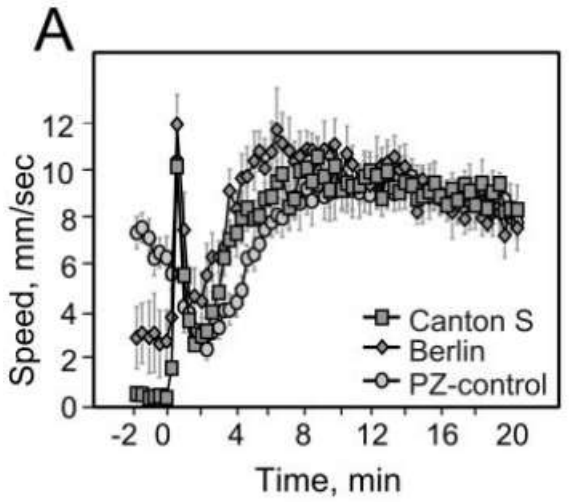


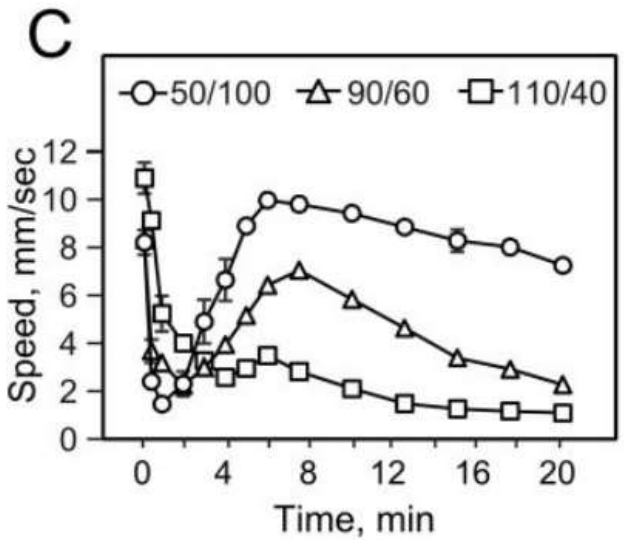
26 TbH expressing neurons of the G3a/AL2 cluster and the VMI-VMIII

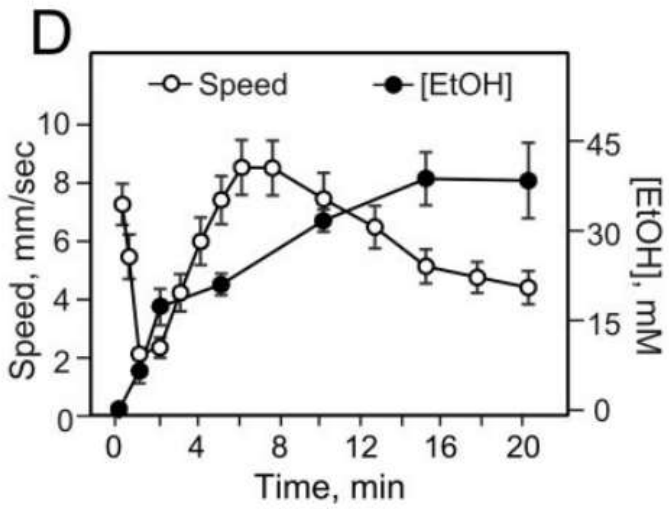
Schneider A.,*et al*., *PLoS ONE*., 2012

The effects of ethanol on locomotion are comparable in flies and mammals



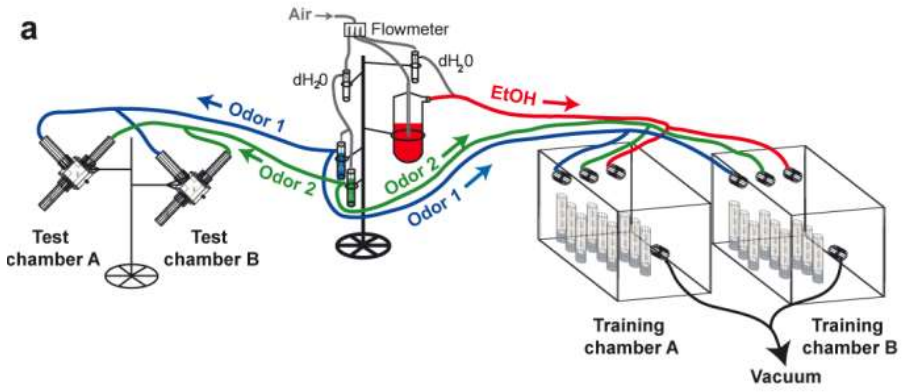


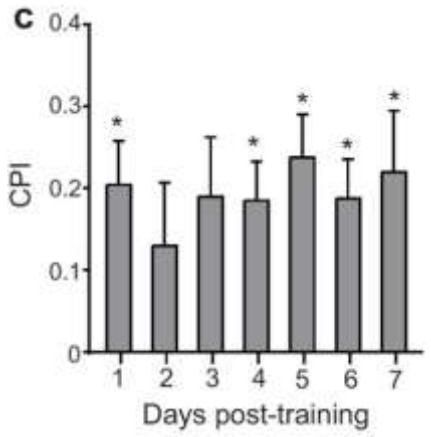


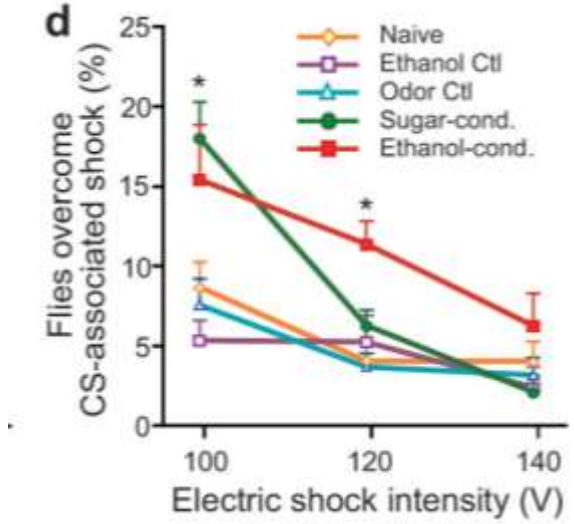


Wolf., *et al*., *J. Neurosci*. 2002

Fruit flies overcome electric shocks to get ethanol

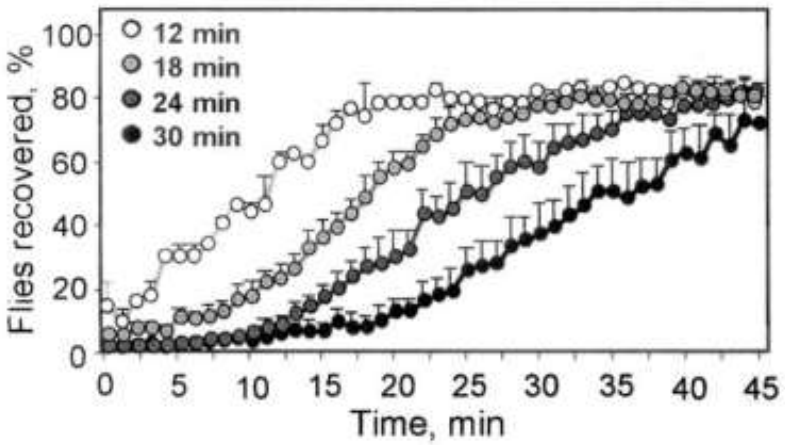


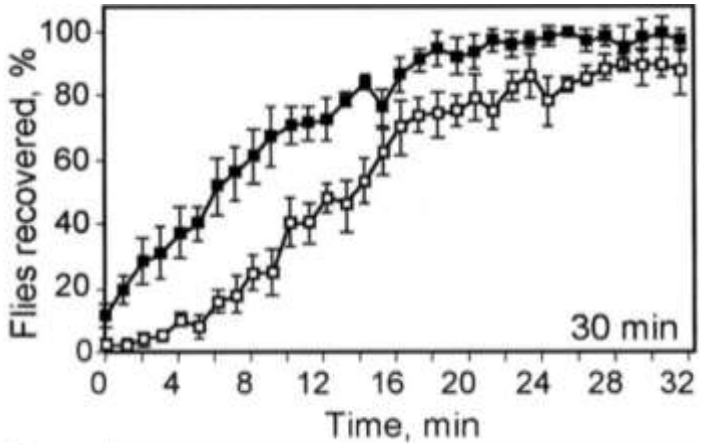




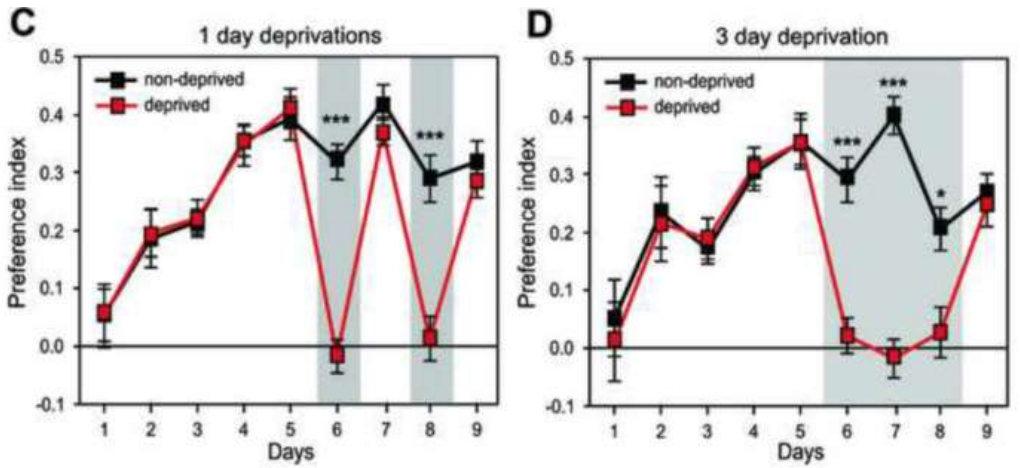
Kaun, et al., *Nat. Neurosci.,* 2011

Ethanol tolerance and withdrawal reaction





□ air
■ ethanol



Berger., *et al*., *Alcohol Clin Exp Res*., 2004

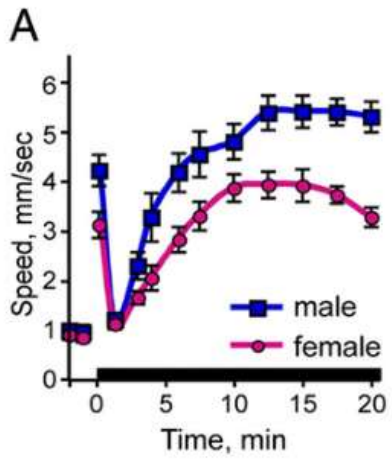
Devineni1 and Heberlein., *Curr Biol*., 2009

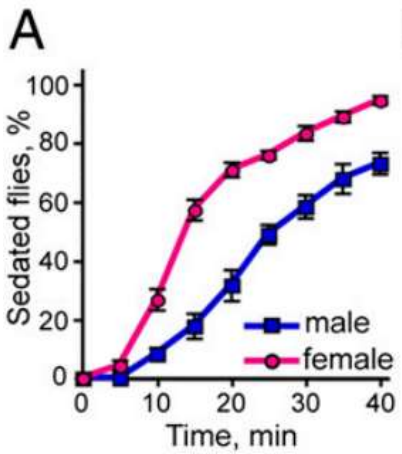
Conclusions:

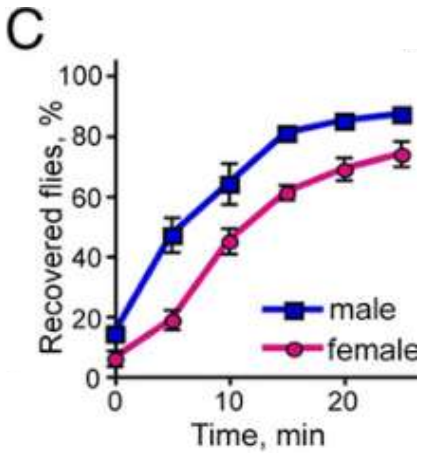
- Q: Why use fruit flies as model organisms to study ethanol addiction?
- A: Fruit flies sense ethanol through smell and exhibit similar characteristics of alcohol addiction to mammals.

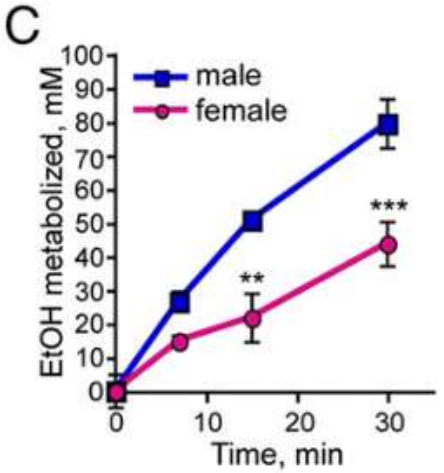
Whether alcohol addiction is sexual dimorphism ?

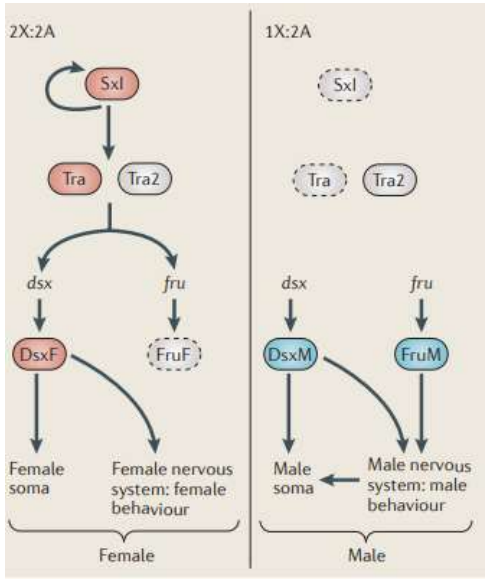
Tra acts in developing nervous system to regulate sexually dimorphic ethanol sedation sensitivity

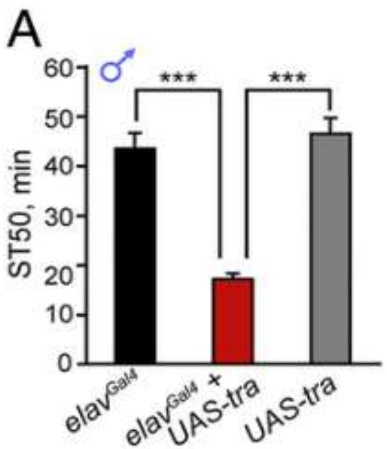


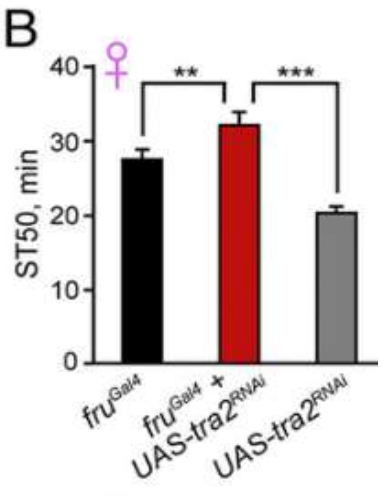


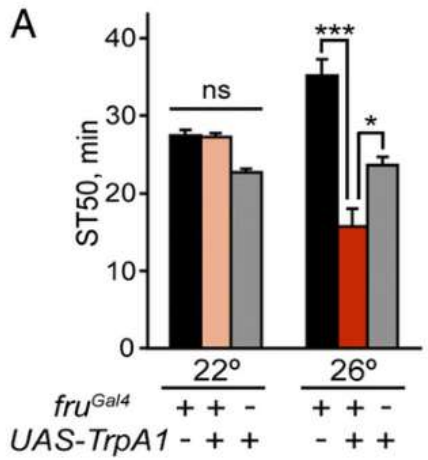


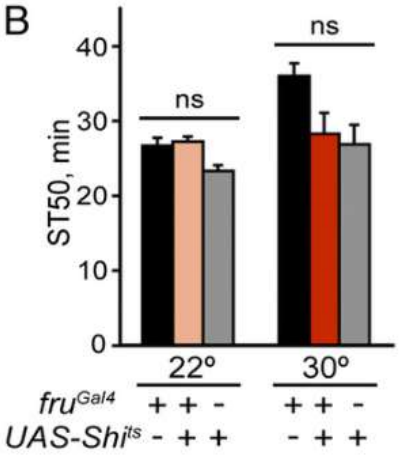












Devineni and Heberlein., *PNAS*., 2012

Conclusions:

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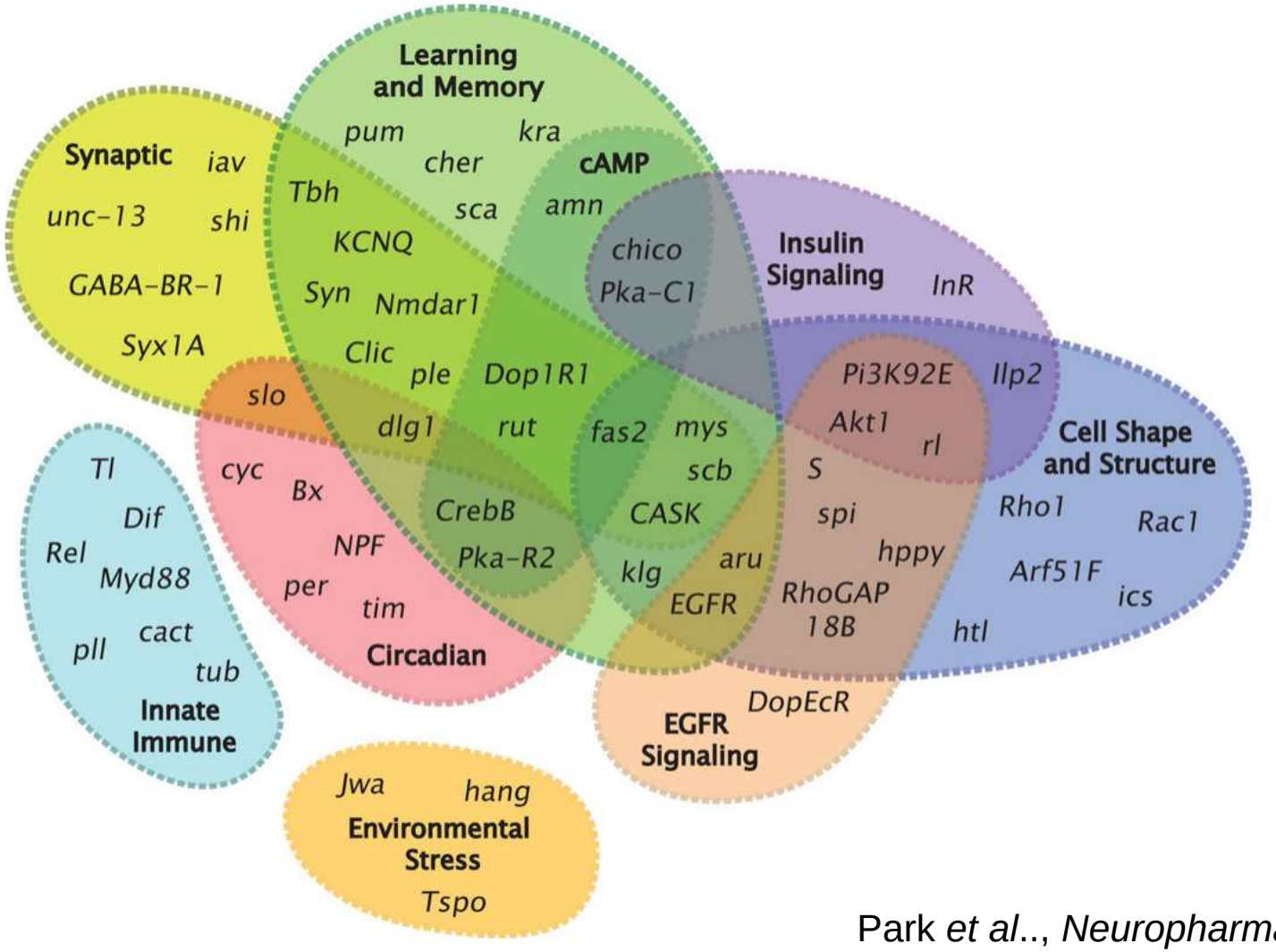
- Q: Whether alcohol addiction is sexual dimorphism ?
- A: Sexually dimorphic ethanol sedation sensitivity is regulated by Tra in developing nervous system

Ethanol resistance, tolerance, and preference genes identified by one-gene-at-a-time genetics organize into GO-like gene categories

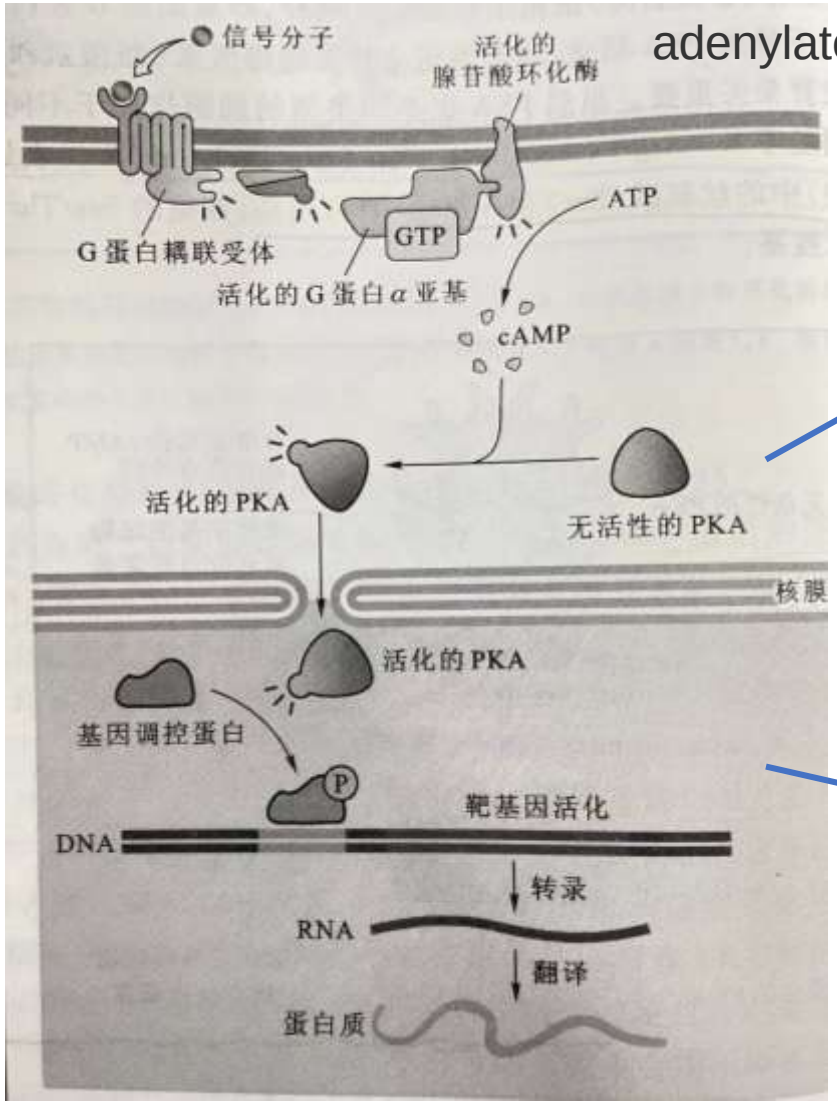


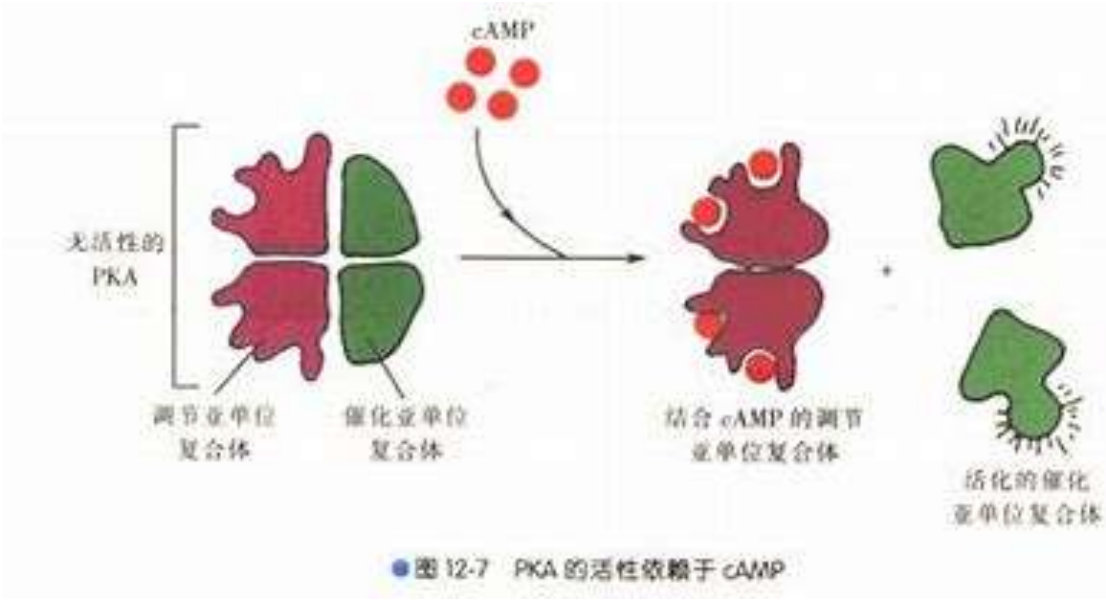
Park *et al*.., *Neuropharmacology*., 2017

