Role of the Drosophila's small lateral ventral neurons in the regulation of behavioral responses to alcohol

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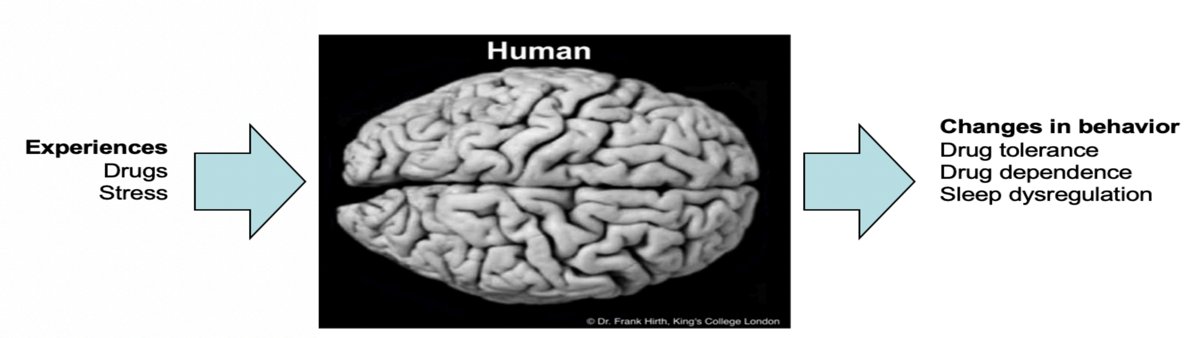
1. Abstract

Alcohol consumption is known to disturb a variety of biological processes that affect normal physiological function. In the nervous system, alcohol is known to affect several molecular targets leading to an overall suppression of neuronal activity. In response, the organism produces a series of neuroadaptive changes that help restore neuronal homeostasis and that lead to the development of alcohol tolerance, dependence and ultimately addiction. These adaptations are also believed to be the root of a series of sleep disturbances, which often manifest during the development of alcoholism. As both, alcohol addiction and sleep regulation are under homeostatic control, we hypothesize that these processes share a common mechanism. Here, we use Drosophila melanogaster as a biological model to understand the molecular underpinnings of the effects of alcohol on the neuronal substrates that control sleep. We show that in Drosophila, a single acute alcohol exposure causes long-term sleep disruptions that resemble those described in mammals. These disturbances include an increase in total sleep duration, decrease sleep latency, and a significant reduction in morning anticipation. Furthermore, we show that the lateral ventral neurons (LNv), a small set of neurons known to control sleep/wake cycles through the secretion of the neuropeptide PDF, is an important regulator of the behavioral responses to alcohol. Silencing of the LNv neurons, either through a mutation in PDF or through a genetic block of synaptic release, significantly increases resistance to alcohol and prevents the development of tolerance. Our results suggest that sleep and alcohol tolerance share common regulatory mechanisms. We believe that the integration of genetic analyses with physiological modulation of neural activity within specific sleep circuits has tremendous potential to uncover the functionally relevant molecular targets whose action contributes to the deleterious effect of alcohol on sleep.

2. Introduction



Alcohol's effect on Sleep Human Studies

TABLE 1

General Effects of Alcohol Use and Cessation on Sleep Architecture.

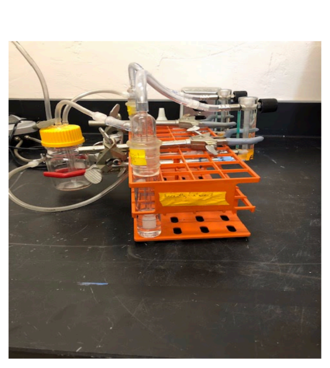
Drinking Behavior	REM Sleep	Slow Wave Sleep	Sleep Continuity	Sleep Latency	Total Sleep Time
Acute Use					
High dose	↓↓	↑↑	↓	↓	↓
Low dose	↓↑	↓↑	↓↑	↑	↑
Chronic Use	↓	↑	↓	↑	↓
Cessation After Chronic Use	↑↑	↓	↓	↑↑	↓

