Serotonin Signaling and Sleep in *Caenorhabditis elegans*

Presented by Jennifer Ju

Introduction:

An Introduction to Sleep (for a review, see Andretic *et al.*, 2008; Dauvilliers *et al.*, 2005; Mahowald and Schenck, 2005)

Importance :
- Ubiquitous across all mammals and some invertebrates
- Linked to learning and memory
- Sleep disturbance is one of the most common reasons for why people seek medical attention
- Lack of sleep can lead to death as seen in fatal familial insomnia

Definition:
- Increased arousal threshold (decreased responsiveness to external stimuli)
- Rapid reversibility to wakefulness
- Homeostasis (rebound sleep after deprivation)
- Decreased activity
- Species-specific posture

Figure 1. *C. elegans* under magnification

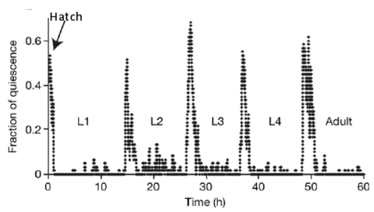
Caenorhabditis elegans as a Model System

Usefulness (for a review, see Hodgkin, 2005; Richmond, 2007):
- Relatively simple behaviors
- Well-characterized nervous system
- Inexpensive
- Short life cycle (~50 hours)
- Evolutionarily conserved pathways: aging, apoptosis, axon guidance

Lethargus (Raizen *et al.*, 2008):
- Between developmental stages (from L1 to L2 to L3 to L4 to Adulthood (A)) (Figure 2)
- Molts
- Exhibits quiescence

Quiescence is a sleep-like state (Raizen *et al.*, 2008; Ghosh and Emmons 2008; Van Buskirk and Sternberg, 2007):
- Has characteristics of sleep
- Concentrated bouts (10-90 seconds each) during lethargus periods (Figure 3)
- Evolutionarily conserved molecular machinery for regulation

Figure 2. Quiescence occurs during lethargus periods between developmental stages (Raizen *et al.*, 2008)



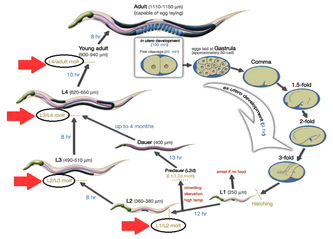
Serotonin (for a review, see Bastiani and Mendel, 2006; Chase and Koelle, 2007; Perez-Mansilla and Nurrish, 2009):

Serotonin in sleep
- Serotonin is important in the regulation of mood and behavior
- In humans, implicated in the formation of circadian rhythm (Kennaway *et al.*, 2001)
- Highest during wakefulness, decreases during NREM sleep, and almost nonexistent during REM sleep (Portas *et al.*, 2000)
- However, shown to promote sleep in *Drosophila* flies and mammals (Yuan *et al.*, 2006; Jouvet, 1968)

Pathway for serotonin biosynthesis is conserved across species

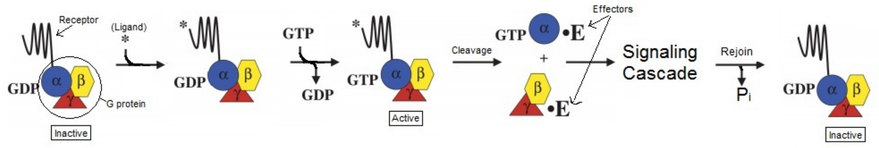
Tryptophan —(TPH-1: tryptophan hydroxylase)→ 5-Hydroxy-tryptophan (5-HTP) —(BAS-1: aromatic amino acid decarboxylase)→ Serotonin (5-HT)

Figure 3. A diagram of the life cycle of *C. elegans* (Altun and Hall, 2005). The red arrows highlight the circled molts when quiescence occurs.



Some serotonin receptors are G protein-coupled

G Protein Signaling (for a review, see Bastiani and Mendel, 2006; Perez-Mansilla and Nurrish, 2009):



Gα subunit is divided into four families: Gαs, Gαi/o, Gαq, and Gα12
- In *C. elegans*, these are GSA-1, GOA-1, EGL-30, and GPA-12 respectively

An example of a downstream Effector is phospholipase C (PLC)
- PLC cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG)
- IP_3 and DAG are second messengers which increase calcium in the cell to activate other proteins
- DAG can be cleaved by diacylglycerol kinase (DGK) to produce phosphatidic acid (PA), another second messenger

G protein-coupled serotonin receptor
- GOA-1 (Gαo) is a direct effector
- DGK-1 is downstream of or in parallel with GOA-1
- Act to decrease acetylcholine release by motor neurons thereby reducing locomotion

G protein signaling in sleep
- Increased GOA-1 signaling induces quiescence and increases arousal threshold in *Drosophila* flies (Guo *et al.*, 2011)
- In *C. elegans*, decreased *goa-1* mutants were hyperactive and increased *goa-1* mutants were lethargic (Mendel *et al.*, 1995)
- Predict that *dgk-1* (loss of function (lof)) mutants would be similar
  - Because downstream of or in parallel with GOA-1 (Mendel *et al.*, 1995; Nurrish *et al.*, 1999; Ségalat *et al.*, 1995; Miller *et al.*, 1999)