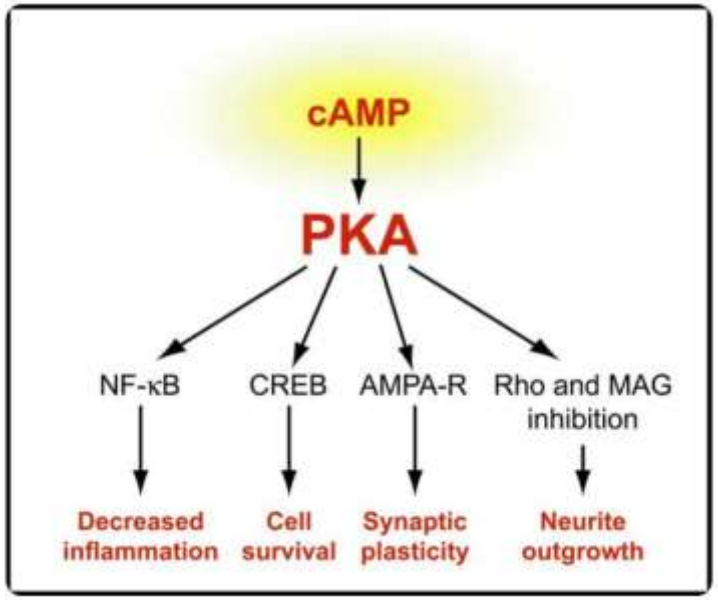
How does alcohol affect memory？

cAMP signaling pathway

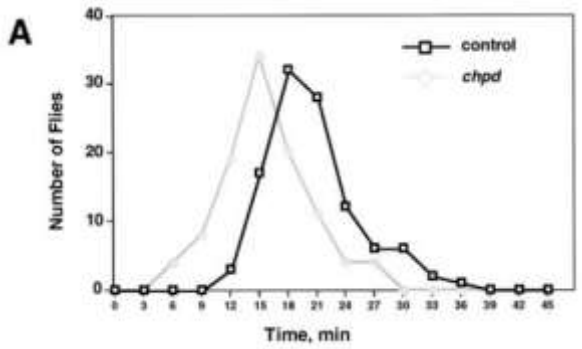
adenylate cyclase, AC



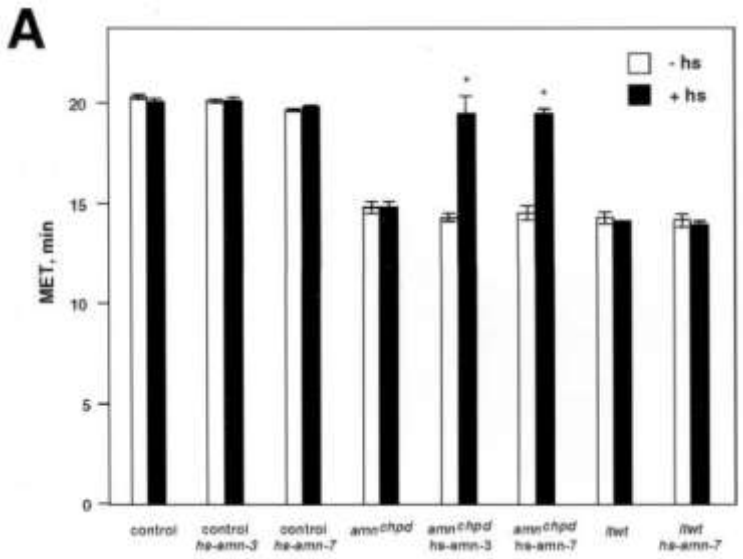




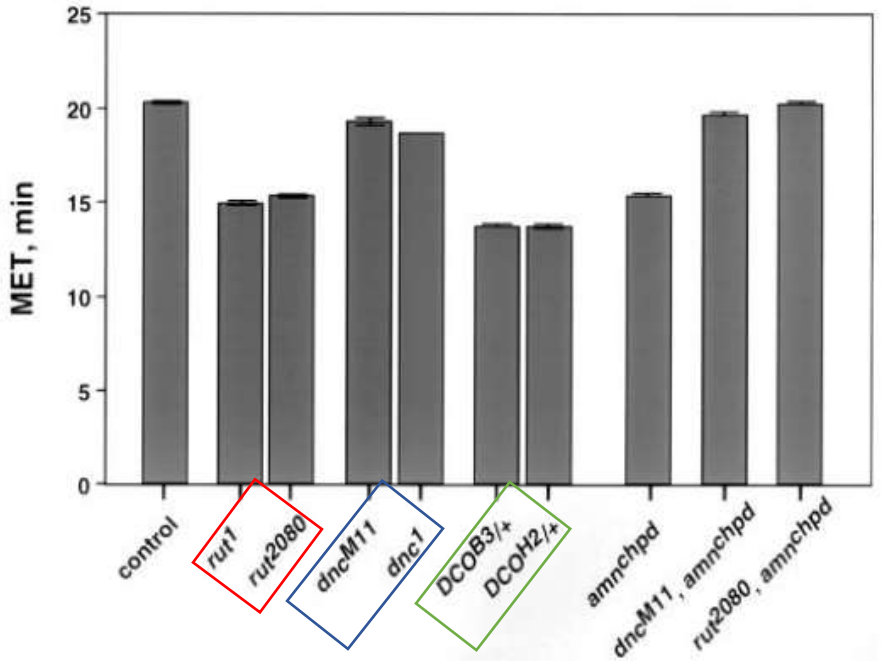
amnesiac and *rutabaga* regulate ethanol sensitivity by modulate cAMP levels



chpd is an allele of *amn*



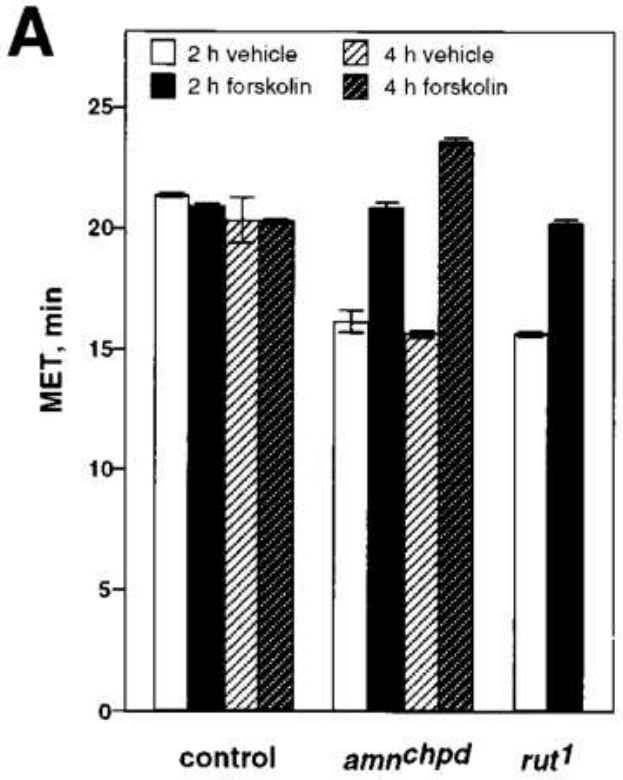
the mean elution time (MET)



encoding the Ca^{2+}calmodulin-sensitive AC

encoding the major cAMP- phosphodiesterase (PDE)磷酸二酯酶

encoding the major catalytic subunit of cAMP-dependent protein kinase (PKA-C1)



Moore., *et al*,. *Cell*., 1998

PKA inhibition in IPCs increases ethanol sensitivity in the nervous system

Science

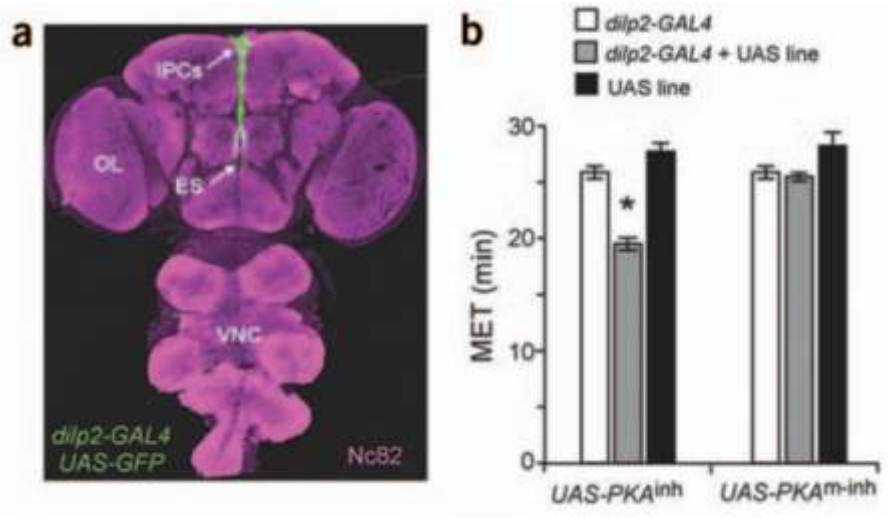
Ablation of Insulin-Producing Neurons in Flies: Growth and Diabetic Phenotypes

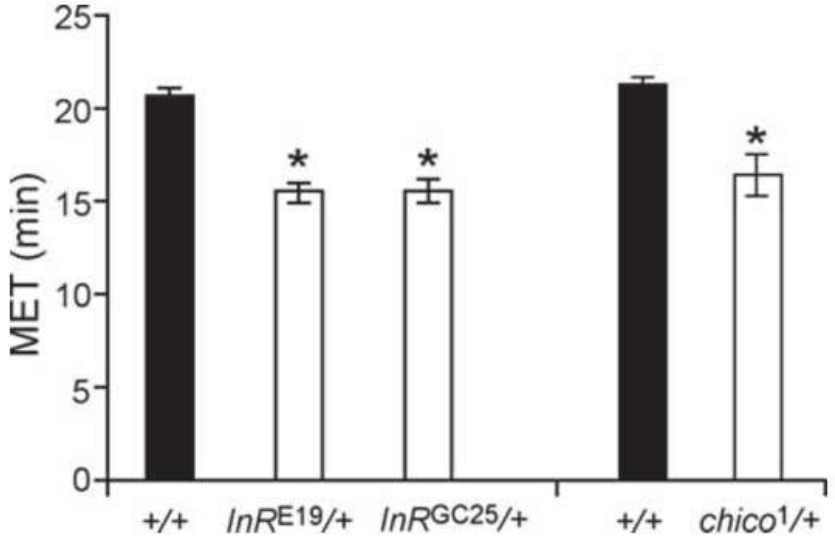
The Journal of Neuroscience
The Official Journal of the Society for Neuroscience

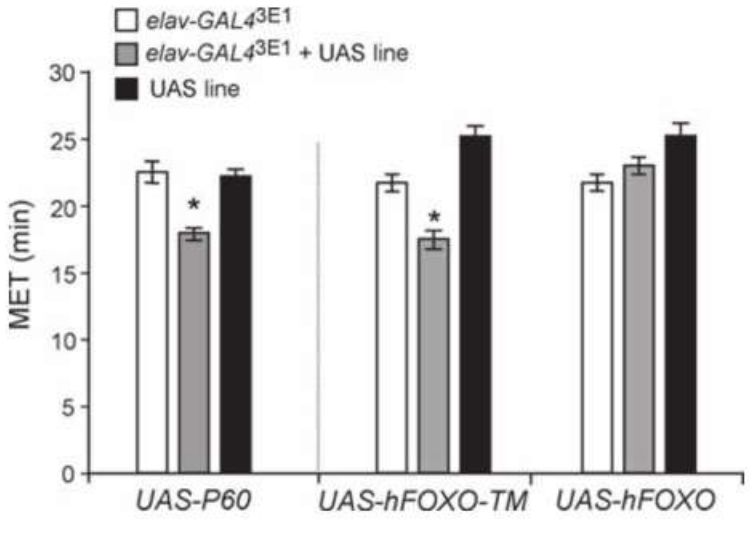
J Neurosci. 2002 Nov 1; 22(21): 9490–9501.
doi: 10.1523/JNEUROSCI.22-21-09490.2002
PMCID: PMC6758036
PMID: 12417673

Functional Dissection of Neuroanatomical Loci Regulating Ethanol Sensitivity in *Drosophila*

Aylin R. Rodan,[1,3] John A. Kiger, Jr.,[4] and Ulrike Heberlein[1,2,3]

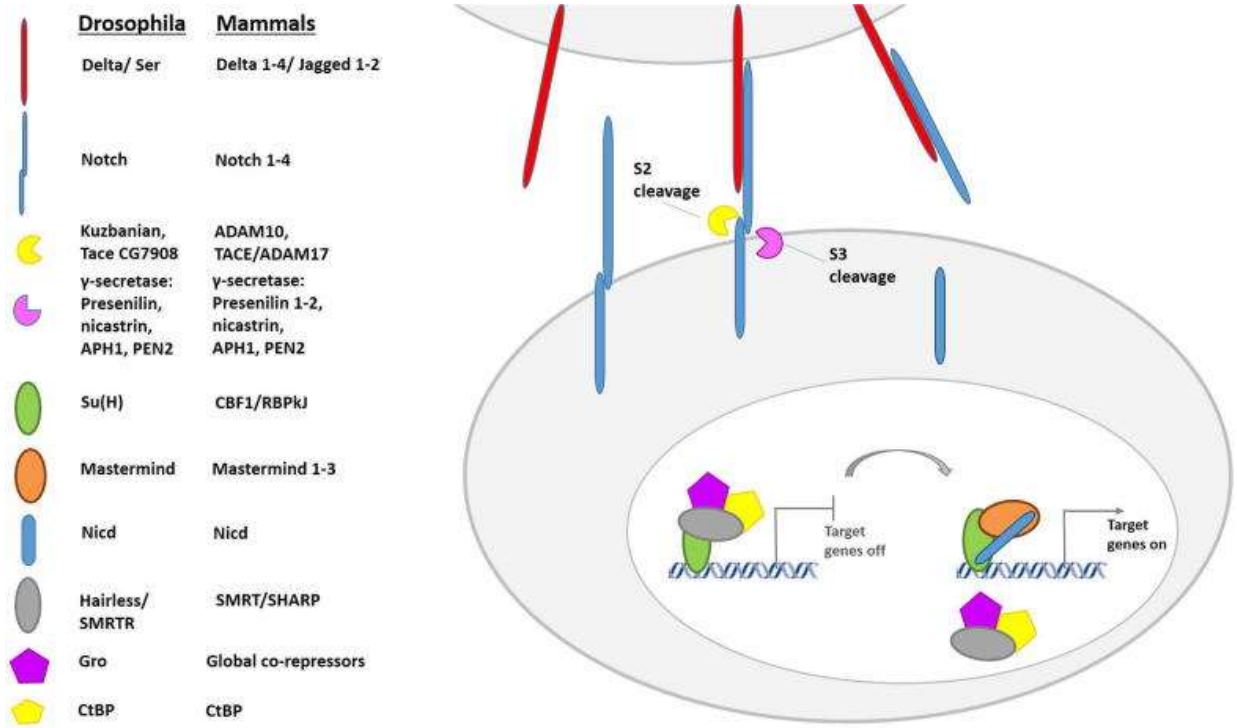






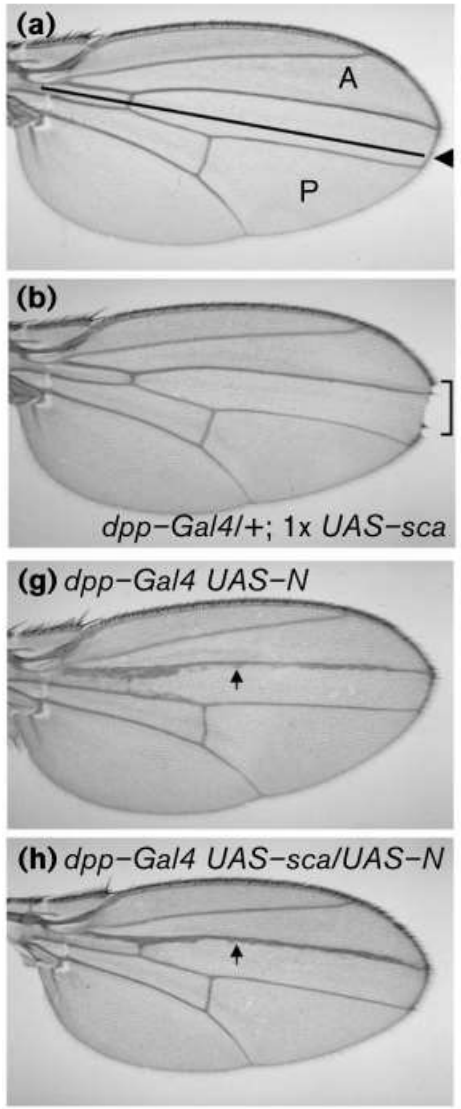
Corl., *et al*., *Nature Neuroscience*., 2005

Notch Signaling pathway

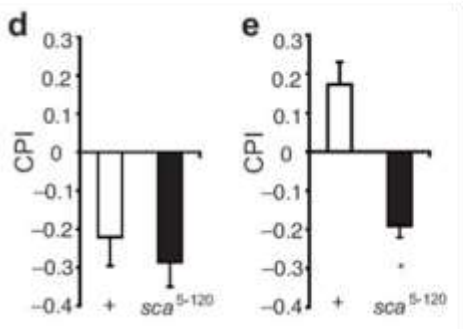


Zacharioudaki and Bray., *Methods*., 2014

scabrous regulates conditioned ethanol preference assay by Notch signaling pathways



Lee., et al., *Curr Biol*., 2000



Kaun, et al., *Nat. Neurosci.,* 2011

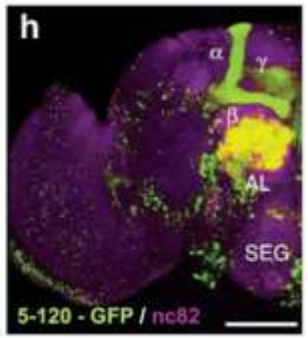
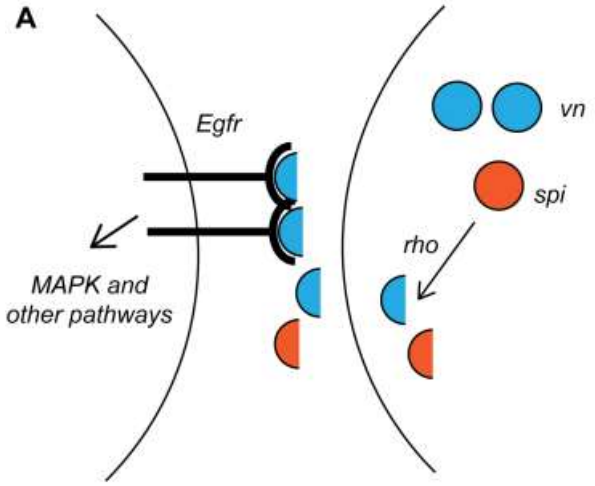


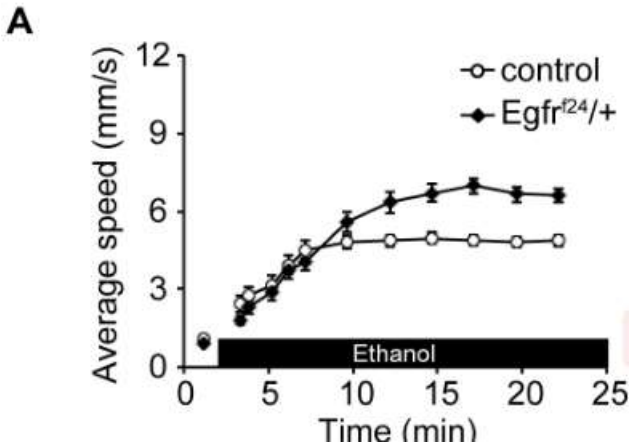
Table 1 Common candidate genes for DGRP and extreme QTL GWA analyses previously associated with alcohol-related phenotypes in humans