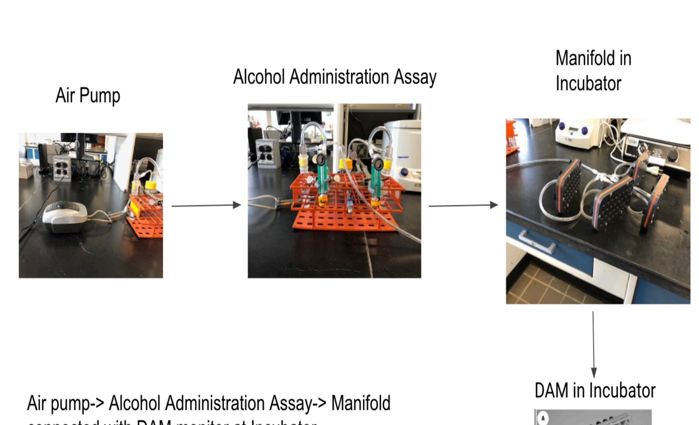
Stein, M. D., & Friedmann, P. D. (2006). Disturbed Sleep and Its Relationship to Alcohol Use. *Substance Abuse,26*(1), 1-13. doi:10.1300/j465v26n01_01

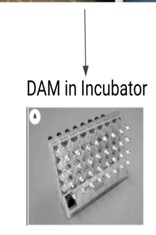
Many genes involved in alcohol responses also involved in circadian rhythms

Gene Symbol	Gene Name	Alcohol Phenotype Affected	Alcohol Reference	circadian rhythm	circadian behavior	Circadian locomotor rhythm	circadian sleep/wake cycle	sleep	circadian regulation of gene expression
Akt1	Akt1	Resistance	Eddison et al., 2011	√					
Bx (dLMO)	Beadex	Resistance	Lasek et al., 2011	√	√	√			
CrebB	Cyclic-AMP response element binding protein B	Tolerance	Wang et al., 2007	√	√	√	√	√	
cyc	cycle	Tolerance	Pohl et al., 2013	√	√	√	√	√	√
dlg1	discs large 1	Tolerance	Maiya et al., 2012	√	√	√			
Drat	Death resistor Adh domain containing target	Resistance	Chen et al., 2012					√	
homer	homer	Tolerance	Urizar et al., 2007	√	√		√	√	
InR	Insulin-like receptor	Resistance	Corl et al., 2005	√	√		√	√	
nej	nejire	Tolerance	Ghezzi et al., 2013	√	√	√			√
NPF	neuropeptide F	Resistance	Wen et al., 2005	√	√	√			
per	period	Tolerance	Pohl et al., 2013	√	√	√	√	√	
Pka-C1	Protein kinase A catalytic subunit 1	Resistance	Moore et al., 1998	√	√	√	√	√	
Pka-R2	Protein kinase A regulatory subunit type 2	Resistance	Park et al., 2000	√	√	√			
slo	slowpoke	Tolerance	Cowmeadow et al., 2005	√	√				
tim	timeless	Tolerance	Pohl et al., 2013	√	√	√	√	√	

3. Experimental Design





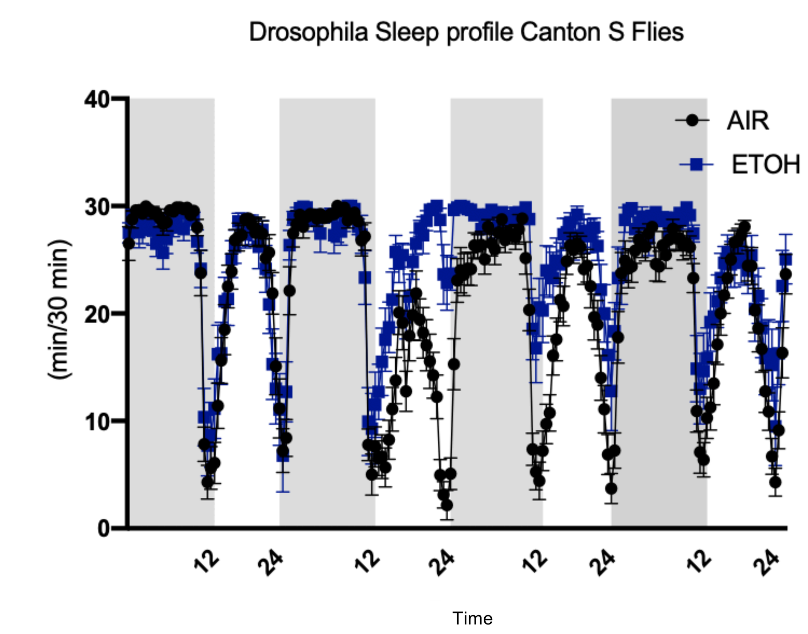


Overall Experiment:

