CHAPTER 6:

Conserved Molecular Mechanisms of Sleep Homeostasis

6.1 ABSTRACT

Rest and repair of any system from a single cell to a large organism is essential for proper maintenance and function. The idea of sleep as a homeostatic process is based on this general concept and is bolstered by its necessity in human and animal health.[1] Deprivation studies in both mammalian and non-mammalian species have shown that a prolonged period of wakefulness results in increased sleep pressure that can only be dissipated during sleep. There are changes in gene expression corresponding to deprivation and the following recovery sleep.[2] However, many questions still remain about the cause of this homeostasis, the pertinent molecular correlates of sleep propensity, and the origin of these signals. Here we explore issues of rest and homeostasis during lethargus, the sleep-like state in the nematode *Caenorhabditis elegans*. We find that aspects of adenosine signaling, an important regulatory pathway of sleep homeostasis, is conserved. By measuring and altering the downstream signaling components of this pathway, we explore the ideas of the specificity and the multi-faceted effects of targeted depression in the neuronal network.

6.2 INTRODUCTION

The two-process model of sleep regulation asserts that sleep pressure results from the combination of both circadian and homeostatic processes. The circadian regulation of sleep ensures that an organism rests at the appropriate and evolutionarily beneficial time, whereas homeostatic regulation is driven by the need for rest induced by the duration and intensity of activity.[3] Sleep homeostasis refers to the maintenance of sleep amount or depth following sleep deprivation, and is a reflection of the essential nature of this sleep. The cause of this homeostasis is under debate, and there are three contending hypotheses: the energy depletion hypothesis, the neural plasticity hypothesis, and the immune defense hypothesis.

The concept of energy metabolism is that neuronal activity during waking consumes energy while sleep allows energy restoration.[4] Adenosine triphosphate (ATP) is the primary energy carrier in the cell, and local energy depletion through prevention of ATP synthesis and increase in the degradation products of ATP were shown to promote sleep. Adenosine is one of these products, and extracellular adenosine in the basal forebrain (BF) increases during prolonged wakefulness and decreases during recovery sleep.[5] Neuronal activity and consumption of ATP during waking is the cause of elevated adenosine concentration, which

in turn feeds back to adenosine receptors primarily as an inhibitory neuromodulator to induce sleep.[6,7] Adenosine binds to the A1, A2, A3, and A4 adenosine receptors. The primary mediator of sleep is the adenosine A1 receptor, which works through the G_{i3} G-protein signaling to activate potassium channels, inactivate calcium channels, and inhibit adenylate cyclase (Figure 1a).

The main targets for vigilance state-modulating effects of adenosine are the cholinergic cells in the basal forebrain. Selective activation of cholinergic BF neurons using neurotensin promotes EEG gamma activity and state of wakefulness.[8] Corresponding cell-specific lesions of cholinergic cells in the BF abolished extracellular adenosine.[9]

Sleep is conserved and present in essentially all animal species tested.[10,11] One sleep-like state in the nematode *C. elegans* is lethargus.[12,13] Lethargus is a quiescent state during which locomotion and feeding are suppressed[12] and sensory arousal is decreased.[13] Lethargus reliably occurs during *C. elegans* development before each of its four larval molts, and the timing of lethargus corresponds to upregulation of LIN-42, homolog of circadian regulator PER.[14] Lethargus exhibits characteristics similar to sleep homeostasis, and following deprivation of rest by increased sensory stimulus and motor activity during lethargus animals show anachronistic rebound quiescence.[13] Daf-16/FOXO has been shown to play a role in the regulation of homeostatic sleep in the muscle.[15] Anachronistic quiescence can also be induced by expression of epidermal growth factor (EGF)[12], a function conserved in mammals[16], and sensory arousal can be depressed by PKG[13], another well-conserved signaling protein. This conservation suggests that the lethargus state in *C. elegans* could prove insightful in understanding sleep regulation.

6.3 ADENOSINE RECEPTOR ANTAGONIST PARTIALLY SUPPRESSES LETHARGUS

Adenosine is an inhibitory neuromodulator that binds to the adenosine receptors to promote sleep. The primary mediator of sleep is the adenosine A1 receptor. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) is a selective adenosine A1 receptor antagonist[17] that has been shown to increase activity and arousal during sleep when injected into the mouse prefrontal cortex[7]. We performed six to eight hour incubations with DPCPX or control (1% DMSO) during the L4 stage preceding lethargus, and found that DPCPX treatment significantly increased activity (Figure 1b) and sensory arousal as measured by 1-octanol response (Figure

1c) at higher concentrations. Acute assays immediately before or during lethargus showed no significant change (data not shown), but it was unclear whether it was because feeding activity was required for entry of the drug or because entry and equilibration of drug concentration is a slow process.