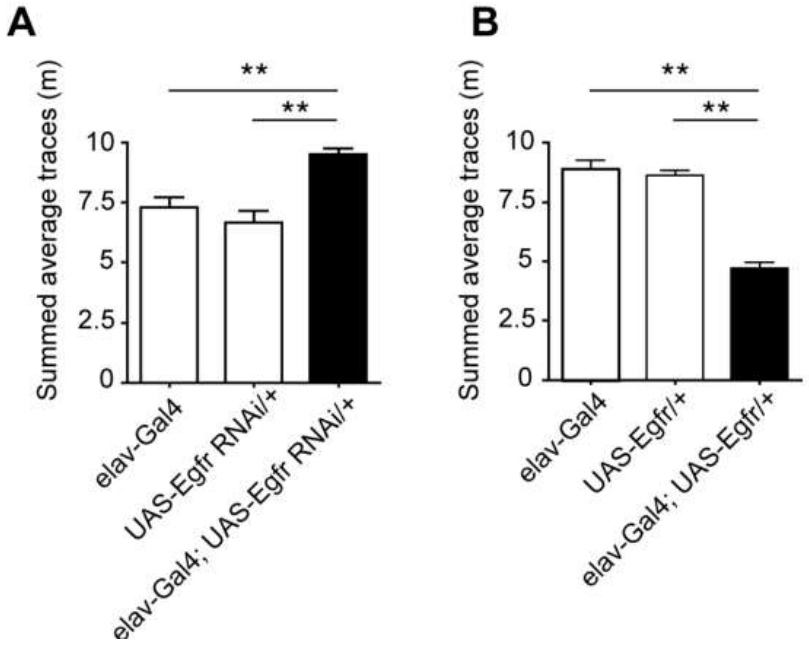
Drosophila gene symbol	Biological process	Human gene symbol
CAP	Muscle attachment; sensory perception of sound	*CAP2* [82]
CG31690	-	*TMTC2* [63]
Dys	Imaginal disc-derived wing vein morphogenesis; neuromuscular synaptic transmission; muscle organ development	*DMD, UTRN* [14, 63]
fz	Wnt signaling pathway; signal transduction; axon extension; heart development; negative regulation of Notch signaling pathway; establishment or maintenance of cell polarity	*FZD9* [82]
Ilp-2	Protein dephosphorylation; regulation of secretion	*PTPN2* [14]
KCNQ	Potassium ion transport; embryonic development via the syncytial blastoderm; regulation of heart rate	*KCNQ3* [63, 64]
luna	Regulation of transcription, DNA-templated; mitotic sister chromatid segregation	*KLF7* [110]
mAt	Axon transport of mitochondrion	*TRAK2* [63]
mtt	Phospholipase C-activating G-protein coupled receptor signaling pathway; response to insecticide; adult feeding behavior	*GRM5* [12]
Nrv-IV	Dorsal closure; protein localization; synaptic vesicle targeting; cell-cell junction organization; establishment or maintenance of cell polarity	*CHRNA5, CHRNA 7* [10, 83, 111]
Pde1c	cGMP metabolic process; cAMP metabolic process	*PDE1C* [63, 64]
Rtgdl	Stem cell development	*MSD* [112]
rhea	Cell adhesion; muscle attachment; regulation of cell shape; larval somatic muscle development; negative regulation of transcription, DNA-templated; phagocytosis	*TLN2* [63]
shn	Ectoderm development; cell proliferation; learning or memory; olfactory learning; transforming growth factor beta receptor signaling pathway; peripheral nervous system development; positive regulation of transcription from RNA polymerase II promoter	*HIVEP1* [63]
stan	Establishment of planar polarity; cell adhesion; axonogenesis; mushroom body development; Wnt signaling pathway; negative regulation of Notch signaling pathway	*CELSR1* [113]

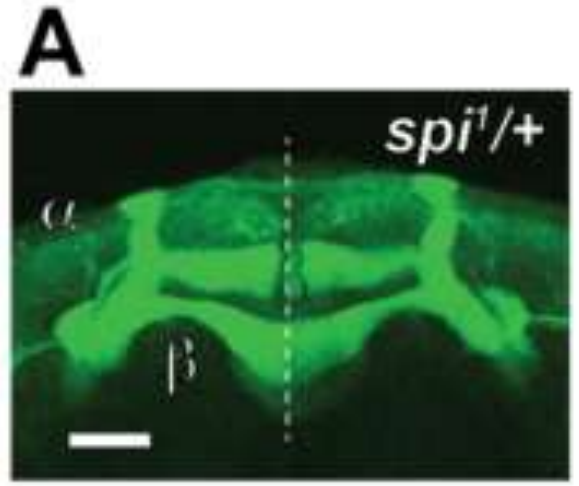
Morozova., et al., *BMC Genomics*., 2015

Epidermal Growth Factor Receptor (EGFR) signaling affects ethanol-induced locomotion maybe by mushroom body









anti-Fasciclin II (FasII)：stains the MB α/β and γ axon lobes

Spi：activator of EGFR

King., *et al*., *PLoS ONE*., 2014

Conclusions:

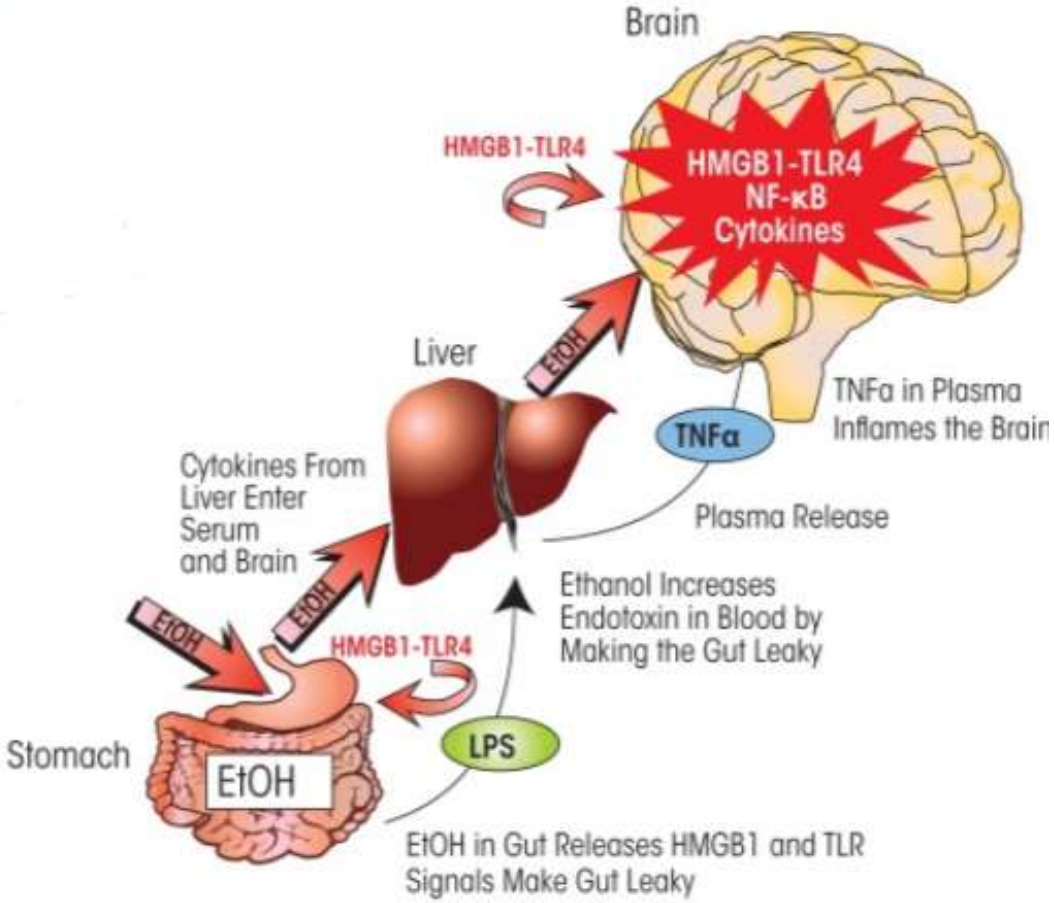
- Q: Why use fruit flies as model organisms to study ethanol addiction
- A: Fruit flies sense ethanol through smell and exhibit similar characteristics of alcohol addiction to mammals.
- Q: Whether alcohol addiction is sexual dimorphism ?
- A: Sexually dimorphic ethanol sedation sensitivity is regulated by Tra in developing nervous system
- Q: How does alcohol affect memory ?
- A:Through cAMP、notch and EGFR signaling pathways in the mushroom body

过度饮酒的危害:

1、营养不良：消化性溃疡病及其他并发症。
2、引发精神障碍：
3、呼吸系统疾病
4、引发酒精性肝病。
5、可引起小脑变性，发生共济失调。
6、体温紊乱。

How does alcohol affect health?

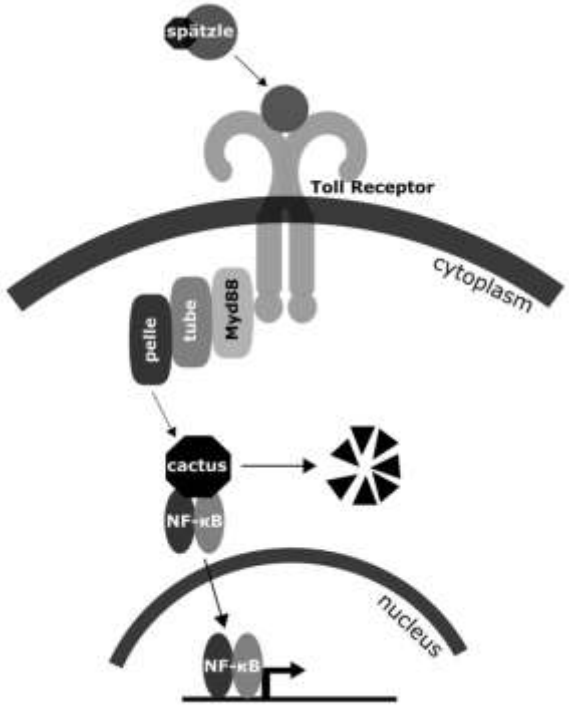
In mammals, the innate immune TLR signaling pathways are activated by ethanol

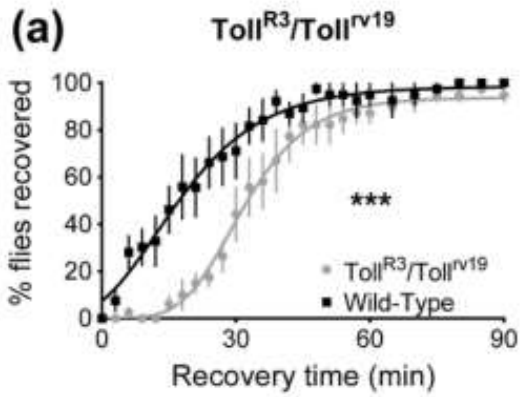


Crews., *et al*., *Alcohol Res*., 2015

In flies, the response of the homologous **Toll pathway** is well-conserved

Alcohol resistance in Drosophila is modulated by the Toll innate immune pathway





Summary of Behavior Experiments

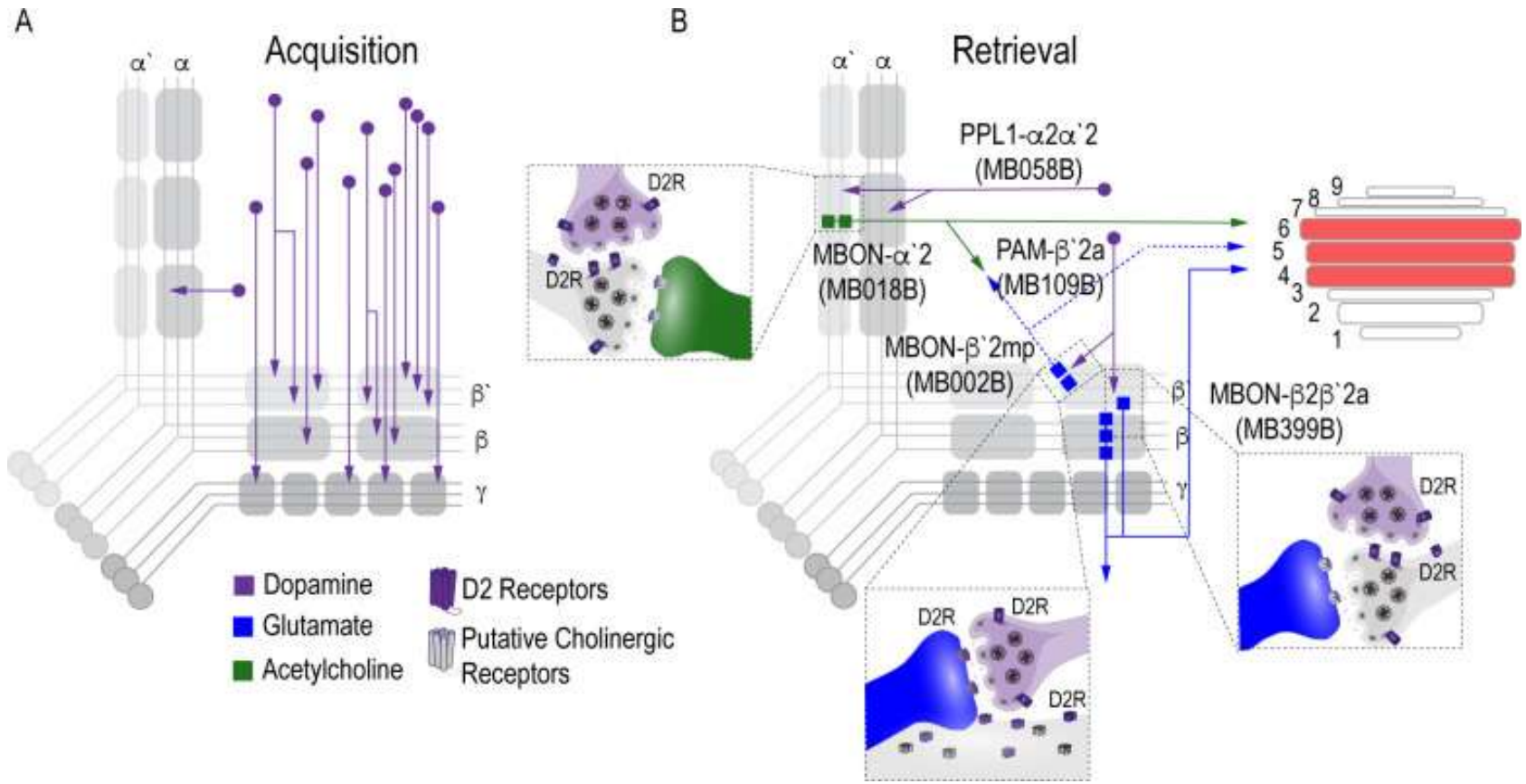
Gene	Mutant Allele	Inducible RNAi	Overexpression		
			Tub-Gal4	Appl-Gal4	Actin-G.S.
spätzle	Sensitive				
Toll	Sensitive	Sensitive	Resistant	Resistant	Resistant
Myd88		Sensitive			
tube	Sensitive	Sensitive			
pelle	Sensitive	Sensitive			
cactus	Resistant	Resistant			
Dif	Sensitive	Sensitive	Resistant	Resistant	n.s.
dorsal	Resistant	Resistant	Lethal	Sensitive	Lethal
Relish	Sensitive	Sensitive	Resistant	Resistant	Sensitive

Troutwine., *et al*., *Genes Brain Behav*., 2016

Conclusions:

- Q: Why use fruit flies as model organisms to study ethanol addiction?
- A: Fruit flies sense ethanol through smell and exhibit similar characteristics of alcohol addiction to mammals.
- Q: Whether alcohol addiction is sexual dimorphism ?
- A: Sexually dimorphic ethanol sedation sensitivity is regulated by Tra in developing nervous system
- Q: How does alcohol affect memory ?
- A:Through cAMP、Notch and EGFR signaling pathways in the mushroom body
- Q: How does alcohol affect health?
- A: The TLR innate immune pathways in mammals;

  The Toll innate immune pathway in Drosophila


A
Acquisition
α`
α
β`
β
γ
Dopamine
Glutamate
Acetylcholine
D2 Receptors
Putative Cholinergic Receptors
B
Retrieval
PPL1-α2α`2
(MB058B)
D2R
MBON-α`2
(MB018B)
PAM-β`2a
(MB109B)
MBON-β`2mp
(MB002B)
MBON-β2β`2a
(MB399B)
1
2
3
4
5
6
7
8
9

PART 2

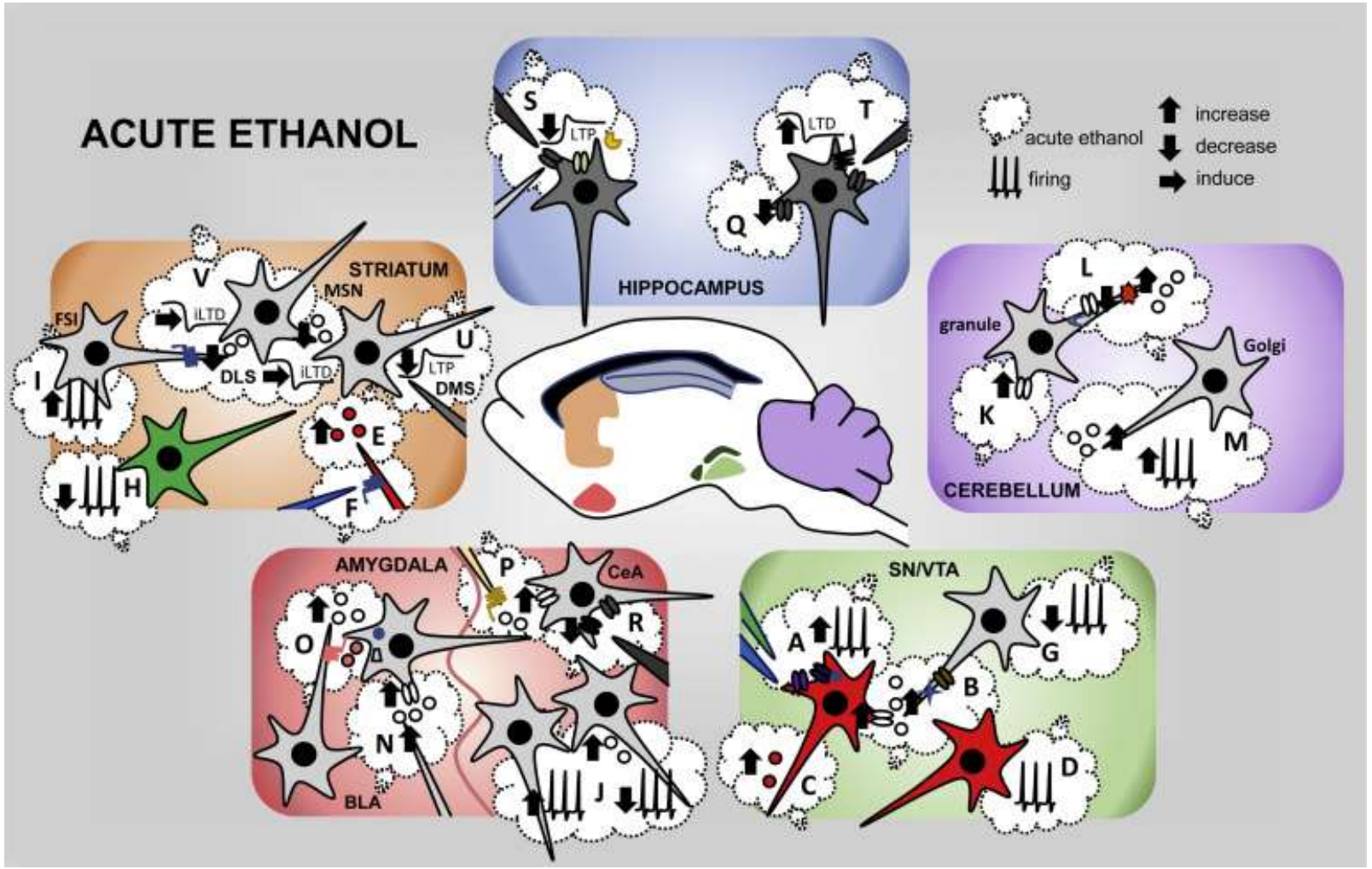
Alcohol and Dopamine in *Drosophila*

—苏祥彬
20211125

The dangers of alcohol

Ethanol’s actions in the brain

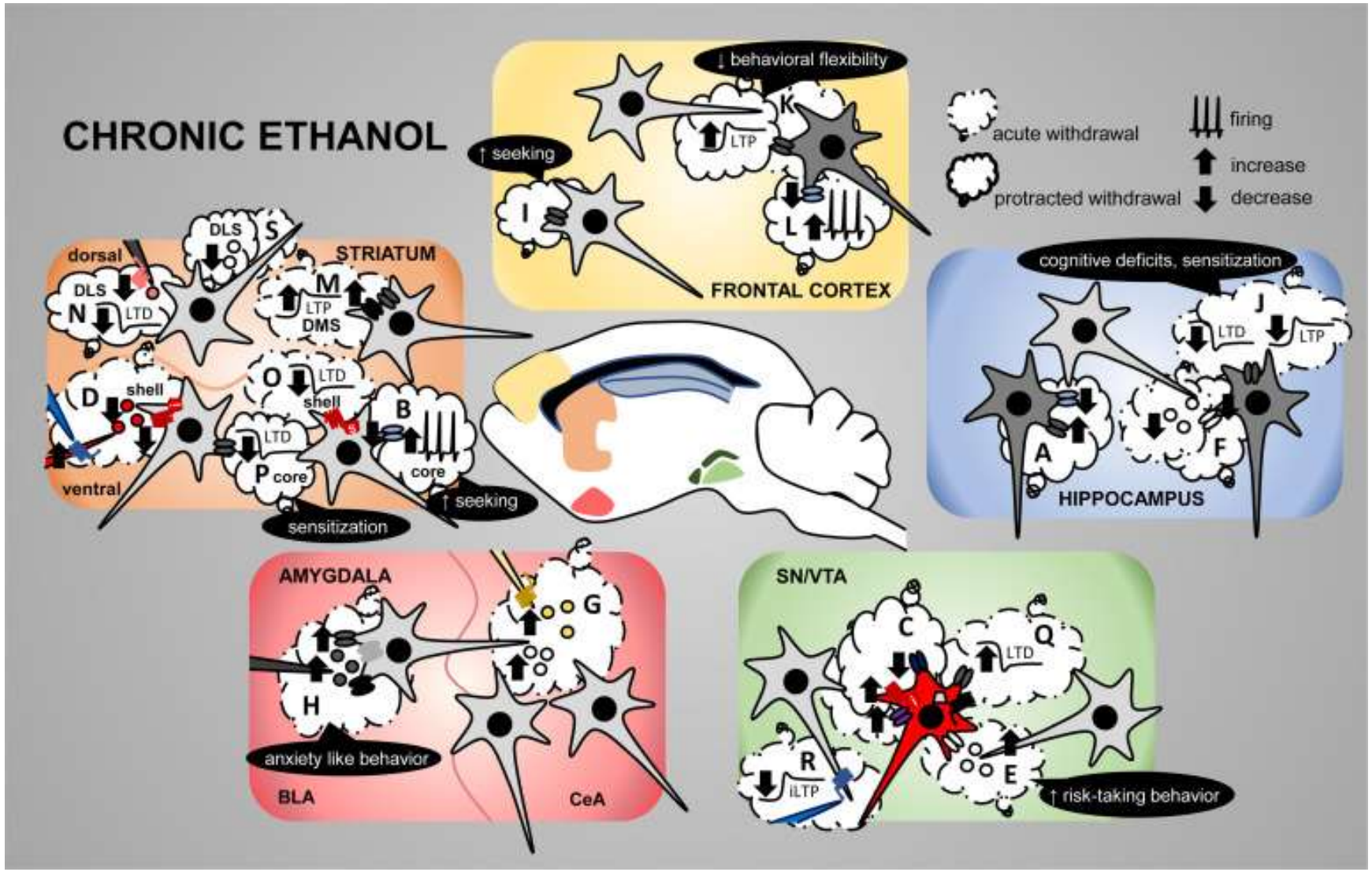
Acute ethanol-related behavioral changes