Therefore, my thesis work examines the roles of TPH-1, GOA-1, and DGK-1 in quiescence

Abstract

Sleep is ubiquitous and inescapable across species, and yet remains a mystery. Serotonin and G protein signaling have been previously implicated in sleep in species such as *Drosophila* flies. Using as a model the nematode *Caenorhabditis elegans*, which undergo quiescence (a sleep-like state), this study examines *goa-1* ($G\alpha_o$), *dgk-1* (diacylglycerol kinase (DGK)), and *tph-1* (tryptophan hydroxylase (an enzyme in the synthesis of serotonin)) loss of function (lof) mutants using an image subtraction analysis of movement. This study reports that *goa-1(lof)* and *dgk-1(lof)* mutants almost completely eliminate quiescence, suggesting a role for GOA-1 and DGK-1 in promoting *C. elegans* quiescence. *tph-1*(*lof*) mutants do not significantly increase total quiescence. The roles of TPH-1 and serotonin in quiescence are still unclear, possibly due to antagonistic serotonin receptor effects. GOA-1 is known to act as a direct effector for serotonin in egg-laying, defecation, and locomotion in *C. elegans*, with DGK-1 acting either in parallel with or downstream of GOA-1. If GOA-1 and DGK-1 were to act in the same manner as for the serotonin modulation of cholinergic release by motor neurons in locomotion, then *goa-1(lof)*, *dgk-1(lof)*, and *tph-1(lof)* should all have the same phenotype for quiescence. However, this study finds that GOA-1 and DGK-1 likely act in a pathway for quiescence distinct from that for serotonin modulation of cholinergic release due to the contrast in the direction of change in total quiescence amounts between *goa-1(lof)* and *dgk-1(lof)* versus *tph-1(lof)* mutants.

Materials and Methods:

Genotypes Used

Table 1. All *C. elegans* were reared at 25°C on standard nematode growth medium (NGM) plates seeded with OP50 *Escherichia coli*.

Strain Name	Genotype	Allele type
N2	-	Wild-type
MT363	*goa-1(n363) I*	Loss of function (lof)
PS2627	*dgk-1(sy428) X*	Loss of function (lof)
GR1382	*tph-1(mg280) II*	Loss of function (lof)

Quiescence Assay in L4 to Adult (L4/A) Molt

Total quiescence assayed using the microfluidic chamber method
As outlined in Singh *et al.* (2011):
- Capture image every 10 seconds for L4/A molt
- Subtract images to detect movement
- Calculate fraction quiescent as rolling average over 60 images
- Mutants compared to wild-type N2 using Student's t-test
- Cut-off for significance was $p \leq 0.05$

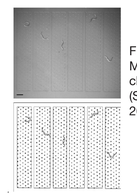


Figure 4. Microfluidic chamber chips (Singh *et al.*, 2011).

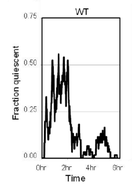


Figure 5. An example of the integral representing total quiescence (Singh *et al.*, 2011).

Results

(Asterisks represent significance with $p \leq 0.05$)

Figure 6. Loss of *goa-1* function causes a dramatic decrease in total quiescence compared to wild-type animals.

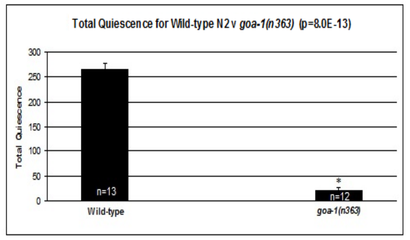


Figure 7. Loss of *dgk-1* function causes a dramatic decrease in total quiescence compared to wild-type animals.

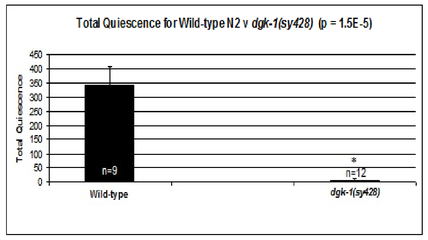
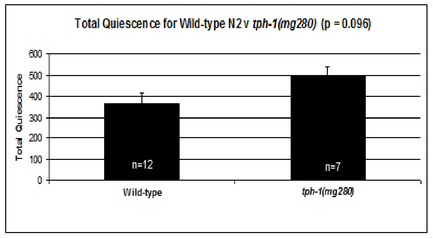


Figure 8. Loss of *tph-1* function has no significant impact on total quiescence compared to wild-type animals.