6.4 DOWNSTREAM EFFECTORS OF A1 RECEPTOR SIGNALING PROMOTE HYPERACTIVITY DURING LETHARGUS

The A1 receptor works through G_{i3} G-protein signaling to activate potassium channels, decrease activity of calcium channels, and inhibit adenylate cyclase. This induces hyperpolarization of the neurons, decreases excitatory response to neurotransmitters that act through $G_{\alpha s}$ signaling, and decreases transcription of genes related to cAMP, which help potentiate excitation. In *C. elegans*, *goa-1* encodes both the main G_o and G_i class of G-protein signaling, and the cAMP catalyzing adenylate cyclase is *acy-1* (Figure 2a).[18]

Loss of downstream effectors of A1 receptor signaling (G_i) should repress neuronal depression mediated by the A1 receptor; we find that loss of *goa-1* indeed results in increased activity and exhibits similar phenotypes to drug treatment. Although *goa-1(lf)* results in hyperactivity in the adult animals[19], there was only a moderate increase in velocity of the animals during lethargus, and this increase was not significant when compared to the wild-type N2 animals (Figure 2b-c). However, the *goa-1(lf)* animals showed a significantly short lethargus whose duration was roughly half that of wild-type N2 animals (Figure 2d). In addition multiple alleles of the *goa-1(lf)* showed increased sensory response during lethargus (Figure 2e), indicating that although animals were not active, they were responsive and had increased sensory arousal.

G_i signaling inhibits adenylate cyclase, which catalyzes conversion of ATP to cAMP. In contrast to the *goa-1(lf)* animals, the mutants of adenylate cyclase, *acy-1(gf),* and mutants of cyclic nucleotide phosphodiesterase, *pde-4(lf)*, increase cAMP (Figure 3a) and showed more general hyperactive phenotypes. The *acy-1(gf)* animals were significantly more hyperactive during lethargus (Figure 2c, 3b-c), as well as during the adult stage[20], and both *acy-1(gf)* and *pde-4(lf)* animals showed increased feeding behavior during lethargus. These animals also exhibited a shorter quiescent period (Figure 2d, 3e), but these periods were not as dramatically short as those seen in the *goa-1(lf)*. Furthermore, we confirmed *acy-1(gf)* and *pde-4(lf)* animals showed no decrease in sensory arousal during lethargus (Figure 2e).[13] The

discrepancy between the two mutants can be explained as increased specificity of lethargus regulation by G_i or *goa-1* and the integration of all general excitatory components by adenylate cyclase or ACY-1 activity. The *goa-1* gene should exhibit more dramatic phenotypes with regard to lethargus-specific phenotypes, whereas *acy-1* should be less specific and exhibit hyperactive phenotypes in general. In addition, the partial suppression of lethargus by *pde-4(lf)* indicate that there is cAMP being produced in the nervous system in the period prior to and possibly during lethargus. It is possible that although adenosine inhibits ACY-1 activity, other pathways that activate ACY-1 may continue to be active.

We wanted to further test this reasoning by measuring the contribution of G_q subtype of G protein signaling. G_q signaling also excites cell signaling and is known to be inhibited by the G_o/G_i class signaling in *C. elegans.*[19] Therefore, overexpression of G_q, *egl-30*, also results in hyperactive phenotype in adult animals.[21] If suppression of activity during lethargus is general and acts through all pathways that decrease neuronal excitability, then overexpression of G_q should also change lethargus phenotypes. Furthermore, since these mutants show a much more dramatic phenotype than the *goa-1(lf)* animals in the adult stage, they should show a stronger effect. Surprisingly, both locomotion of *egl-30(OE)* animals during lethargus and lethargus duration were not significantly different from wildtype (Figure 2b-c). These results suggest that dampening of neuronal activity during lethargus is primarily through the G_i pathways.