Karina P. Abrahao, Armando G. Salinas, and David M. Lovinger. 2017

- Slowed reaction times
- Motor incoordination
- Cognitive impairment occur

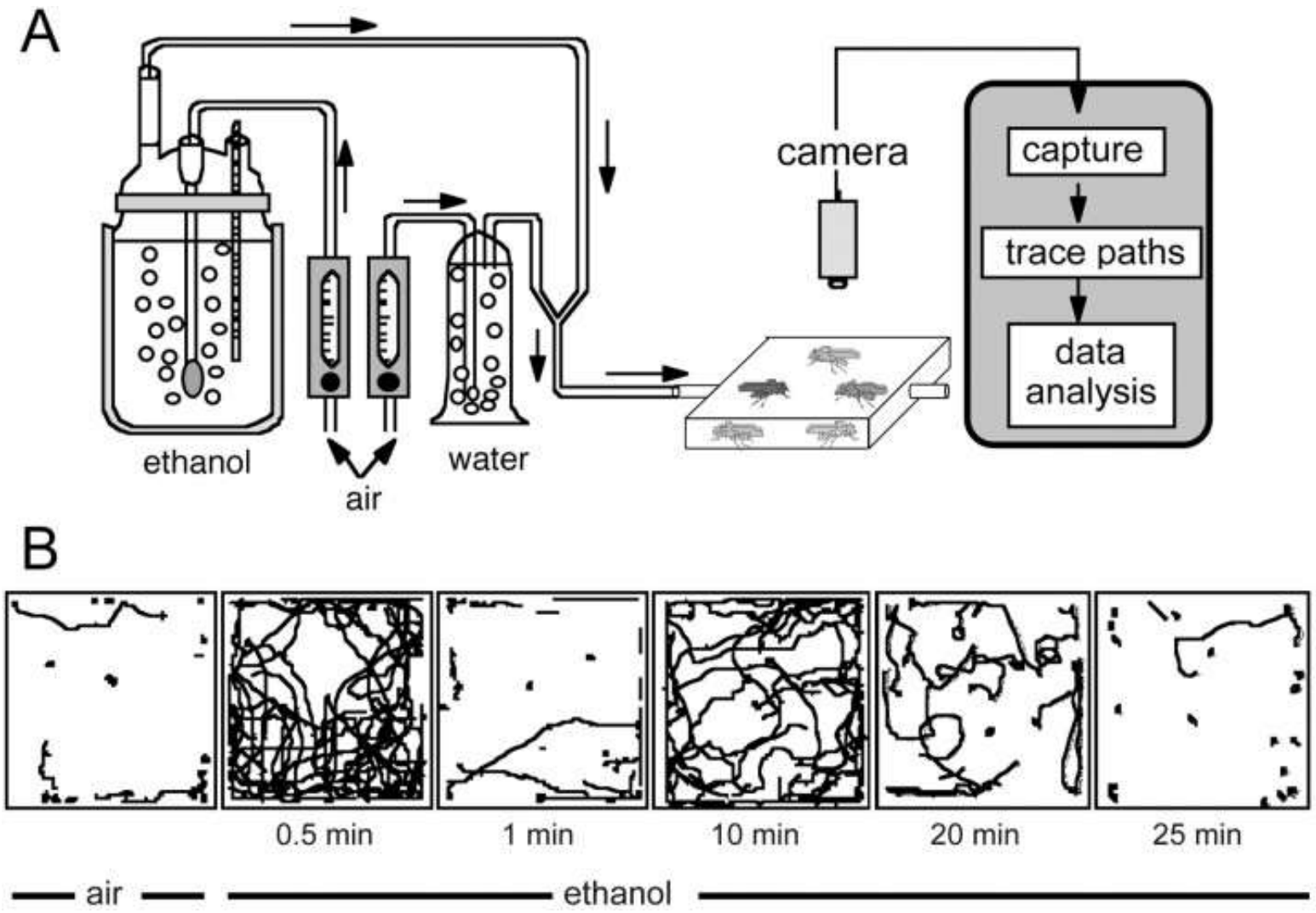
Chronic changes in behavior



- Escalated use
- Tolerance
- Compulsive seeking
- Dependence

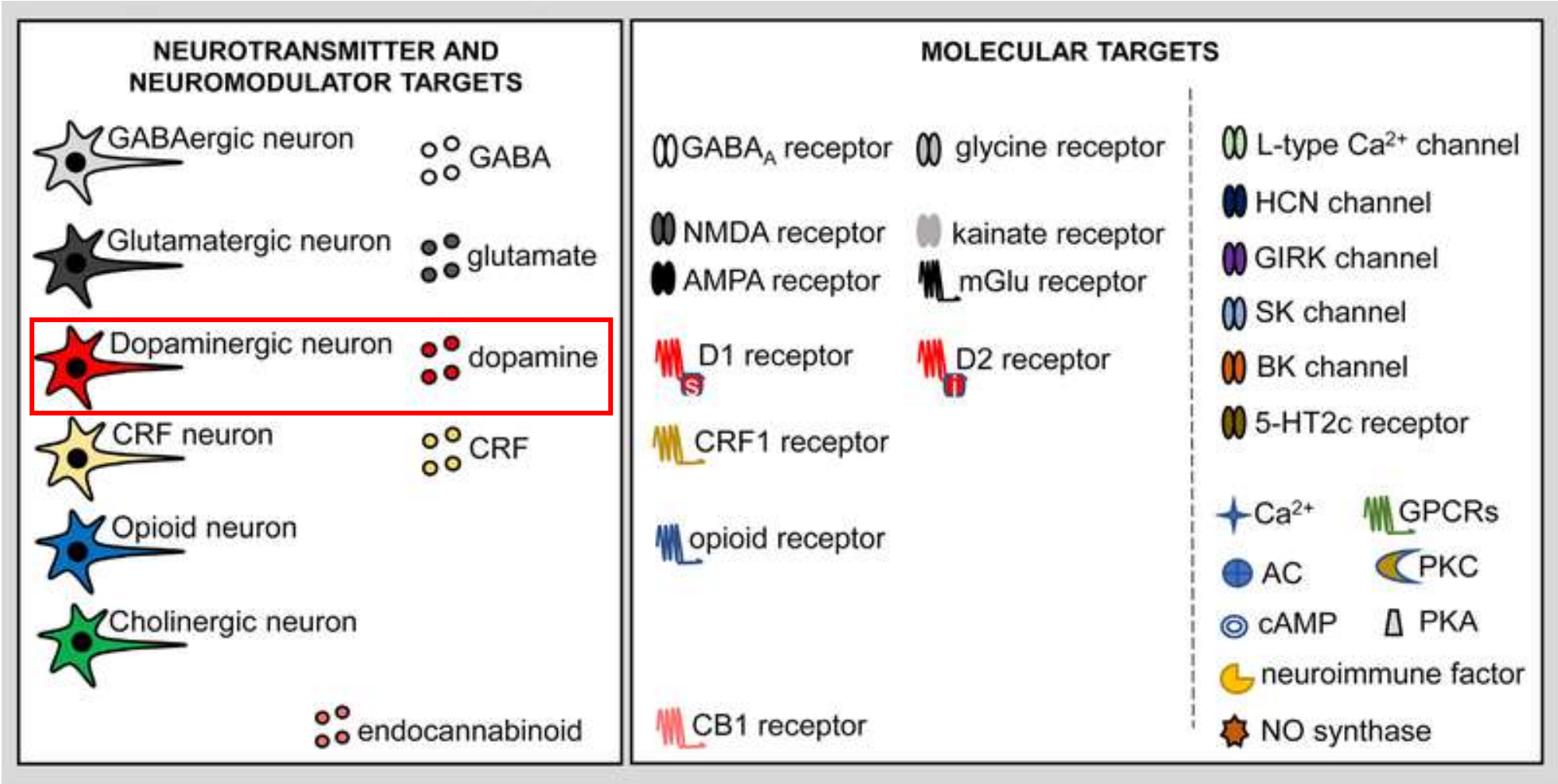
Karina P. Abrahao, Armando G. Salinas, and David M. Lovinger. 2017

Effect of ethanol on locomotion



Fred W. Wolf, et al. 2002

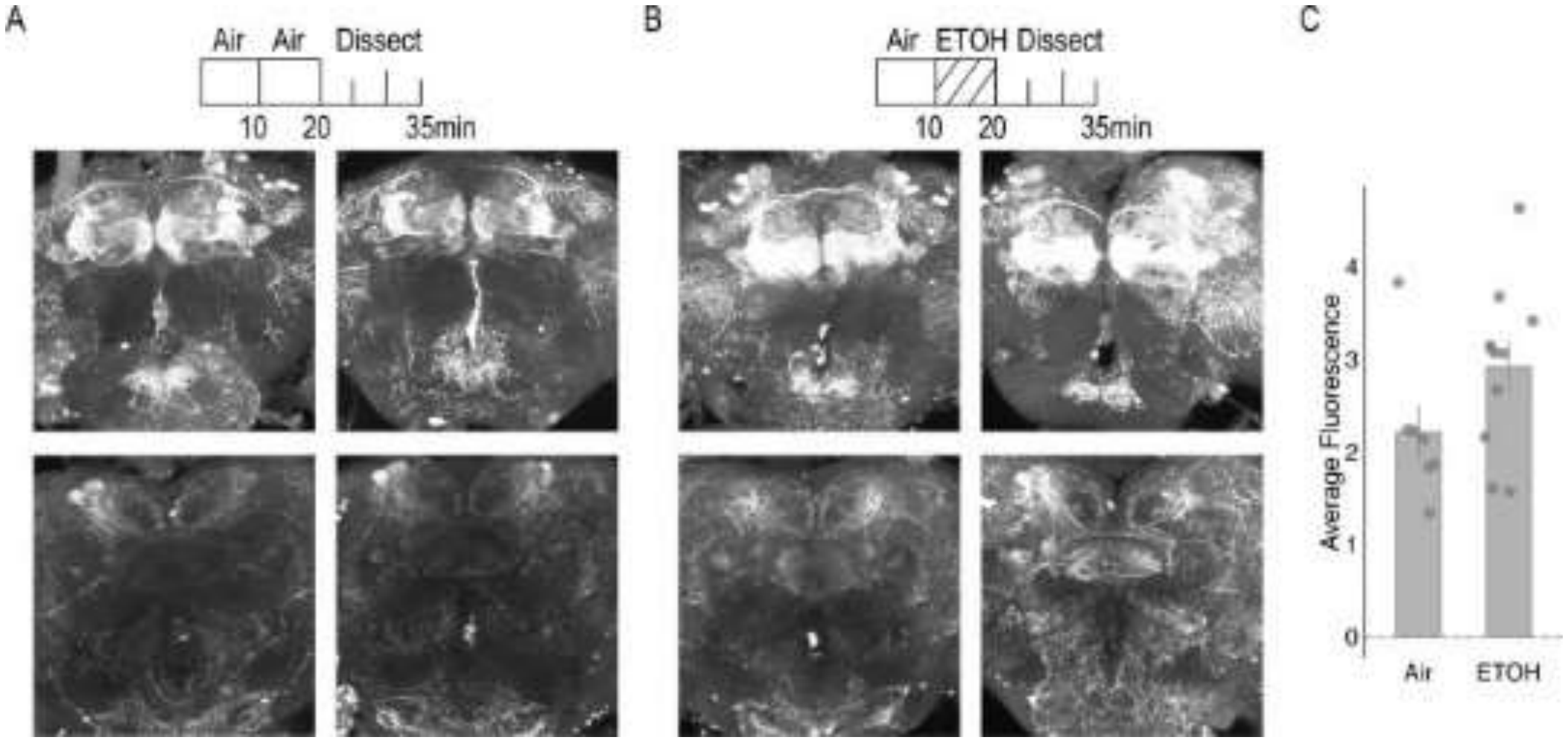
Neurotransmitter/Modulator systems and molecular targets of ethanol



Karina P. Abrahao, Armando G. Salinas, and David M. Lovinger. 2017

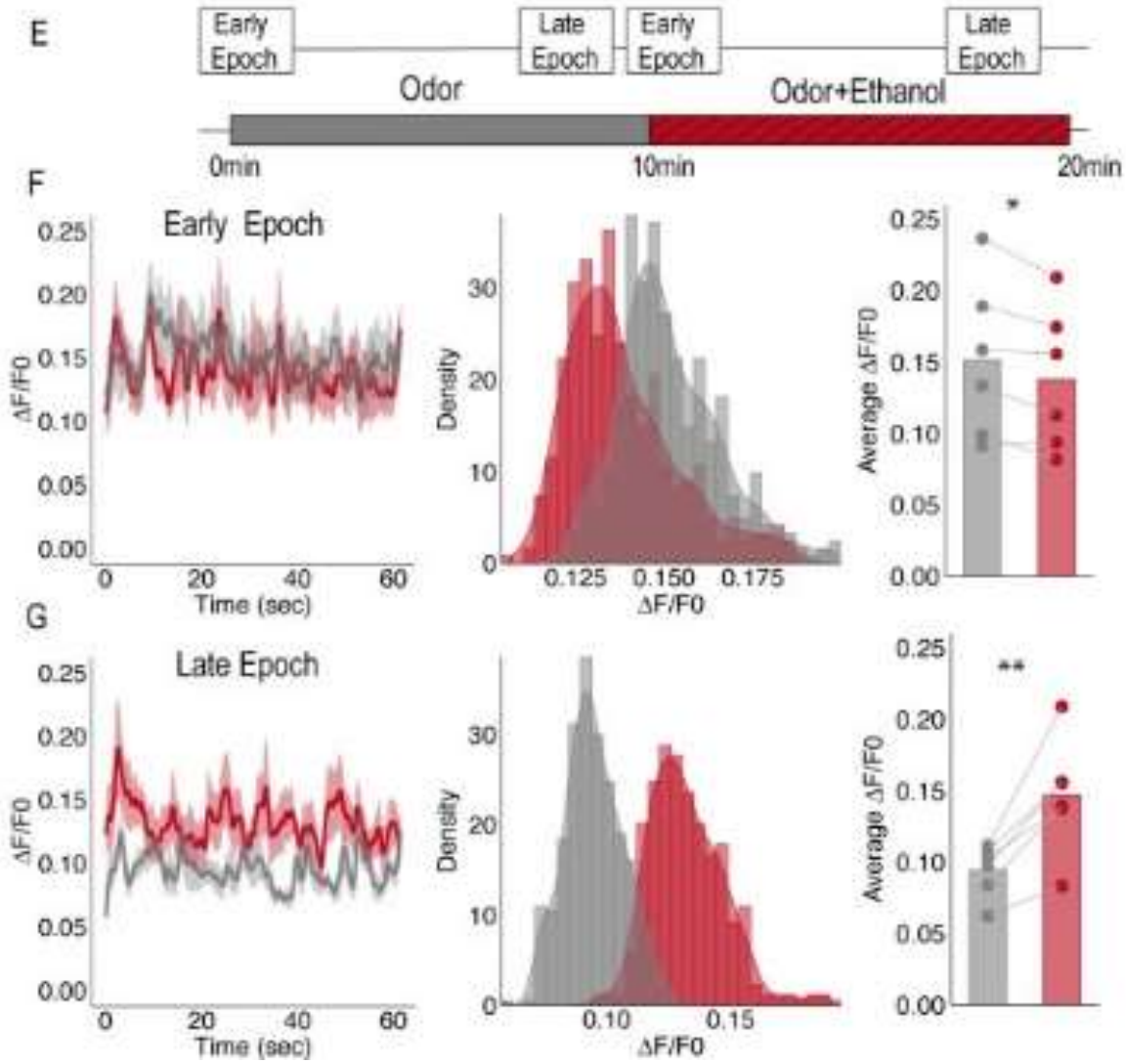
What is the effect of alcohol on dopamine?

Dopamine staining within the brain following 10 min of air or 10 min of ethanol



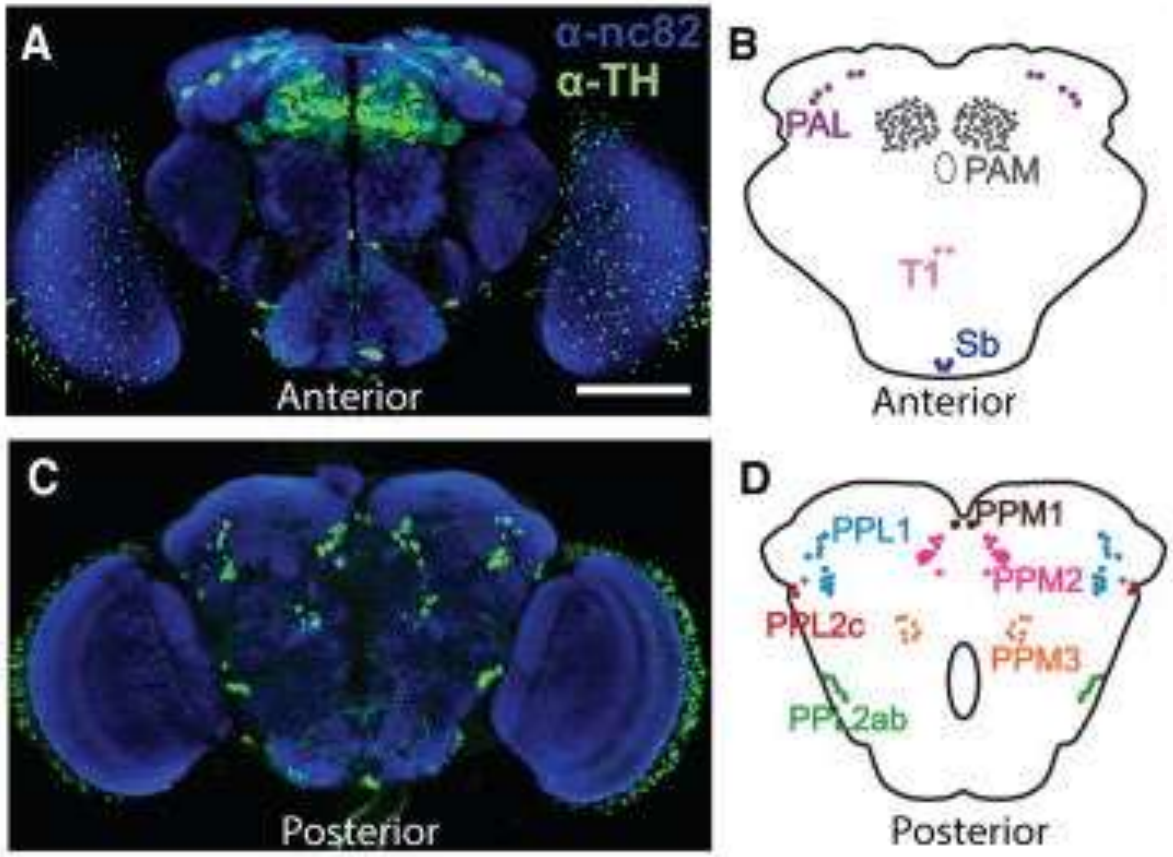
Kristin M Scaplen, et al. 2020

Effects of ethanol on PAM DANs



Kristin M Scaplen, et al. 2020

Drosophila behaviors associated with dopamine



Xie T, et al. 2018

Neuro-transmitter	*Drosophila* behaviors *Indicates behaviors impacted by alcohol	References
Dopamine	Aggression	Alekseyenko et al., 2013
	Associative learning*	Tully and Quinn, 1985; Riemensperger et al., 2005
	Aversive association*	Honjo and Furukubo-Tokunaga, 2009
	Circadian rhythms	Allada and Chung, 2010
	Locomotion*	Yellman et al., 1997; Pendleton et al., 2002; Kume et al., 2005; Kong et al., 2010; Strausfeld and Hirth, 2013
	Male courtship behavior*	Liu et al., 2008; Hoopfer et al., 2015; Zhang et al., 2019
	Memory removal	Berry et al., 2012
	Multisensory processing	Wolff and Rubin, 2018
	Olfactory learning and memory*	Cognigni et al., 2018
	Reward signaling*	Liu C. et al., 2012; Yamagata et al., 2015
	Salience-based decision making	Zhang et al., 2007
	Sleep and arousal*	Foltenyi et al., 2007; Van Swinderen and Andretic, 2011; Strausfeld and Hirth, 2013

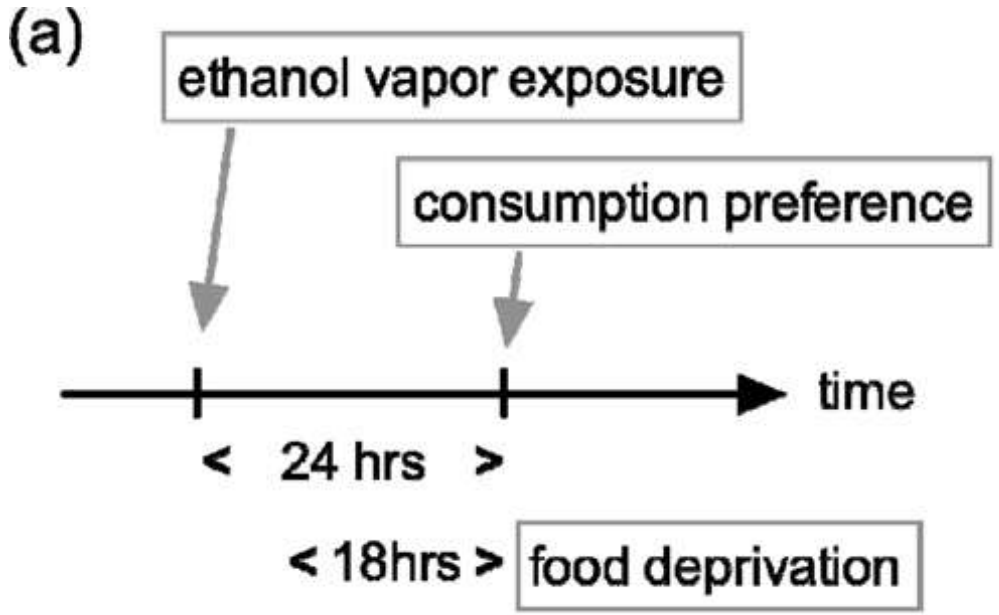
Maggie M. Chvilicek, Iris Titos and Adrian Rothenfluh. 2020

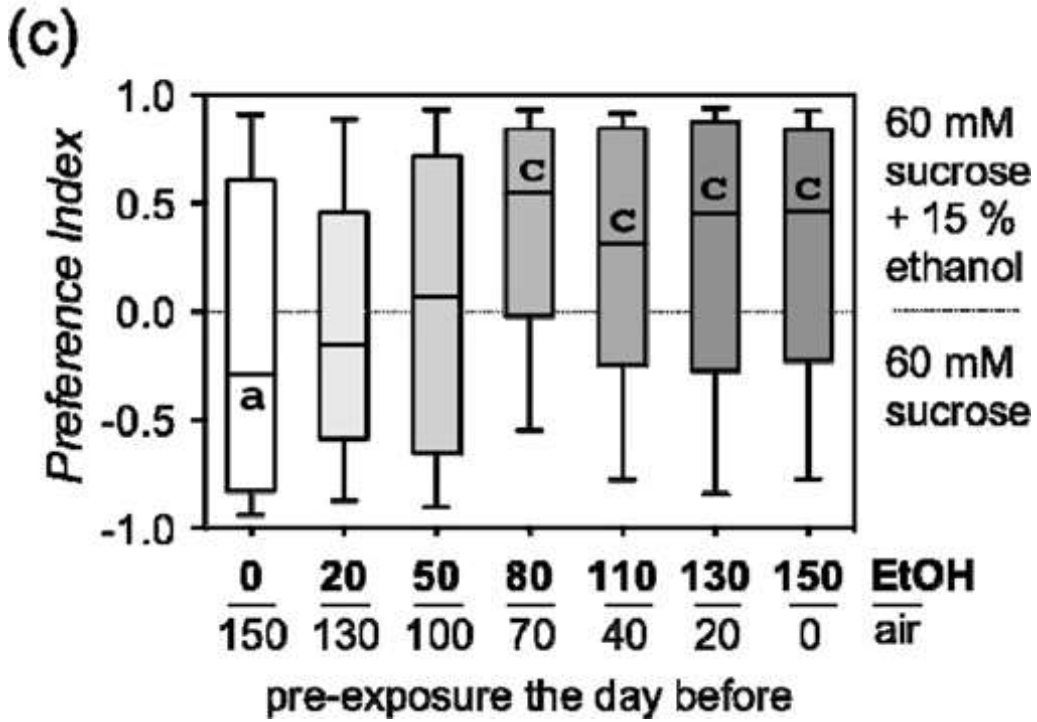
How does alcohol affect the body through dopamine?