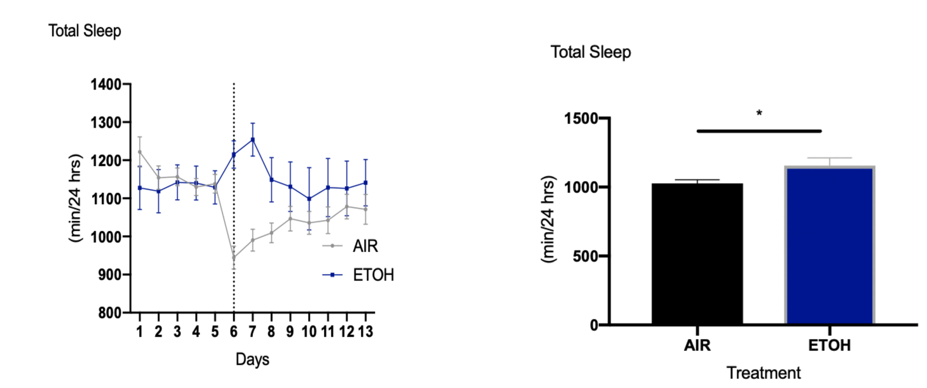
- ✓ 1.5 LPM Water
- ✓ 1.5 LPM ETOH
- ✓ 1.5 LPM Water

4. Effect of a Single Sedating Dose of Ethanol on Sleep on Female Canton S Flies

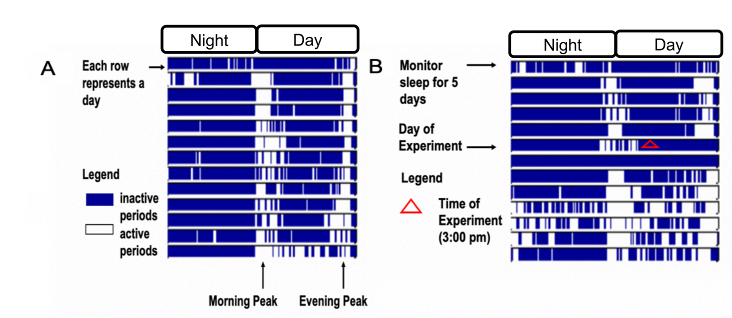
A Effect of a single high dose of alcohol exposure during and after the experiment



B A single high dose ethanol exposure causes a significant increase in sleep.

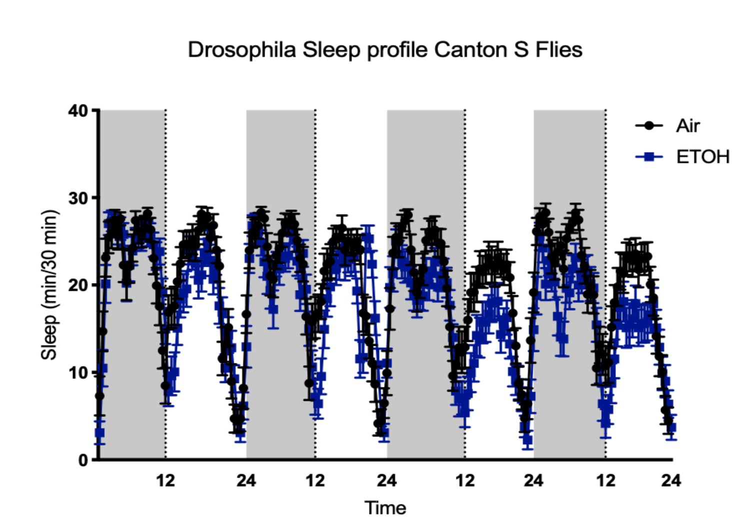


C An individual actogram reveals a short-term block of morning anticipation, the day after alcohol exposure, suggesting a direct effect of alcohol on the wake promoting ventral lateral neurons.