6.5 SUPPRESSION OF SENSORY AROUSAL IS MEDIATED THROUGH THE NERVOUS SYSTEM

GOA-1, EGL-30, ACY-1, and PDE-4 are expressed almost ubiquitously in the neurons and muscles of *C. elegans.*[18] Selective expression of ACY-1(gf) in either muscle or neurons result in adult hyperactivity.[22] However, we found that, although expression of ACY-1(gf) resulted in increased feeding behavior, there was a smaller increase of locomotion that was not significant when compared to the wildtype N2 animals (Figure 4a,c). Furthermore, no significant change in lethargus duration could be detected (Figure 4b). However, there was a significant increase sensory arousal in animals with neuronal expression of ACY-1(gf), and a small trend toward sensory depression in muscle expression of ACY-1(gf) (Figure 4d). These data suggest that sensory arousal is mediated through activity in the nervous system and fatigue induced by increased muscle activity.

cAMP acts through CREB phophorylation to mediate transcription through the CRE enhancer. Measuring CRE-mediated transcription with the CRE::GFP can help suggest neuronal changes of cAMP during lethargus. We used a previously developed marker using multiple CRE domains fused to a destabilized GFP, and confirmed that CRE-mediated transcription is primarily in head neurons and pharyngeal muscle in the adult (Figure 5a-b).[23] We also found that expression persists during lethargus, but changes in intensity of expression varied in neuronal subsets suggest their modulation during lethargus (Figure 5c).

6.6 INTERACTION WITH OTHER SLEEP REGULATORS

The presence of EGF is correlated with activity in the SCN to drive circadian rhythms in mammals.[24] EGF is known to induce anachronistic sleep in *C. elegans* through the ALA interneuron.[12] EGF overexpression suppresses activity, feeding behavior, and sensory arousal. Furthermore, this sensory arousal is reversible, much like it is during sleep. We assessed activity of mutations increasing cAMP in the context of the ALA and EGF overexpression. We found that removal of ALA does not result in additional hyperactivity in the mutant *acy-1* animals. In fact, loss of ALA causes no significant change in velocity or lethargus duration, although there is a small trend toward extending lethargus duration (Figure 6a-b). Locomotor (Figure 6b) but not feeding (Figure 6d-e) phenotype of LIN-3 or EGF overexpression is suppressed by *acy-1(gf)*. In contrast, *pde-4(lf)* no animals showed no suppression of the LIN-3 overexpression phenotypes. The lack of feeding activity as measured by pharyngeal contractions in the *acy-1(gf)* and *pde-4(lf)* mutants with LIN-3 overexpression indicates that LIN-3 regulates this behavior independently by affecting components that are downstream of *acy-1* and *pde-4* signaling. This suggests that *acy-1,* but not *pde-4,* is downstream of *lin-3*. Perhaps in neurons where the LIN-3 receptor (*let-23*), *acy-1* and *pde-4* are expressed together, LIN-3 decreases neuronal excitability by inhibiting genes that promote ACY-1 activity, indirectly decreasing cAMP. With little cAMP to break down, the role of PDE-4 decreases significantly in these cells. It is also possible that *lin-3* and *acy-1* work in parallel pathways that inhibit or potentiate common downstream components of neuronal transmission. In this case, loss of *pde-4* should also suppress LIN-3 overexpression. The fact that it does not suggests that *lin-3* signaling directly or indirectly affects cAMP levels and the activity of *acy-1*.

6.7 DISCUSSION

Here we show conservation of adenosine signaling in the nematode *C. elegans* and its conserved role in promoting quiescence. We find that the downstream effectors of the adenosine signaling pathway show phenotypes consistent with decreased sleep drive: increased activity, lack of sensory depression, and decreased lethargus duration. Downstream effectors specific to the adenosine signaling pathway show more lethargus-specific phenotypes, whereas commonly shared components show more general hyperactive phenotypes. Furthermore, G_i or GOA-1 has been shown to interact with potassium channel, *unc-103*[25,26], which has been shown to be one of the components necessary for quiescence during lethargus (Van Buskirk et Al., unpublished).

The use of G-protein pathways in the regulation of sleep has two advantages: long-term regulation and integration of activity in context with environmental and internal stimuli. G_i interaction with the potassium channel ensures long-term hyperpolarization of the cell, but inhibition of adenylate cyclase ensures general dampening while continuing to allow integration of excitatory signals in the nervous system. If there are sufficient excitatory drivers at a given neuron, then there will be suppression of lethargus with respect to that behavior. However, the identity and the site of activation are important and only suppression of adenosine signaling will allow partial suppression of all lethargus-specific behaviors. Another interesting point is that increasing motor activity by bypassing the nervous system does not increase sensory response, showing that muscle hyperactivity and potential receptivity does not equate to fast response. In fact, animals with muscle-specific ACY-1(gf) show a trend toward further decrease in sensory arousal as compared to wild-type N2. This bolsters the idea that fatigue in the muscles may feed back on the nervous system.