Alcohol preference and avoidance

- Alcohol avoidance

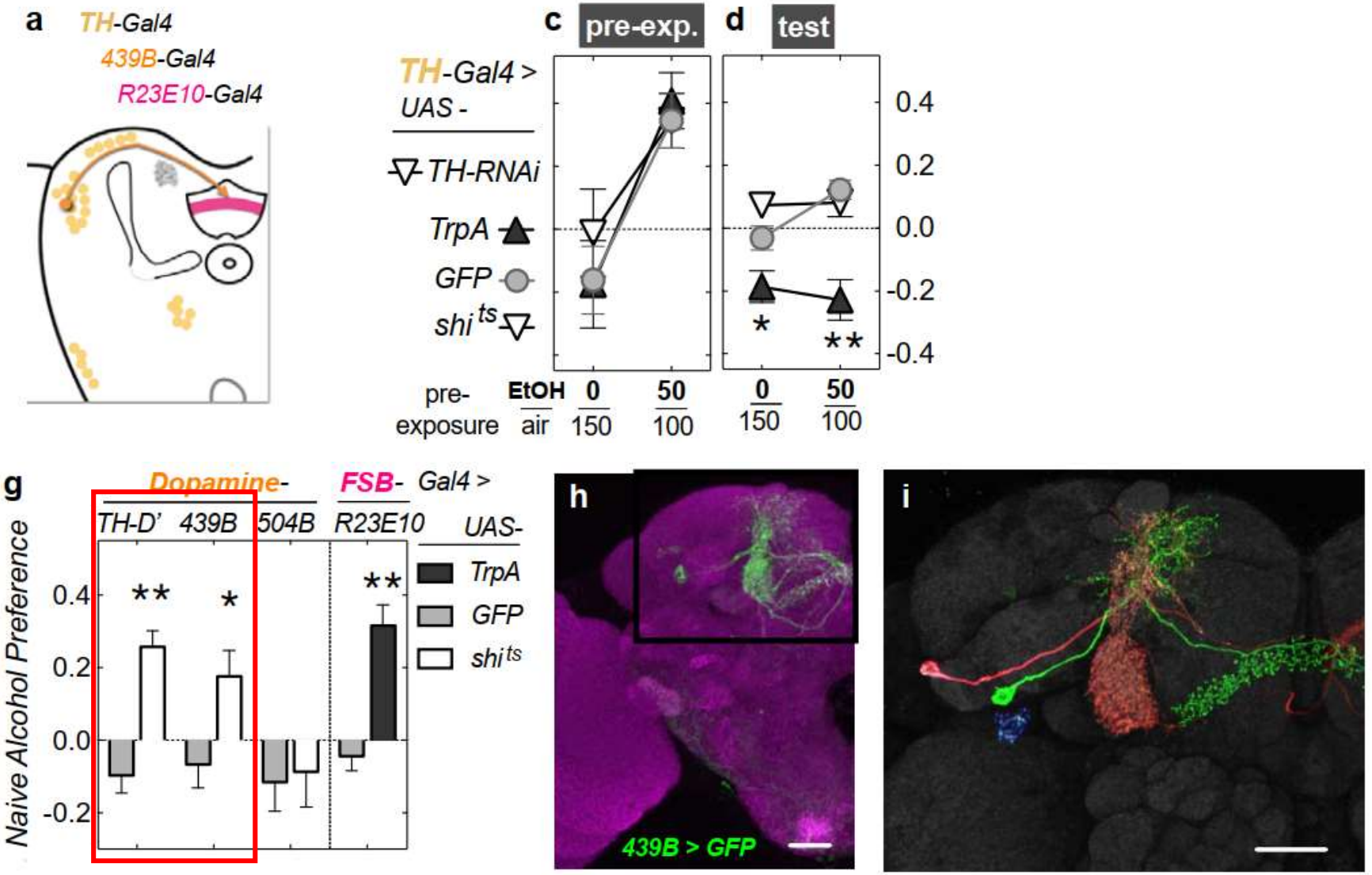
Flies show experience-dependent ethanol consumption preference





Raniero L. Peru y Colón de Portugal, et al. 2014

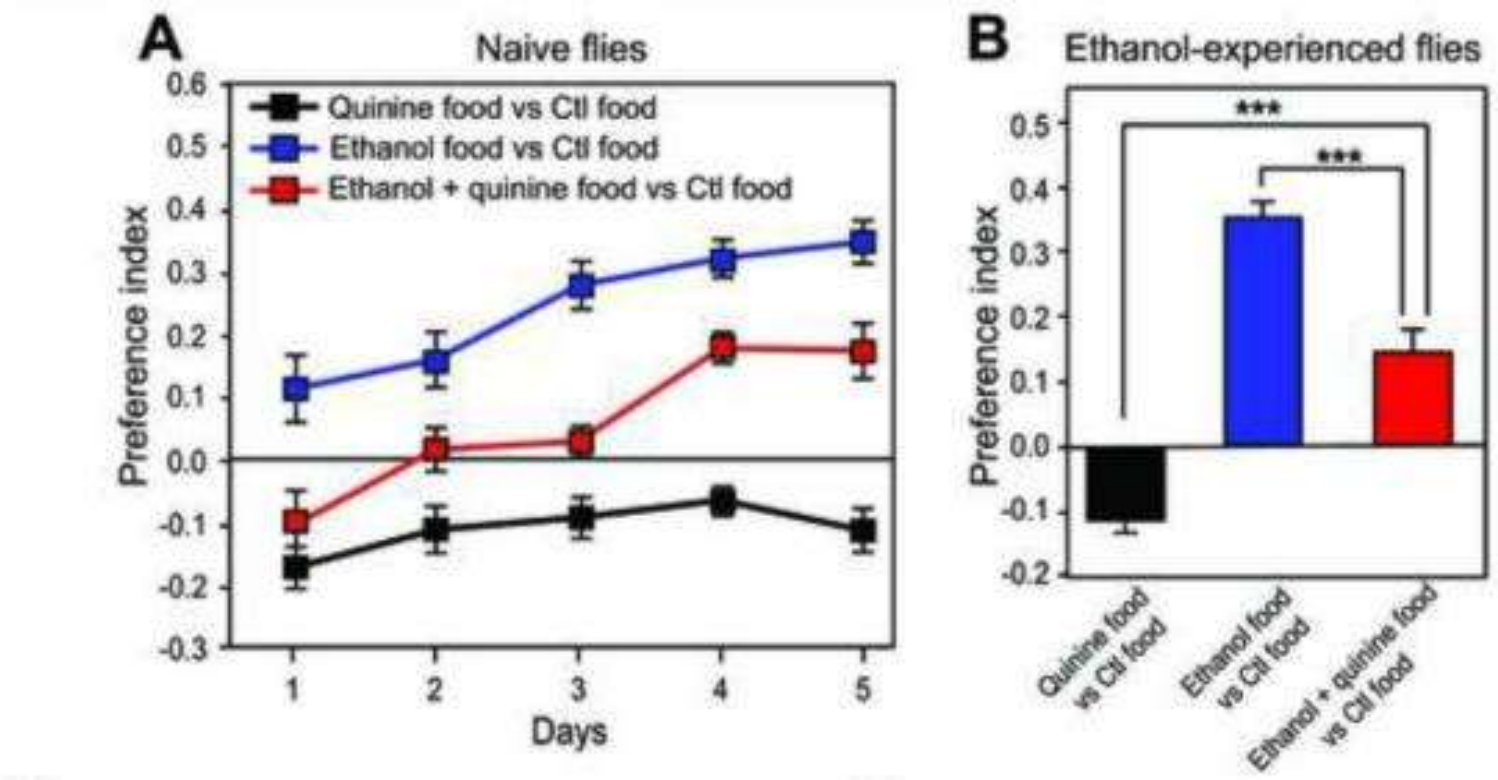
PPL1 dopamine neurons projecting to the fan-shaped body mediate acute naïve alcohol avoidance



Shamsideen A. Ojelade, et al. 2019

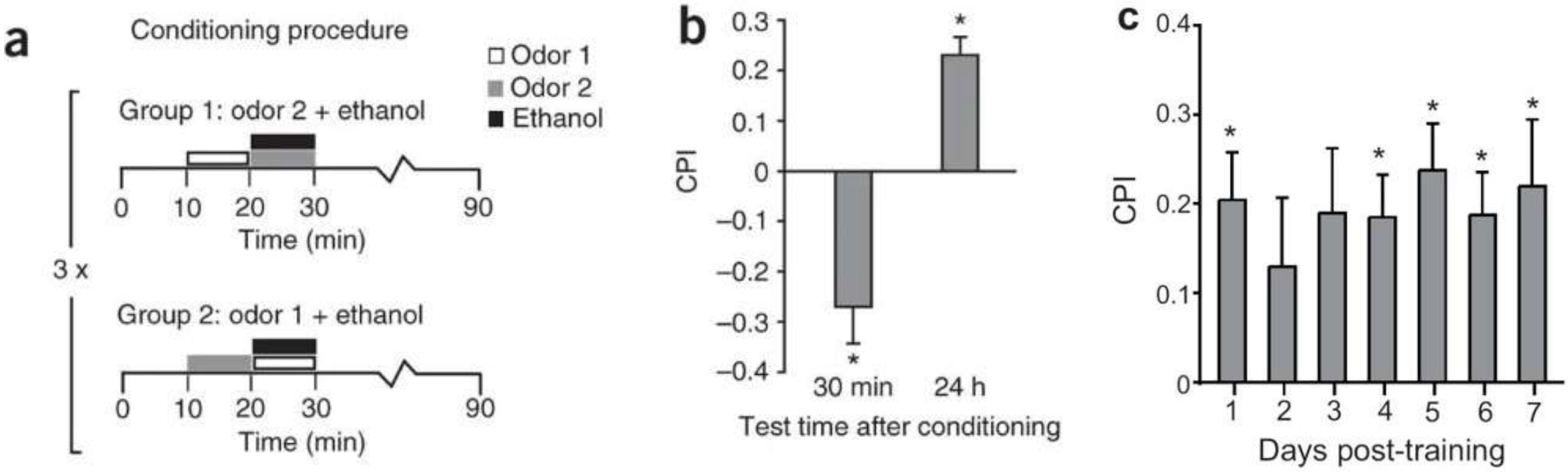
- Alcohol preference

Ethanol preference in flies exhibits features of addiction



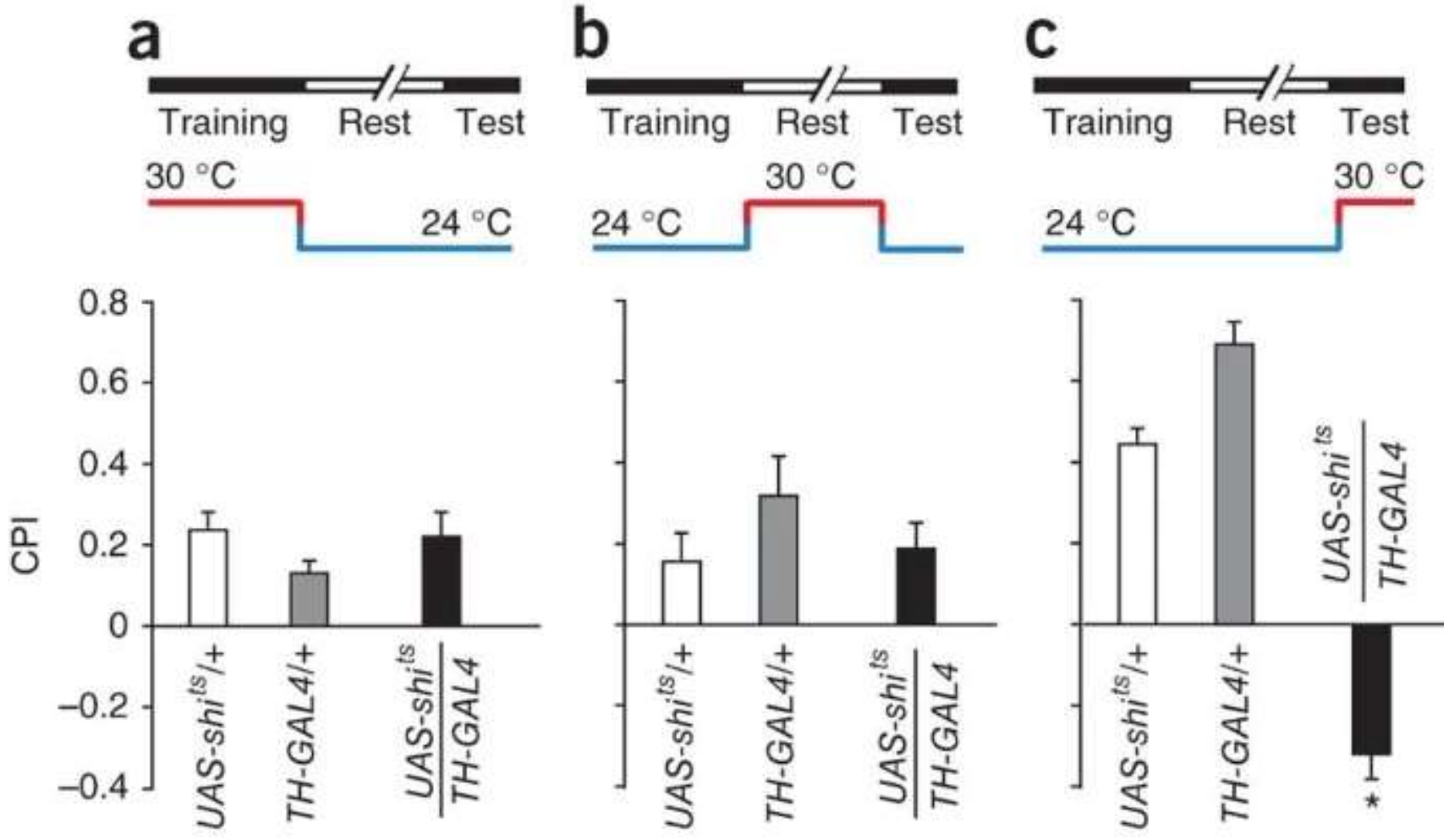
Anita V. Devineni and Ulrike Heberlein. 2009

Ethanol is both aversive and rewarding to flies



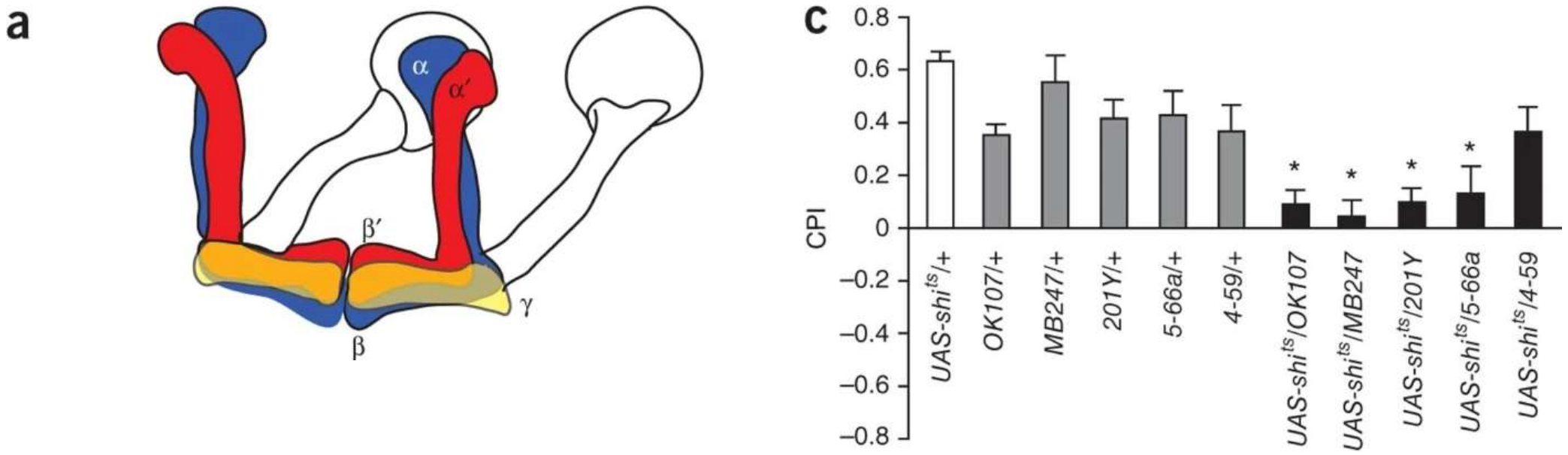
K.R. Kaun, et al. 2011

Dopamine is required for expression of ethanol reward



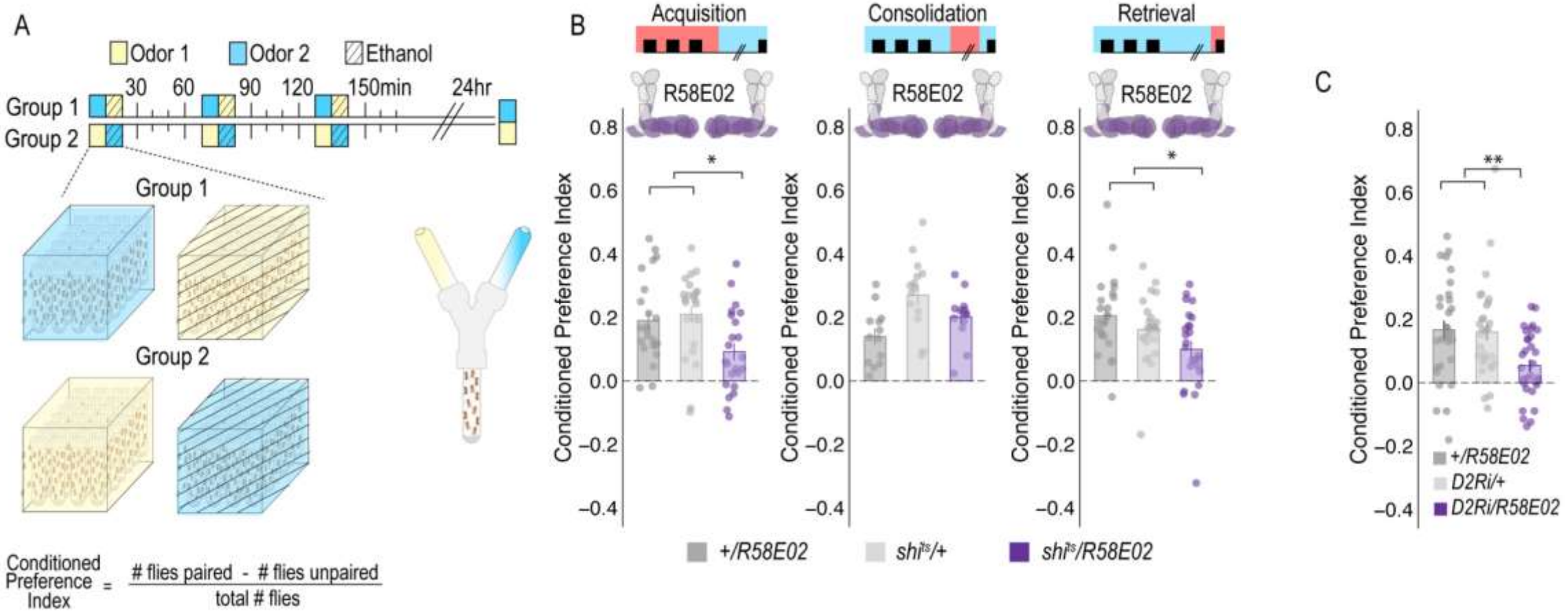
K.R. Kaun, et al. 2011

The mushroom body is required for ethanol reward memory



K.R. Kaun, et al. 2011

PAM DANs are necessary for encoding alcohol-associated preference



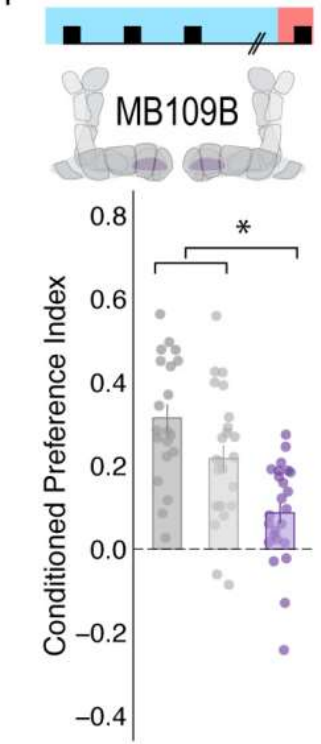
Kristin M Scaplen, et al. 2020

Memory expression during retrieval is dependent on a sparse population of DANs

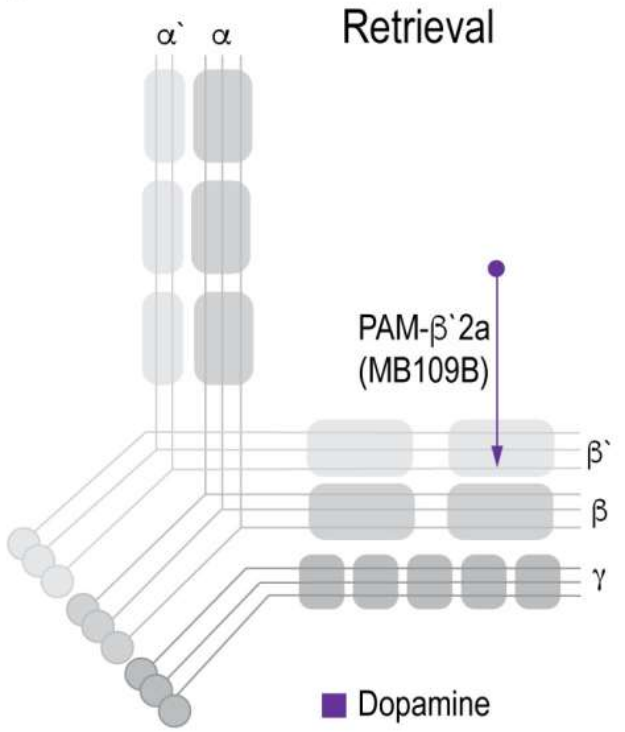
I

Line	Expression Pattern
MB040B	PAM-γ5, PAM-β2, PAM-β`2m, PAM-β`2p, PAM-γ4, PAM-γ3, PAM-α1, PAM-β`1ap, PAM-β`1m
MB042B	PAM-β`2m, PAM-γ3, PAM-γ4, PAM-γ5
MB188B	PAM-β`1ap, PAM-β`1m, PAM-γ3, PAM-γ4
MB032B	PAM-β`2m
MB301B	PAM-β2β`2a
MB109B	PAM-β`2a
MB315C	PAM-γ5
MB299B	PAM-α1