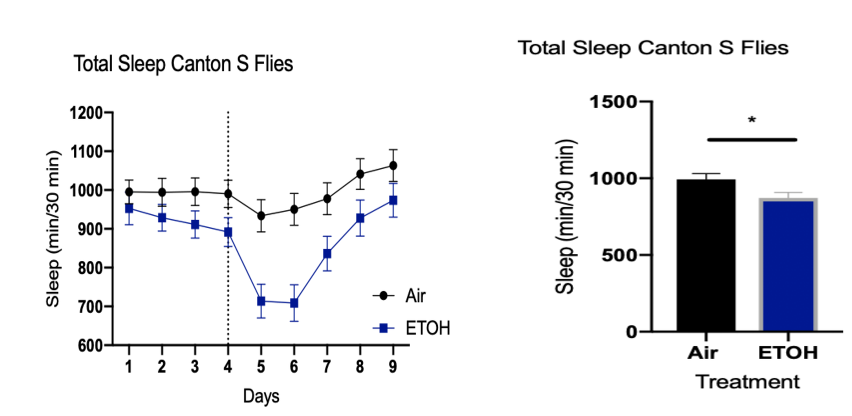


5. Effect of a Non-Sedating Dose of Ethanol on Sleep in Male Flies

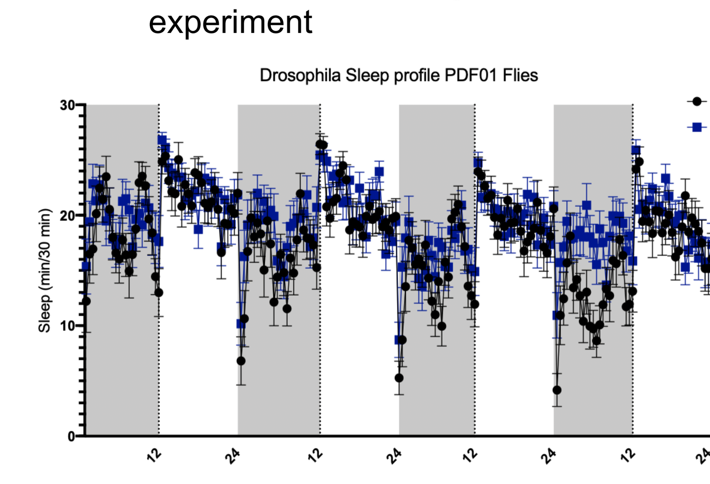
A Effect of a low dose of alcohol exposure during and after the experiment



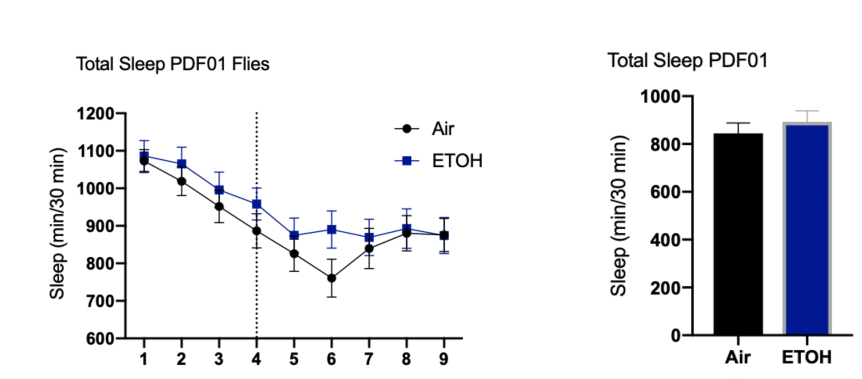
B A single low dose ethanol exposure causes a significant decrease in sleep.



C Effect of a low dose of alcohol exposure in PDF01 Flies during and after the experiment



D A single low dose ethanol exposure causes no effect on total sleep of PDF01 Flies.



7. Future Directions

- ✓ Repeat the single sedating dose of ethanol on sleep experiment with Male Canton S Flies.
- ✓ Repeat the non-sedating dose of ethanol on sleep experiments with Female Canton S Flies.
- ✓ Use ion channels to manipulate the neuronal excitability of small lateral neurons in order to understand their role in alcohol-induced sleep behaviors.

RISE: 5R25GM061151-18.
COBRE: 2P20GM103642