Furthermore, we show a strategy for the integration of EGF signaling with that of the adenosine pathway through downstream effector, *acy-1*. ACY-1 is a good starting point for understanding the molecular components of sleep drive integration by the two-process model.

FIGURES

Figure 1

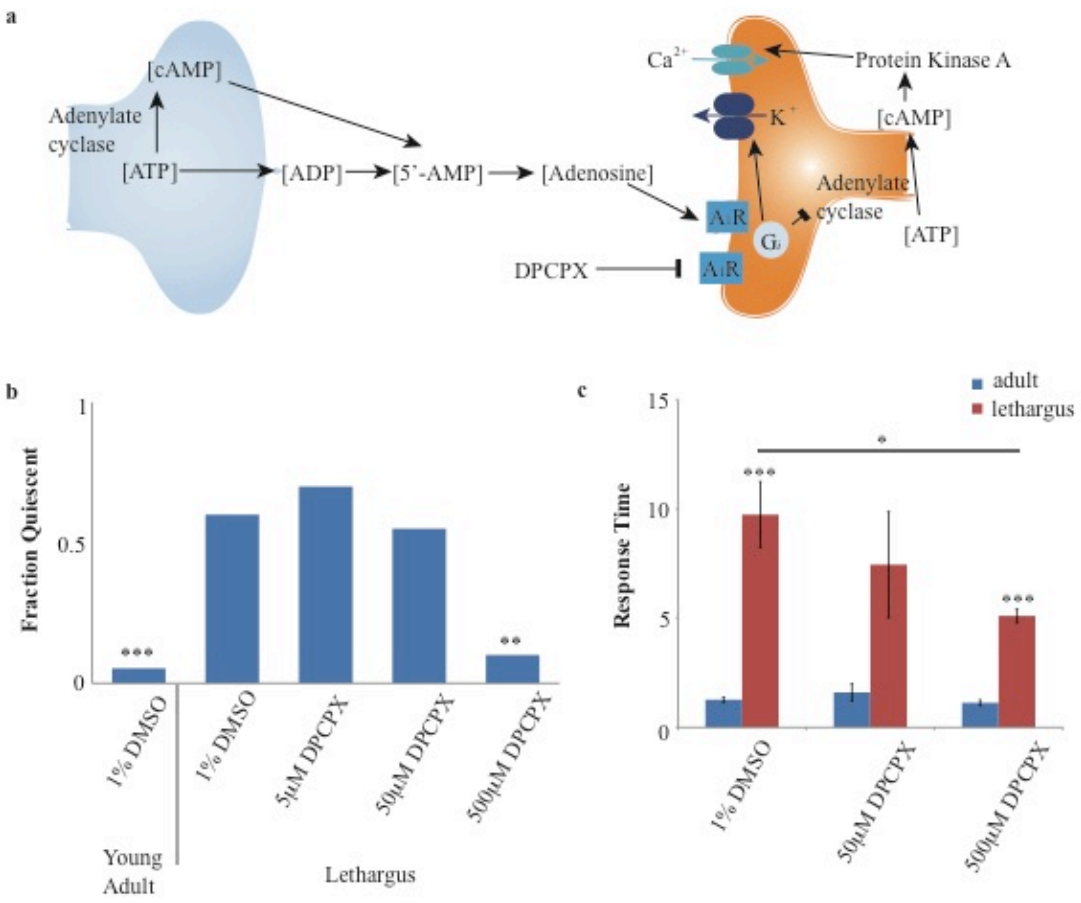


Figure 1. Adenosine anatagonist suppresses the sleep-like state.
(a) Schematic of adenosine signaling**.** (b) Quiescence measurements of animals incubated in labeled concentrations of the adenosine A1 antagonist DPCPX. Fraction of the population exhibiting suppression of locomotion (n=20). Fisher's exact test, **p<0.001, ***p<0.0001. (c) 1-octanol response assays in adult and lethargus animals incubated with 1% DMSO (n=25), 5μM DPCPX (n=15), 50μM DPCPX (n=18), 500μM DPCPX (n=20). Student's t-test *p<0.01, ***p<0.0001.