F

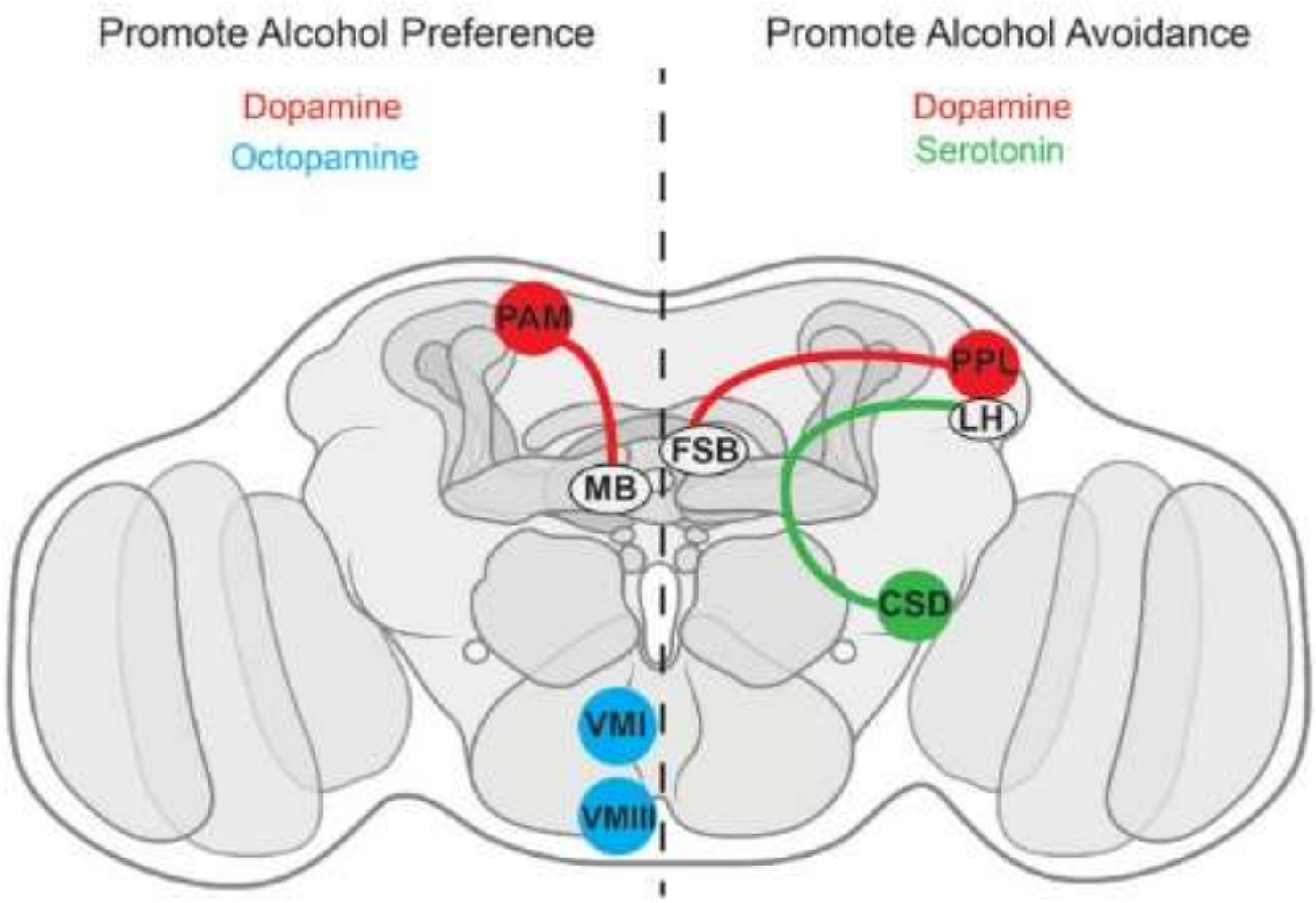


J



Kristin M Scaplen, et al. 2020

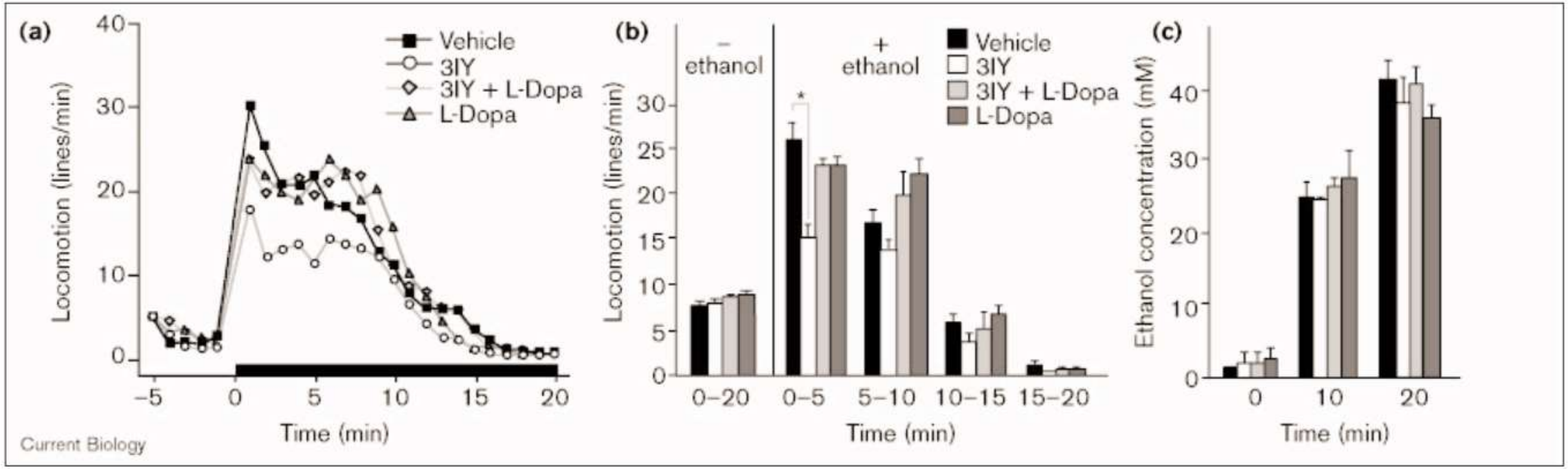
Known circuitry for neurotransmitters mediating alcohol preference and avoidance



Maggie M. Chvilicek, Iris Titos and Adrian Rothenfluh. 2020

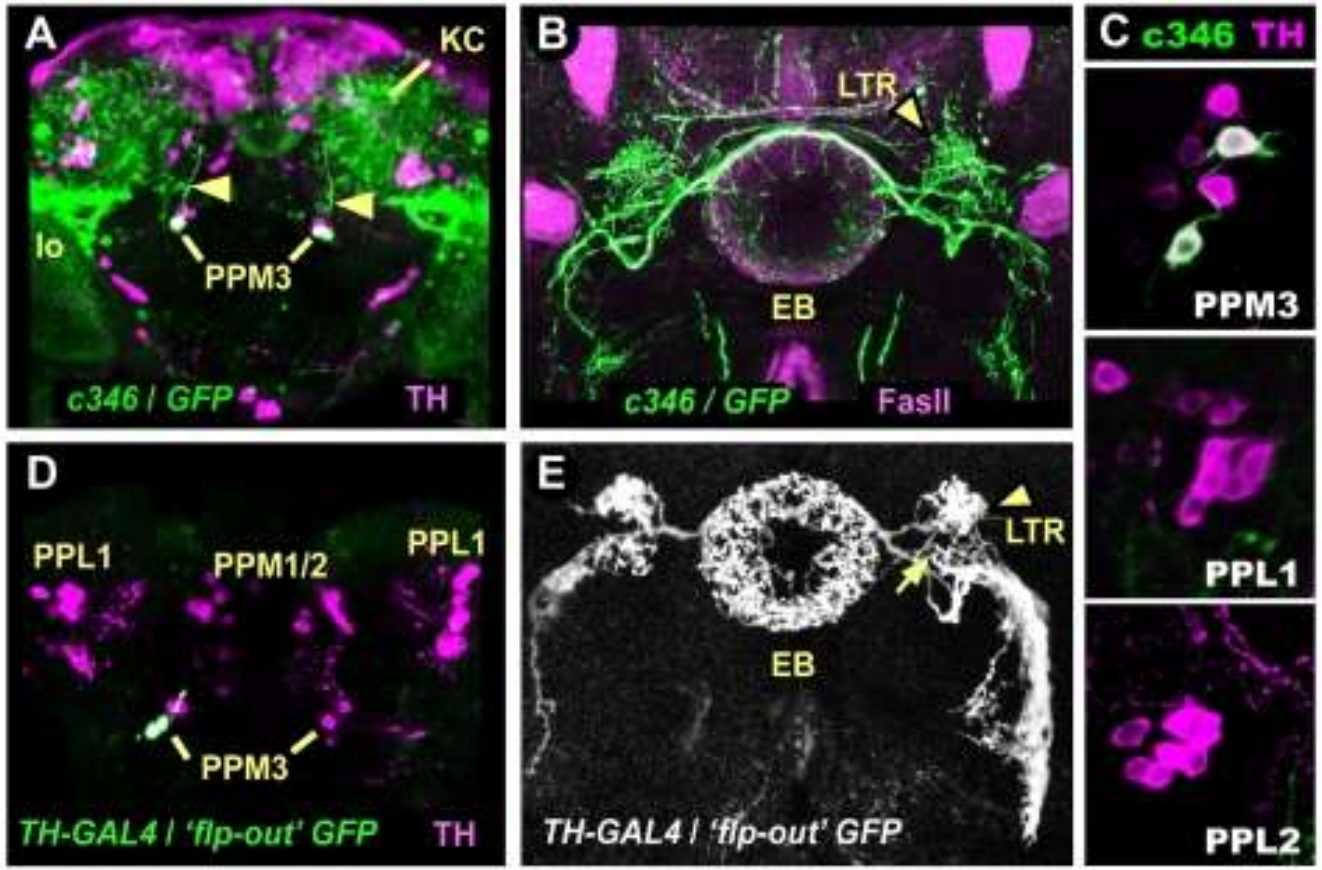
Locomotion and Sedation

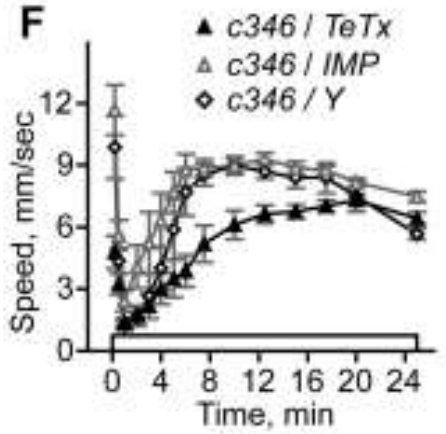
Pharmacological reduction of dopamine levels decreases sensitivity to ethanol-induced hyperactivity

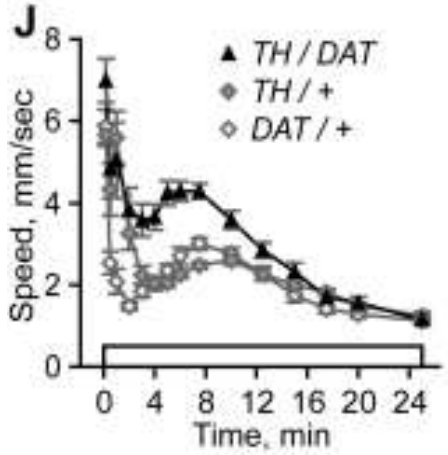


Roland J. Bainton, et al. 2000

TH-positive PPM3 neurons that project to the ellipsoid body promote ethanol-induced hyperactivity

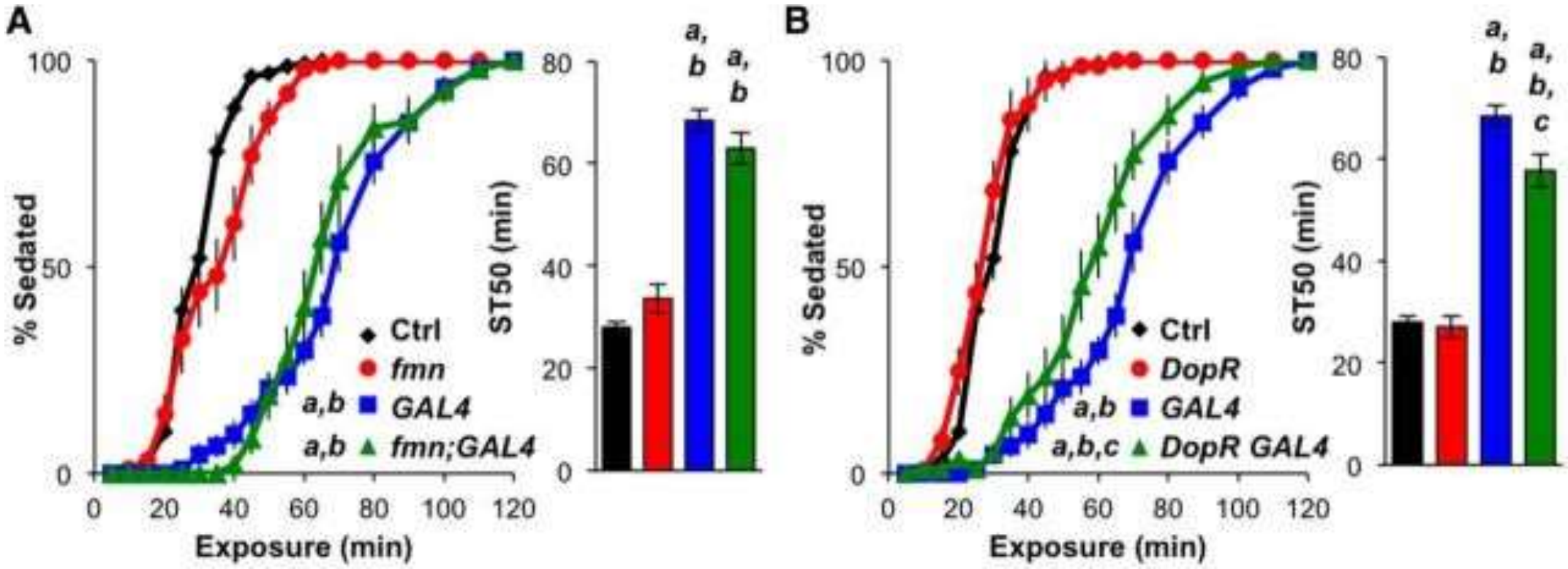






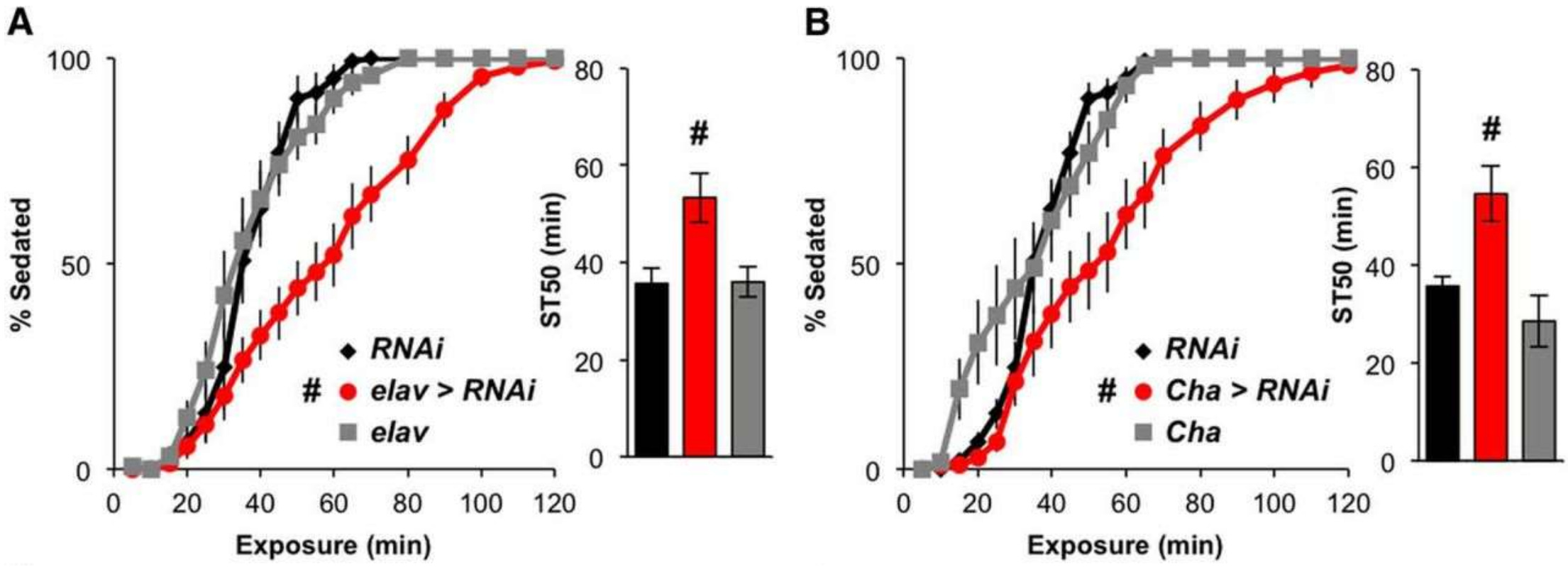
Eric C. Kong, et al. 2010

Ethanol-induced sedation is normal in dopaminergic mutants DAT^{fmn} and $DopR^{PL00420}$



Emily Petruccelli, et al. 2016

Knockdown of DopEcR in neurons enhances resistance to ethanol-induced sedation



Emily Petruccelli, et al. 2016

Conclusions

- Ethanol acts on numerous molecular targets in neurons and synapses throughout the brain.
- The effects of alcohol in fruit flies are similar to mammals.
- Alcohol preference is experience-dependent in Drosophila.
- PPL1 DAN are required for alcohol avoidance, and need PAM DAN to regular alcohol preference.
- PPM3 neurons that project to the ellipsoid body promote ethanol-induced hyperactivity.

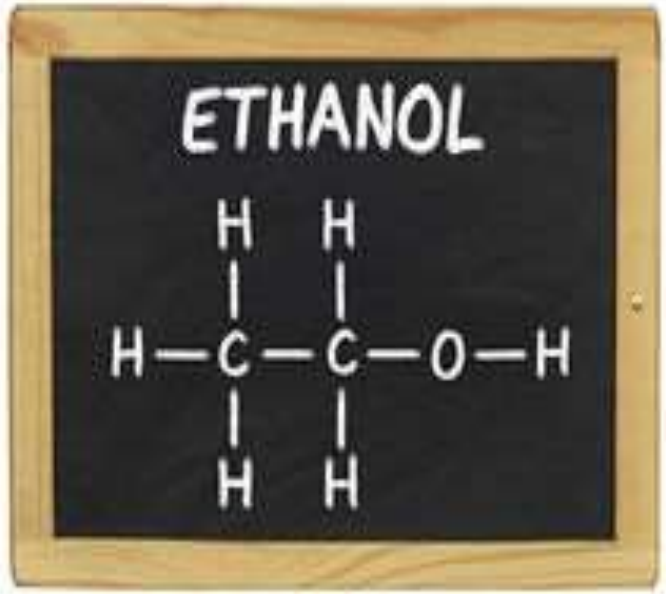
谢谢!