Conclusions

Past Studies:
- -Serotonin is highest in wakefulness, decreases in NREM sleep, and almost nonexistent in REM sleep (Portas *et al.*, 2000)
- However, serotonin is shown to promote sleep in *Drosophila* flies and mammals (Yuan *et al.*, 2006; Jouvet, 1968)
- -TPH-1 => serotonin => GOA-1 => DGK-1 => reduced locomotion (for a review, see Perez-Mansilla and Nurrish, 2009)

New Findings:
- GOA-1 and DGK-1 are needed for quiescence in *C. elegans*
- Roles of TPH-1 and serotonin are less clear
- GOA-1 and DGK-1 may not be downstream of serotonin and TPH-1 for quiescence because
  - * TPH-1 deficient (and therefore serotonin deficient) mutants do not significantly increase total quiescence
  - * GOA-1 and DGK-1 deficient mutants drastically drop in total quiescence
  - * Thus, GOA-1 & DGK-1 do not seem to act downstream of serotonin for quiescence as they do in locomotion

References

Altun ZF, Hall DH (2005) Handbook of *C. elegans* Anatomy. In *WormAtlas*. http://www.wormatlas.org/ver1/handbook/contents.htm.
Andretic R, Franken P, Tafti M (2008) Genetics of sleep. *Annu Rev Genet* **42**: 361-388.
Bastiani C, Mendel J (2006). Heterotrimeric G proteins in *C. elegans*. In WormBook, The *C. elegans* Research Community, ed. 10.1895/wormbook.1.751, http://www.wormbook.org.
Chase DL, Koelle MR (2007) Biogenic amino neurotransmitters in *C. elegans*. In WormBook, The *C. elegans* Research Community, ed. 10.1895/wormbook.1.132.1, http://www.wormbook.org.
Dauvilliers Y, Maret S, Tafti M (2005) Genetics of normal and pathological sleep in humans. *Sleep Med Rev* **9**: 91-100.
Ghosh R, Emmons SW (2008) Episodic swimming behavior in the nematode *C. elegans*. *J Exp Biol* **211**: 3703-3711.
Guo F, Yi W, Zhou M, Guo A (2011) Go signaling in mushroom bodies regulates sleep in *Drosophila*. *Sleep* **34**: 273-281.
Hodgkin J (2005) Introduction to genetics and genomics. In WormBook, The *C. elegans* Research Community, ed. 10.1895/wormbook.1.17.1, http://www.wormbook.org.
Jouvet M (1968) Insomnia and decrease of cerebral 5-hydroxytryptamine after destruction of the raphe system in the cat. *Adv Pharmacol* **6**: 265–279
Kennaway DJ, Moyer RW, Voultsios A, Varcoe TJ (2001) Serotonin, excitatory amino acids and the photic control of melatonin rhythms and SCN c-FOS in the rat. *Brain Res* **897**: 36-43.
Mahowald MW, Schenck CH (2005) Insights from studying human sleep disorders. *Nature* **437**: 1279-1285.
Mendel JE, Korswagen HC, Liu KS, Hajdu-Cronin YM, Simon MI, Plasterk RH, Sternberg PW (1995) Participation of the protein Go in multiple aspects of behavior in *C. elegans*. *Science* **267**: 1652–1655.
Miller KG, Emerson MD, Rand JB (1999) Goalpha and diacylglycerol kinase negatively regulate the Gαqalpha pathway in *C. elegans*. *Neuron* **24**: 323-333.
Nurrish L, Ségalar L, Kaplan JM (1999) Serotonin inhibition of synaptic transmission: Galpha(0) decreases the abundance of UNC-13 at release sites. *Neuron* **24**: 231-241.
Perez-Mansilla B, Nurrish S (2009). A network of G-protein signaling pathways control neuronal activity in *C. elegans*. *Adv Genet* **65**: 145-192.
Portas CM, Bjorvatn B, Ursin R (2000) Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. *Prog Neurobiol* **60**: 13-35.
Raizen DM, Zimmerman JE, Maycock MH, Ta UD, You Y, Sundaram MV, Pack AI (2008) Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature* **451**: 569-573.
Richmond J (2007). Synaptic function. In WormBook, The *C. elegans* Research Community, ed. 10.1895/wormbook.1.69.1, http://www.wormbook.org.
Ségalat L, Elkes DA, Kaplan JM (1995) Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. *Science* **267**: 1648–1651.
Singh K, Chao MY, Somers GA, Komatsu H, Corkins ME, Larkins-Ford J, Tucey T, Dionne HM, Walsh MB, Beaumont EK, Hart DP, Lockery SR, Hart AC (2011) *C. elegans* Notch signaling regulates adult chemosensory response and larval molting quiescence. *Curr Biol* **21**: 825-834.
Van Buskirk C, Sternberg PW (2007) Epidermal growth factor signaling induces behavioral quiescence in *Caenorhabditis elegans*. *Nature Neurosci* **10:** 1300-1307.
Yuan Q, Joiner WJ, Sehgal A (2006) A sleep-promoting role for the *Drosophila* serotonin receptor 1A. *Curr Biol* **16**: 1051-1062.

Acknowledgements:

I would like to thank Dr. Anne Hart and Dr. Komudi Singh for their tremendous support in this project. Dr Komudi Singh also provided the unpublished *goa-1 (lof)* mutant data. In addition, I would like to thank Heather Bennett, Dr. Maria Dimitriadi, Dr. Winnie Huang, Sade Parsons, Altar Sorkac, Melissa Walsh, and Dr. Jill Yersak for their help in the lab. Nematode genotypes used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources. Funding provided for by NIH NINDS.