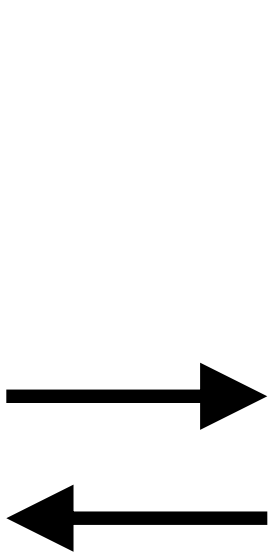
PART3

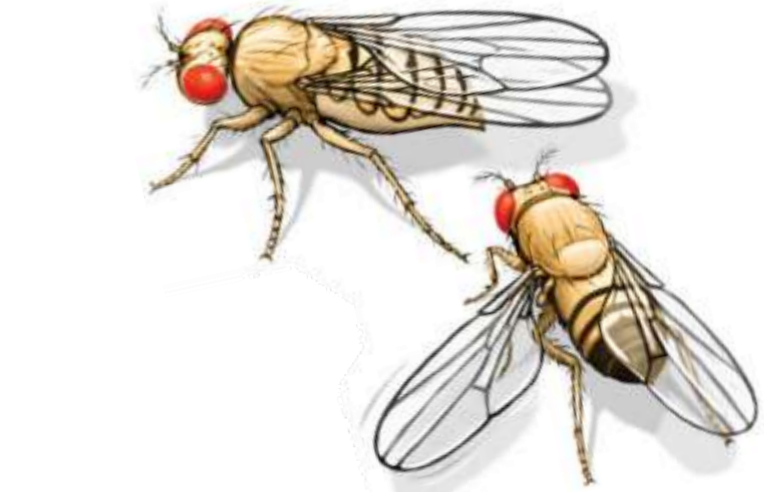
Alcohol and social behaviors

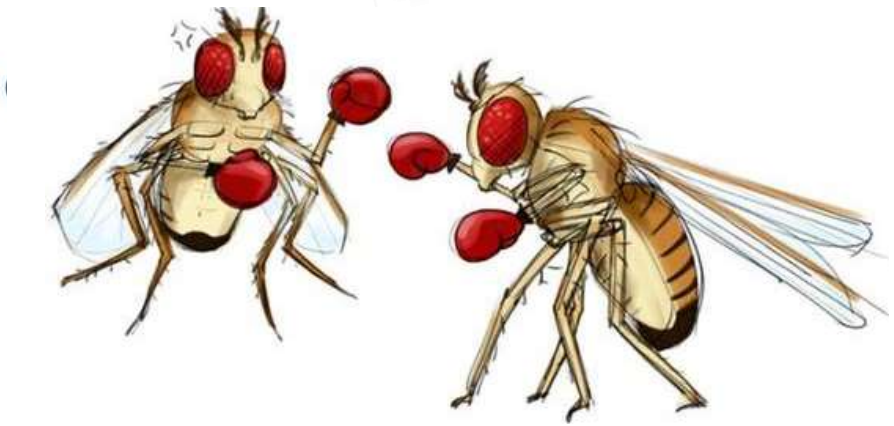
——JXX

Alcohol and social behaviors



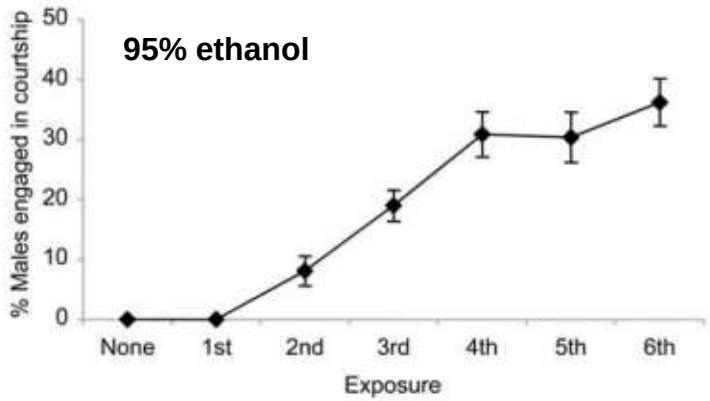


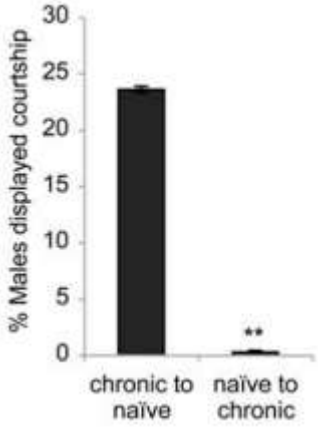




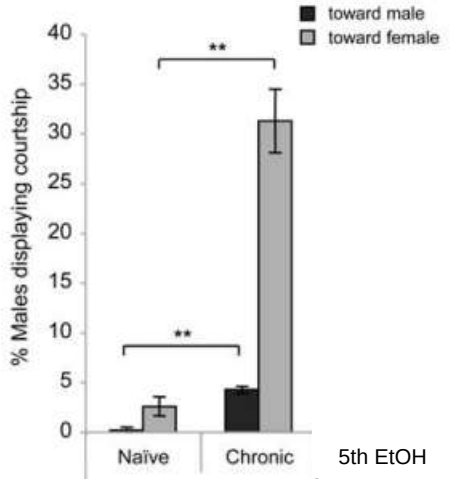
Recurring ethanol exposure induces disinhibited courtship

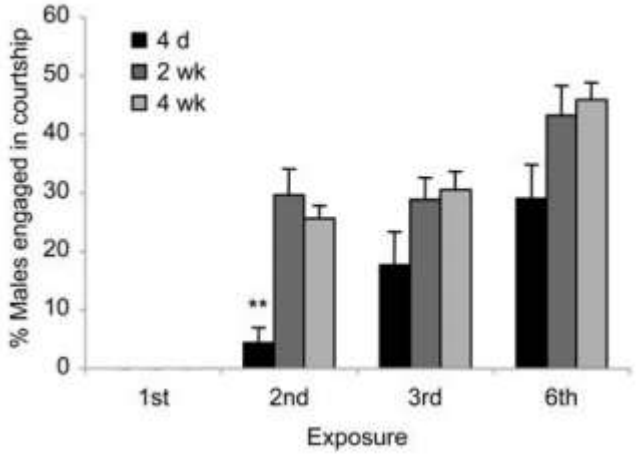
male-male

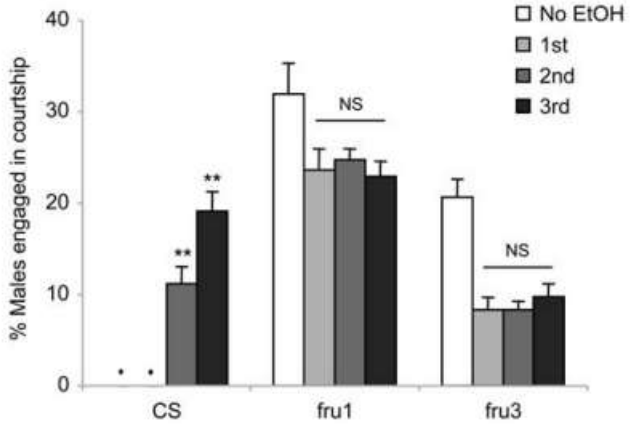


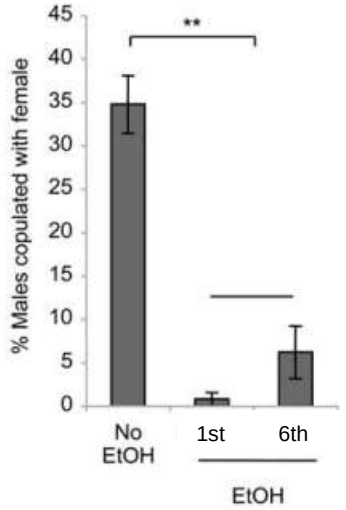


male-female



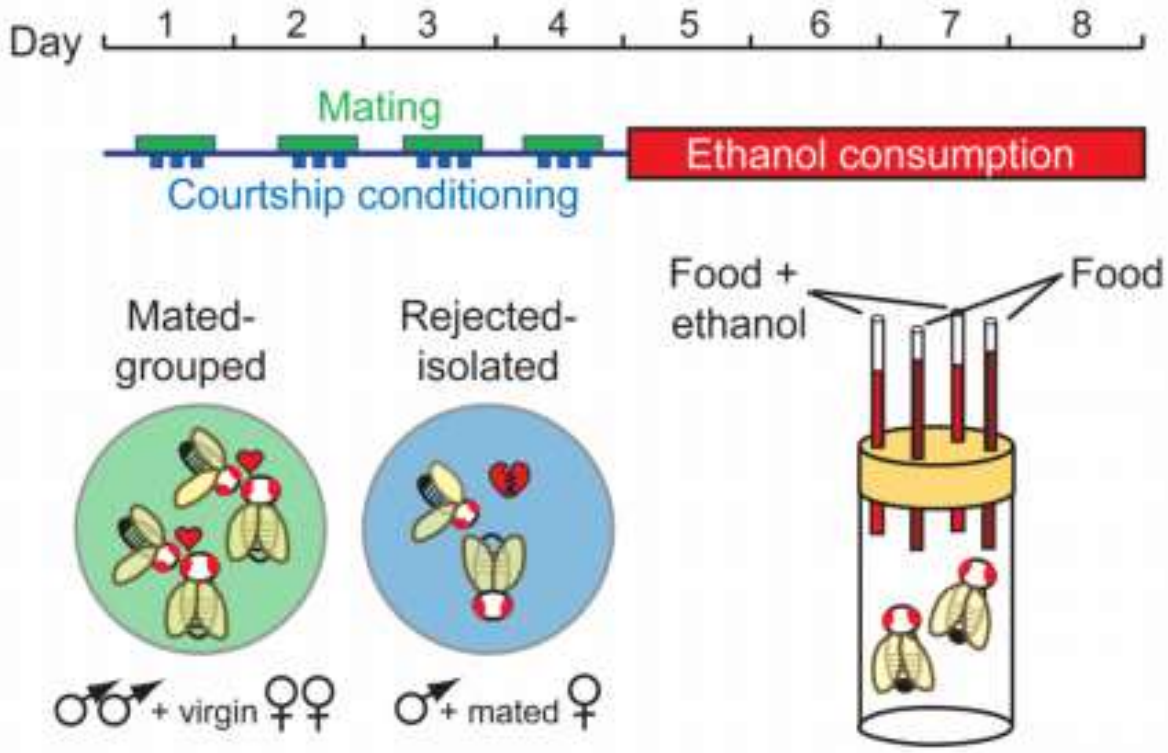


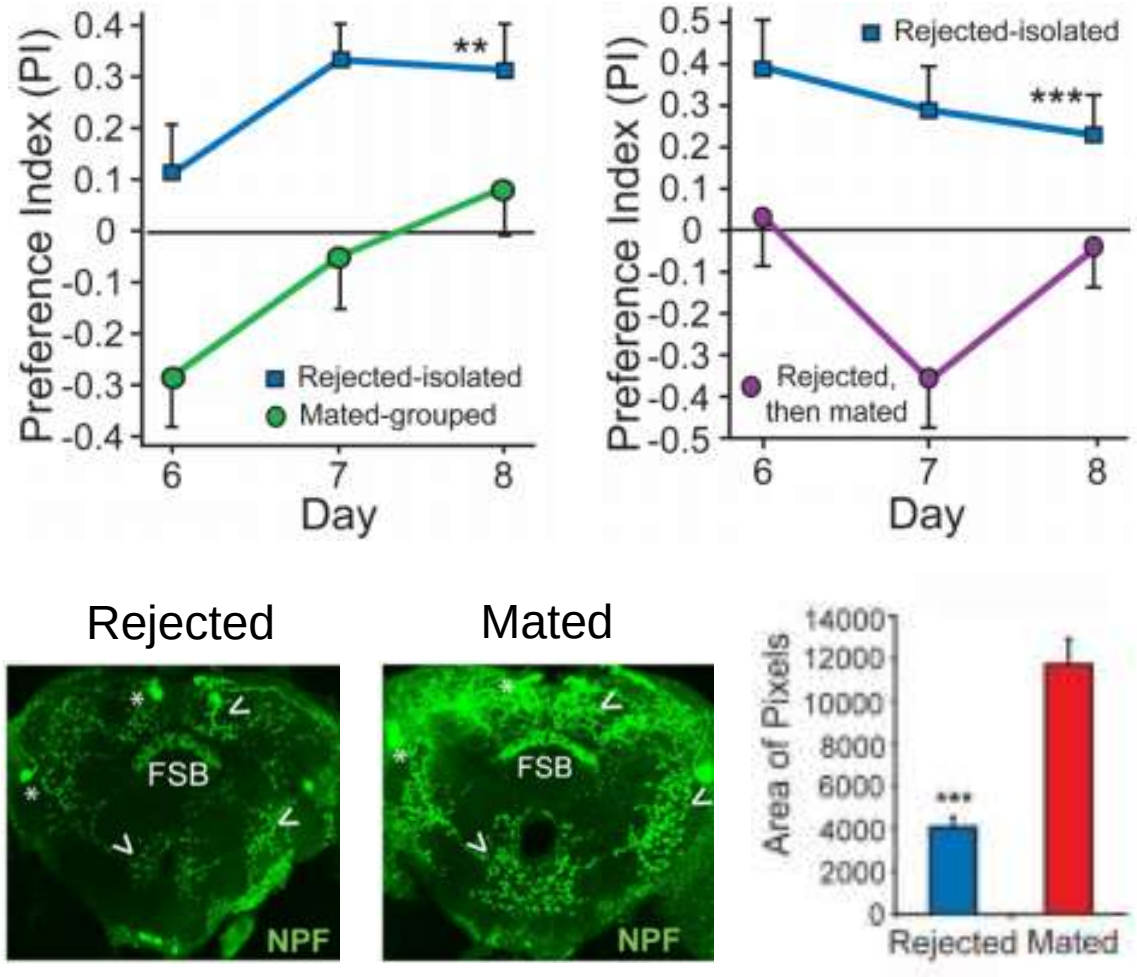




(Lee et al., PLoS One, 2008)

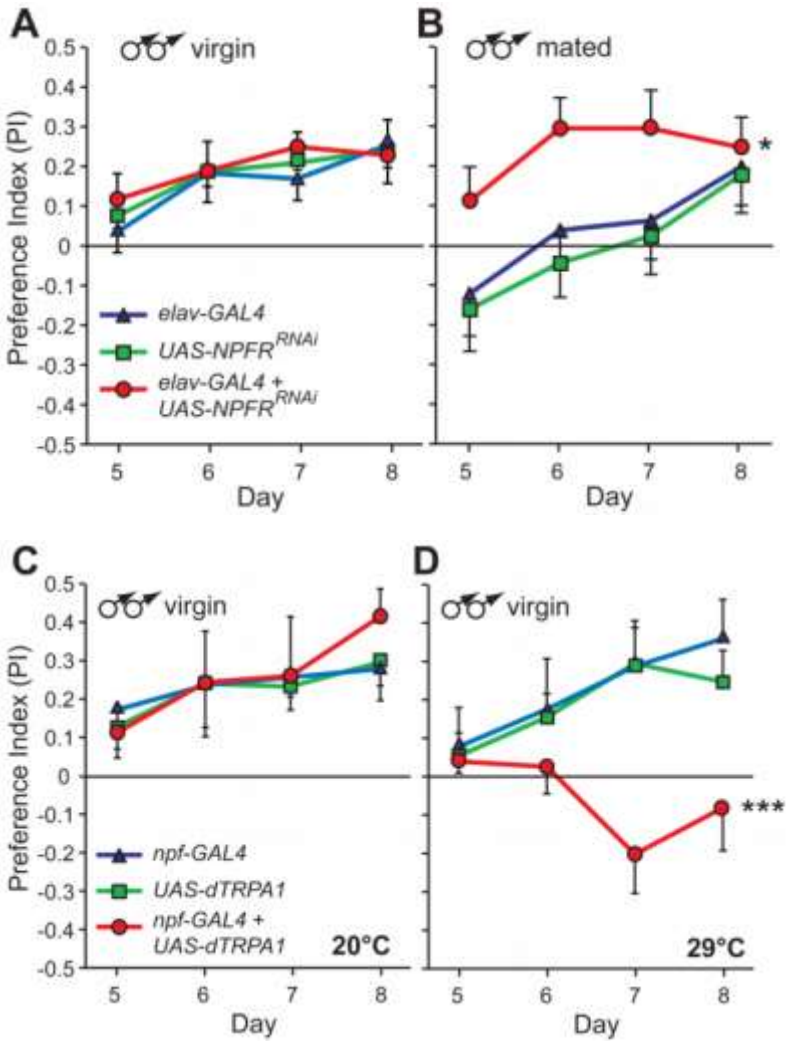
Sexual deprivation increases ethanol intake through Neuropeptide F

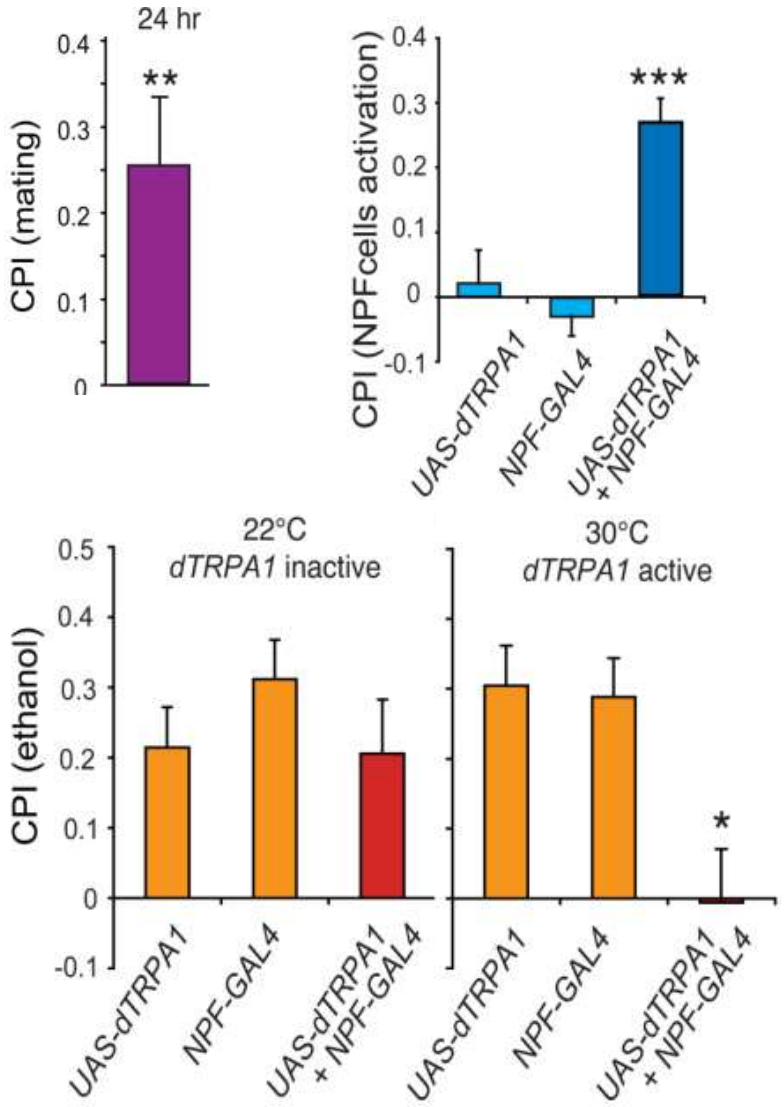




(Shohat-Ophir et al., Science, 2012)

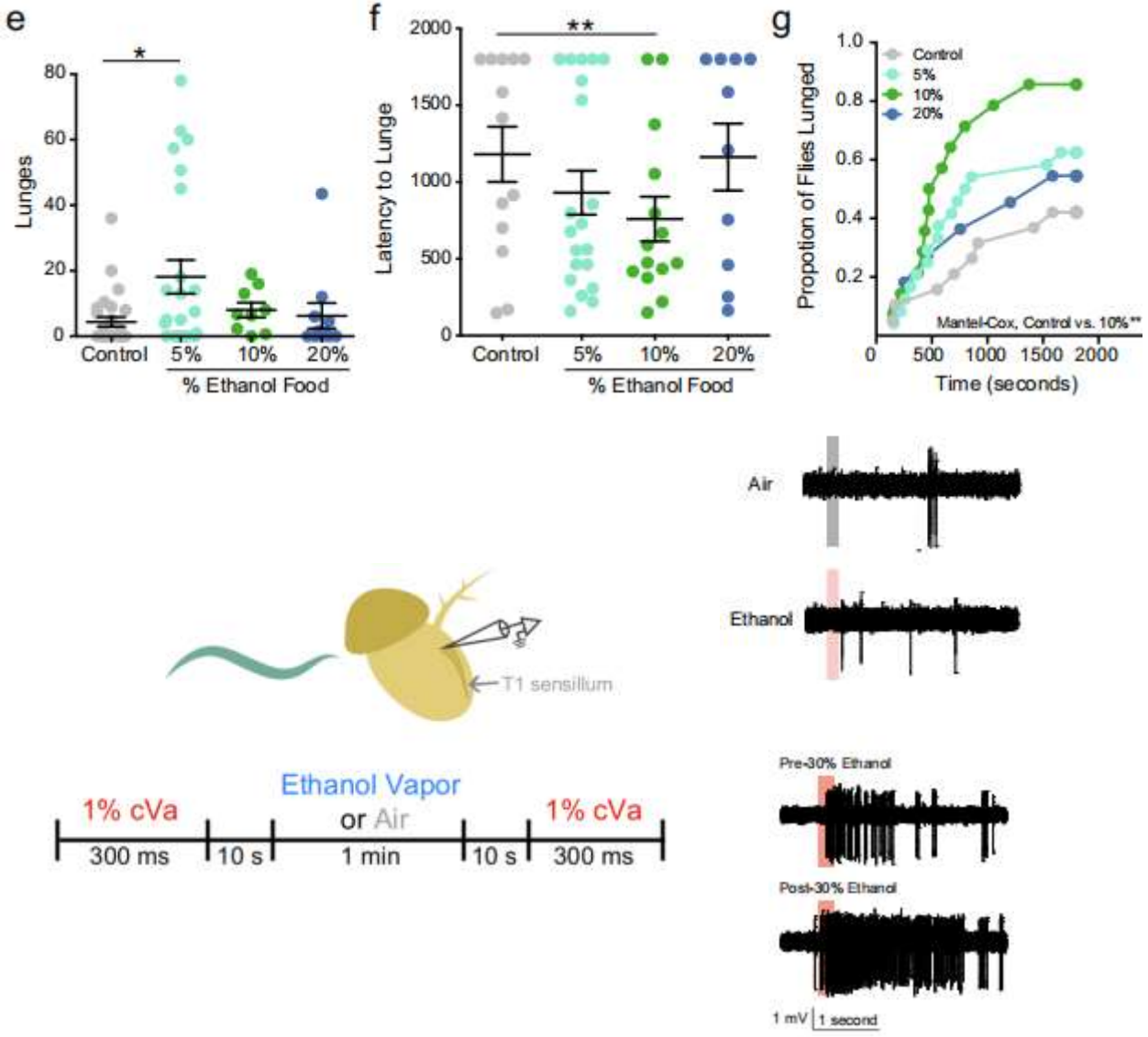
Sexual deprivation increases ethanol intake through Neuropeptide F

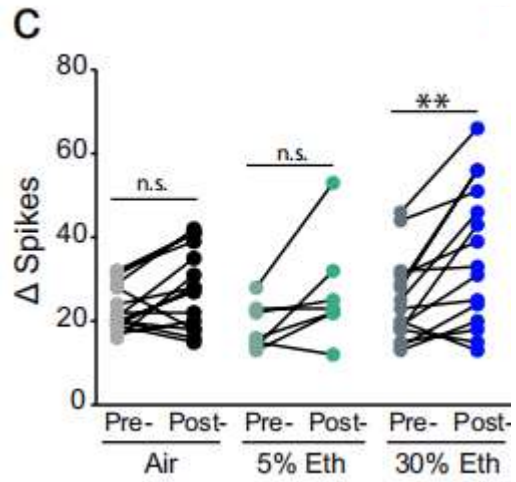


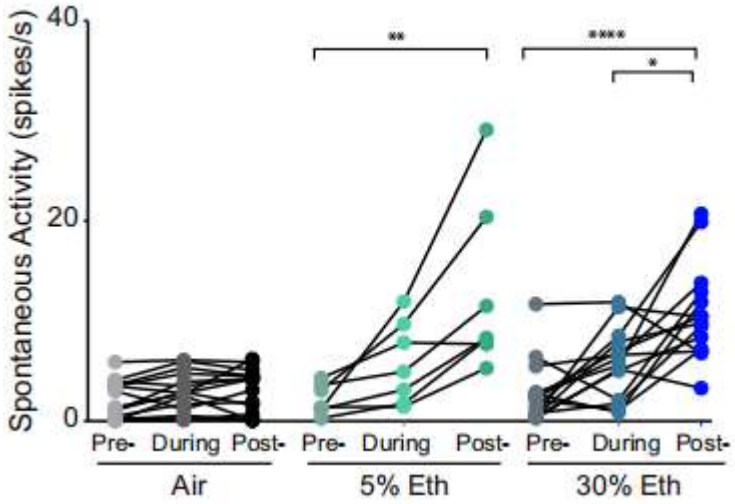


(Shohat-Ophir et al., Science, 2012)

Alcohol potentiates the activity of sensory neurons in response to cVa

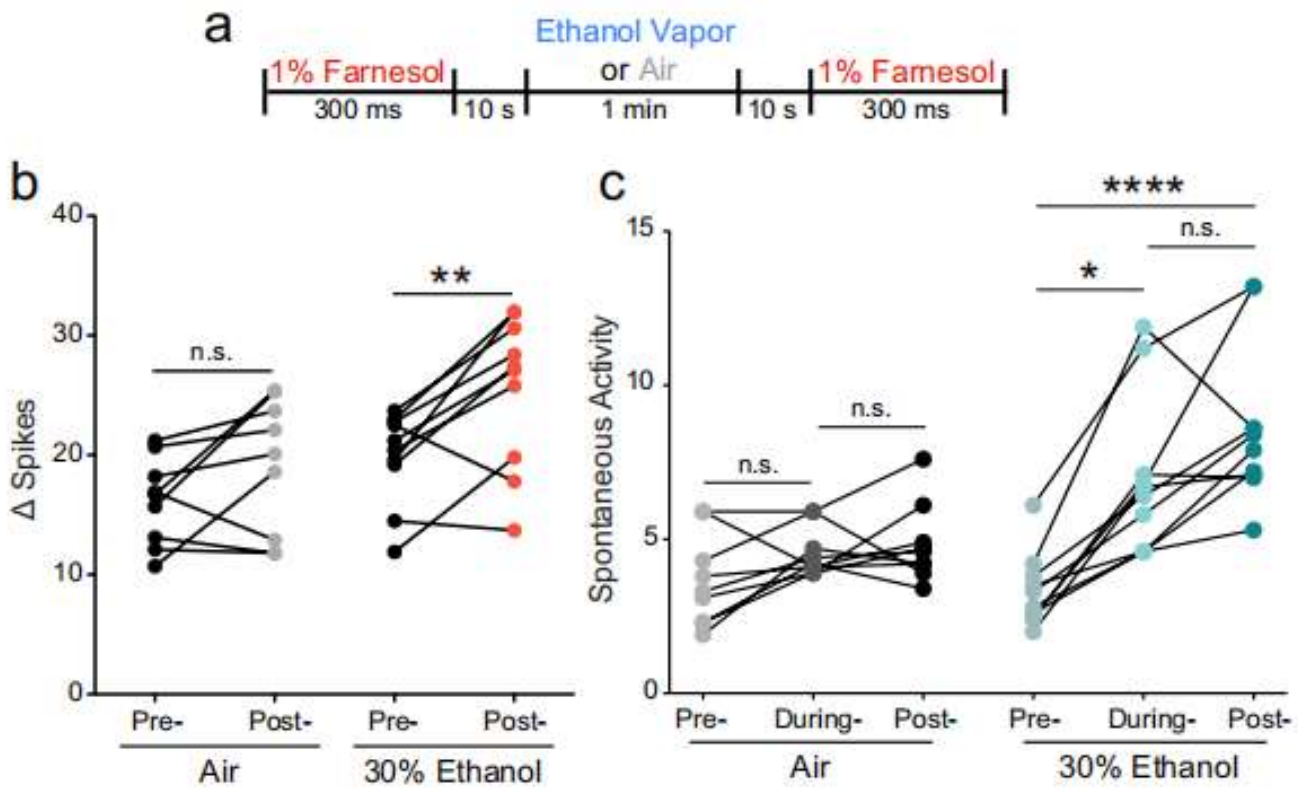


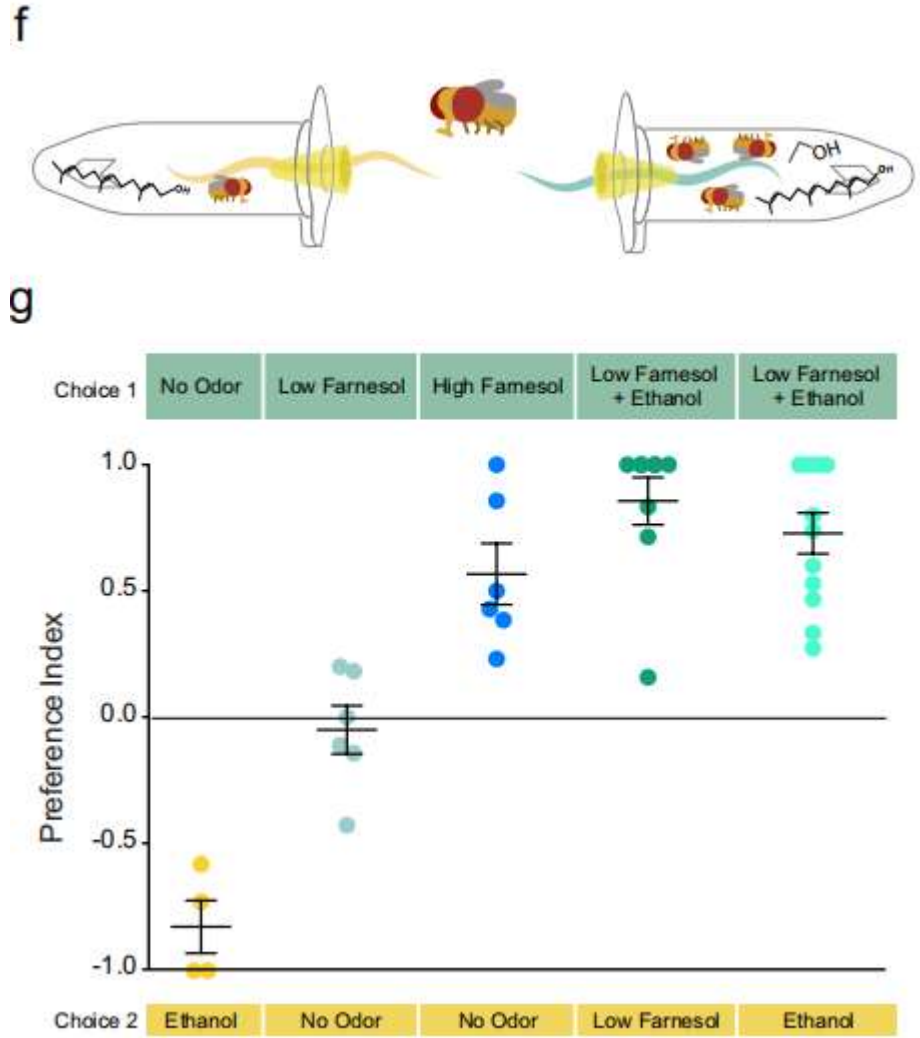




(Park et al., eLife, 2020)

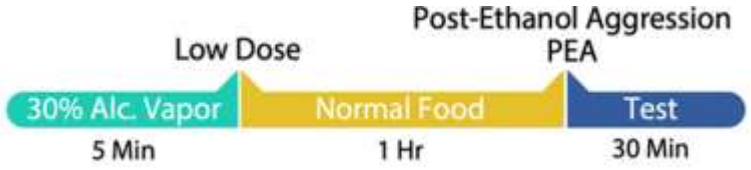
Alcohol potentiates the activity of sensory neurons in response to Farnesol

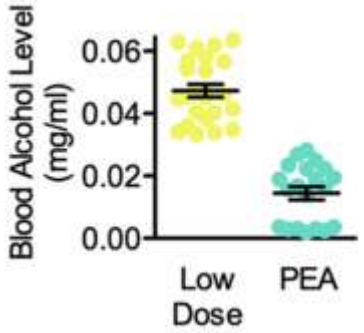


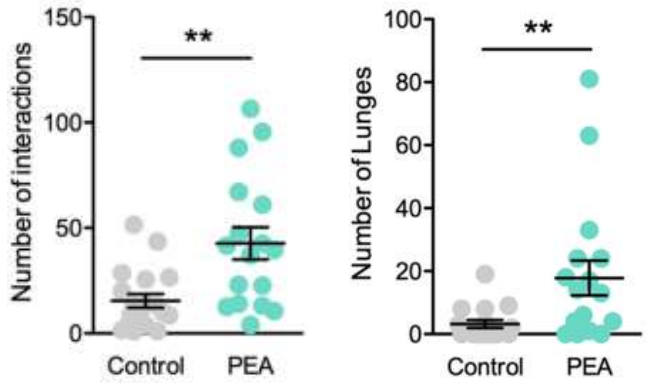


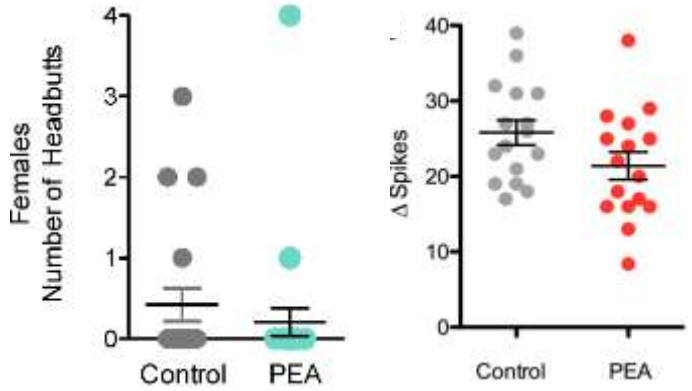
(Park et al., eLife, 2020)

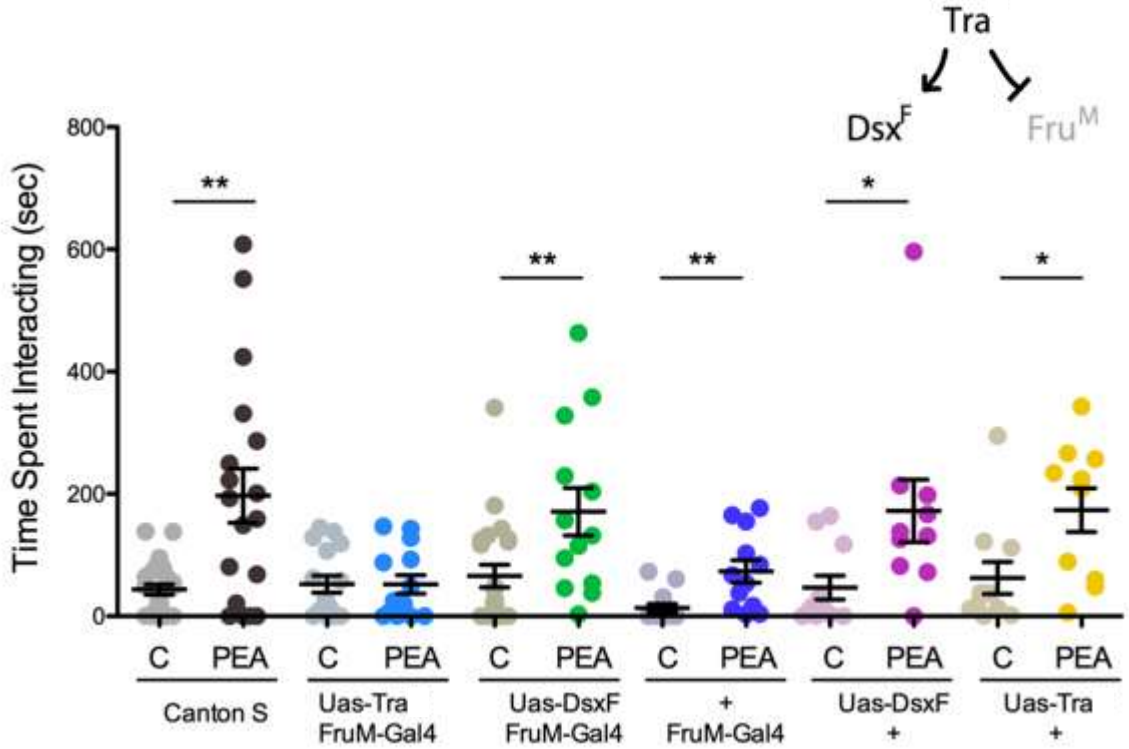
Ethanol treatment affects post-ethanol aggression through Fru^M

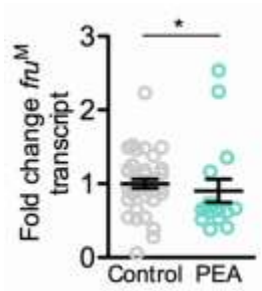


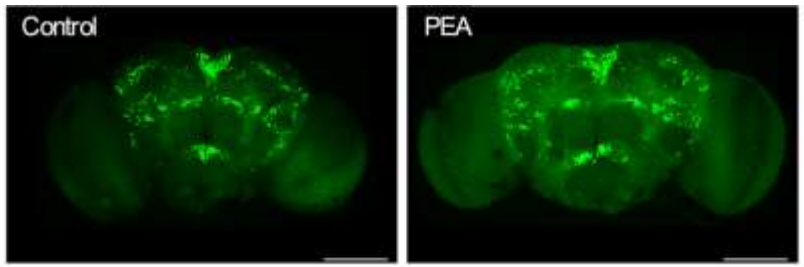


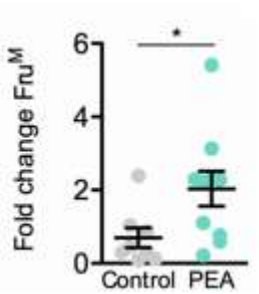






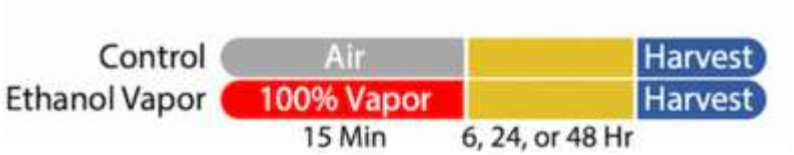


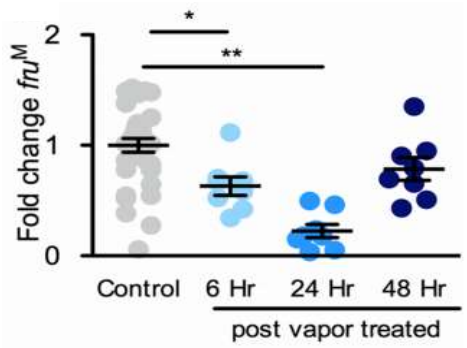


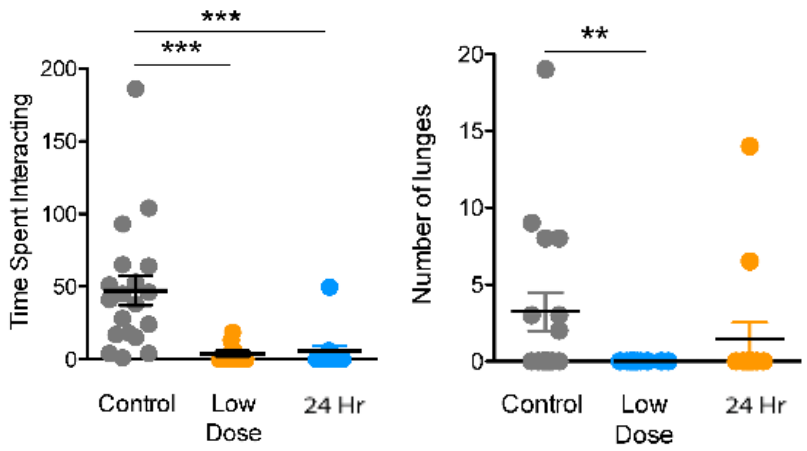


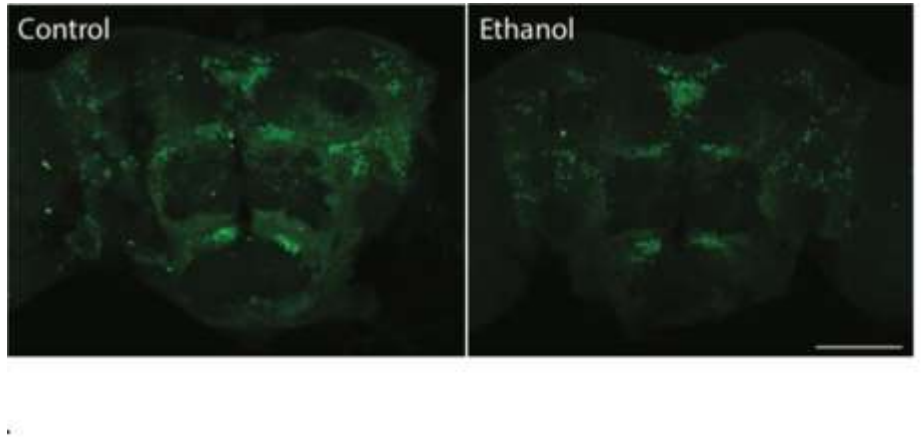
(Park et al., Addiction Biology, 2021)

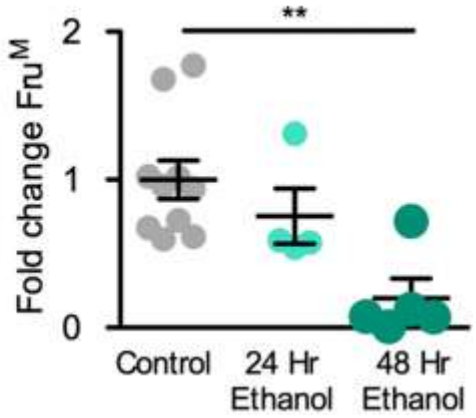
Ethanol treatment affects post-ethanol aggression through Fru^M



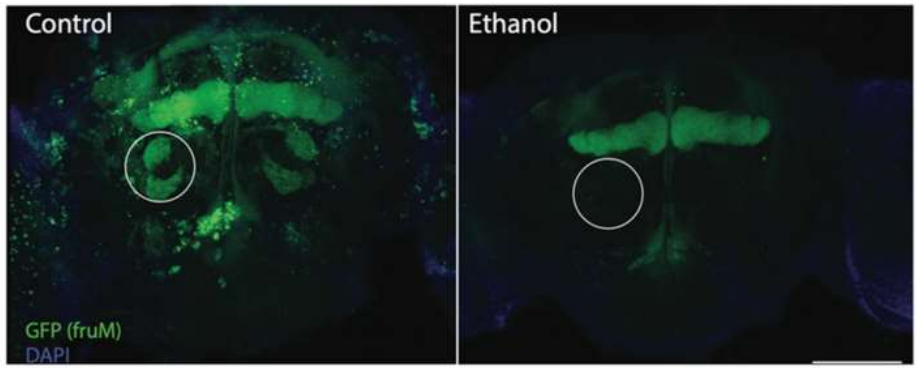


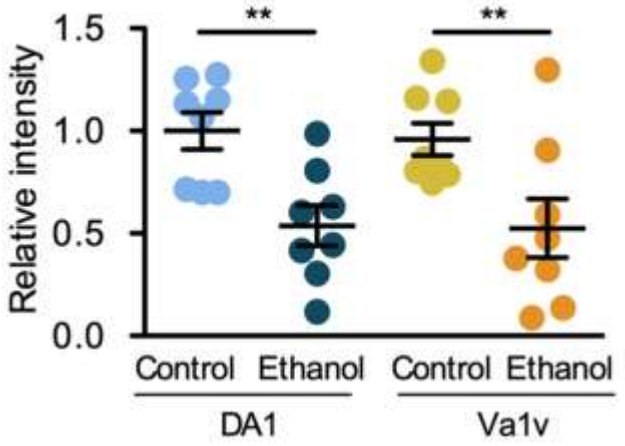






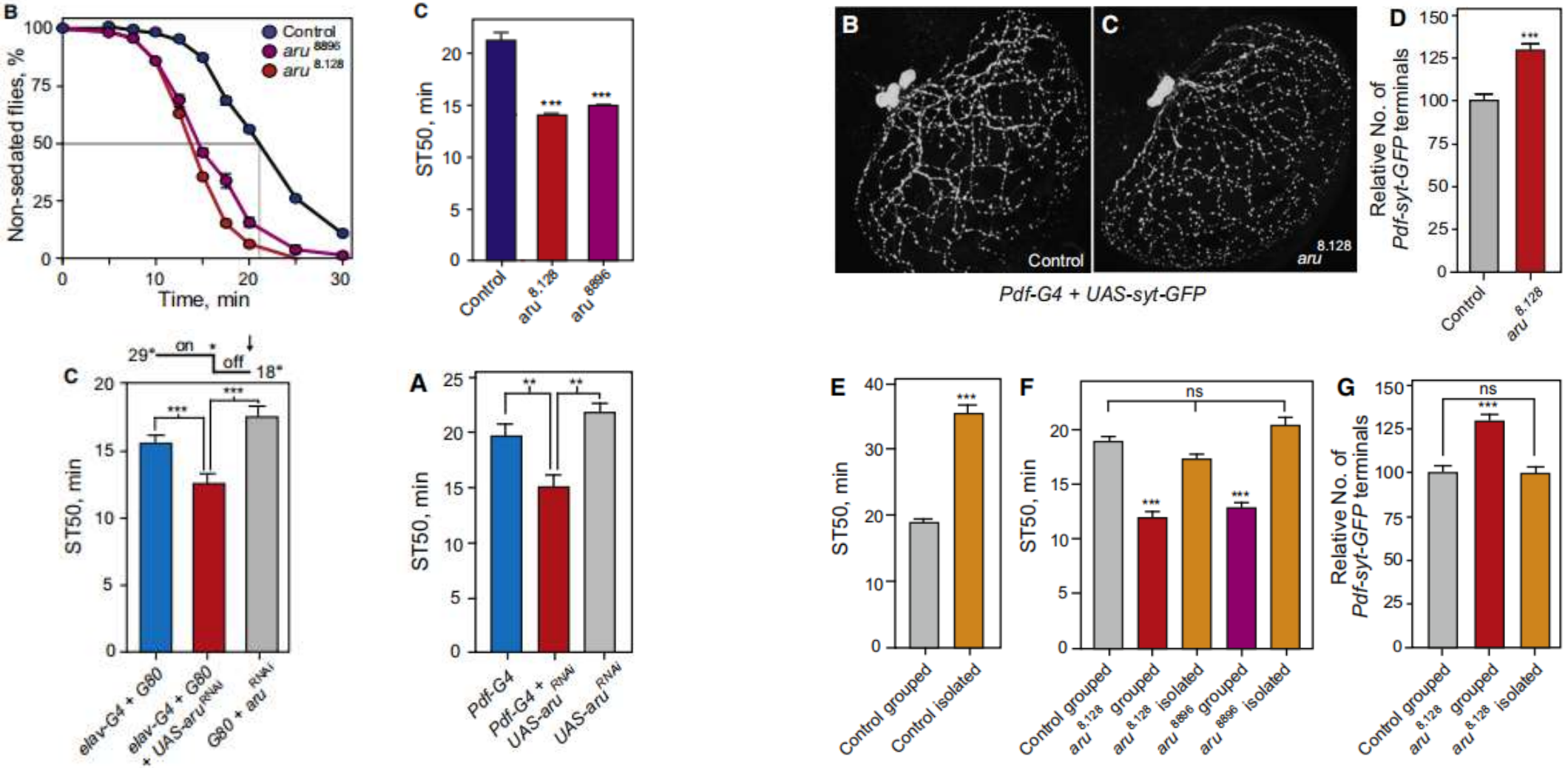
fru^{Gal4} > eGFP





(Park et al., Addiction Biology, 2021)

Social isolation restores normal ethanol sensitivity and synapse number to *aru* mutant flies



(Eddison et al., Neuron, 2011)

Summary

- Male flies showed a strong increase in courtship toward other males after repeated daily ethanol exposures.
- Sexual deprivation increases voluntary ethanol consumption in *Drosophila*, and neuropeptide F serves as a key molecular transducer.
- Alcohol odor increases aggression by potentiating the response to cVa in male flies.
- In male flies, post-ethanol aggression is dependent on the male isoform of the *fruitless* transcription factor (Fru^{M}).
- Synapse number, which is under both genetic and social control, regulates ethanol sensitivity of adult *Drosophila*.

Conclusions:

- Sexually dimorphic ethanol sedation sensitivity is regulated by Tra in developing nervous system
- Alcohol-induced memory problems are partly regulated by cAMP, Notch and EGFR signaling pathways in the mushroom body
- The Toll innate immune pathway can be affected by alcohol

- PPL1 DAN are required for alcohol avoidance, and need PAM DAN to regular alcohol preference
- PPM3 neurons that project to the ellipsoid body promote ethanol-induced hyperactivity

- Sexual deprivation leads to increased alcohol consumption, and acute alcohol consumption enhances M-M courtship
- Ethanol-enhanced aggression is associated with cVA and Fru^{M} in males


HAPPY
THANKSGIVING