Figure 2

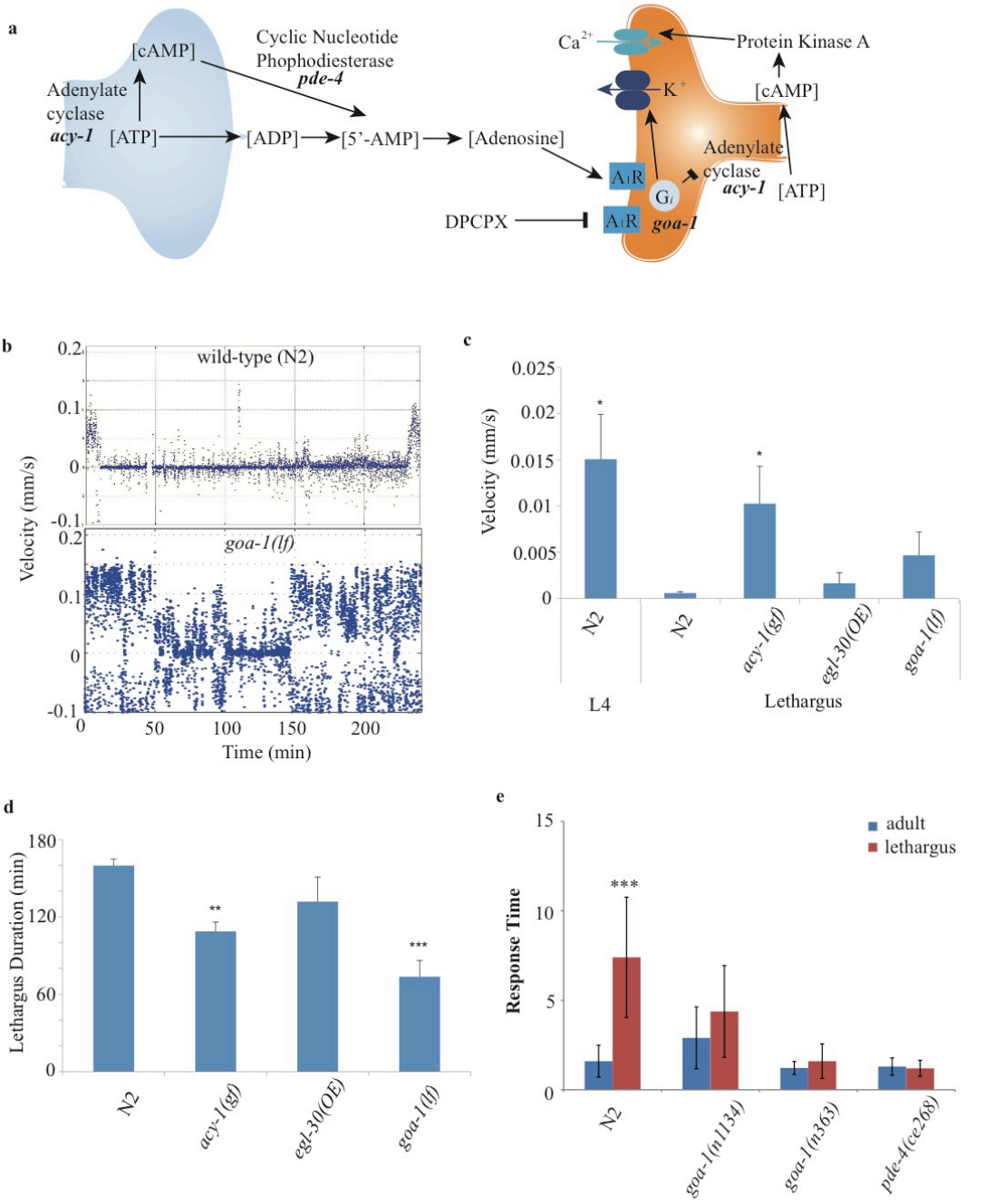


Figure 2. Lethargus phenotypes are preferentially promoted by the $G_{i/o}$ pathway.
(a) Schematic of candidate *C. elegans* genes in the adenosine signaling pathway. (b) Representative activity traces during lethargus in both wildtype N2 and *goa-1* animals. (c) Velocity measurements of wildtype N2 animals in the L4 stage (n=10), lethargus (n=10) as well mutants in lethargus: *acy-1* (n=9), *egl-30* (n=16), and *goa-1* (n=9). ANOVA *p<0.01. (d) Lethargus duration in wildtype N2 (n=20), *acy-1* (n=9), *egl-30* (n=16), and *goa-1* (n=9). ANOVA **p<0.001, ***p<0.0001. (e) 1-octanol response assays in adult and lethargus animals: wildtype N2 (n=20), *goa-1(n1134)* (n=14), *goa-1(n363)* (n=9), and *pde-4(ce268)* (n=9). Student's t-test of unequal variance ***p<0.0001.

Figure 3

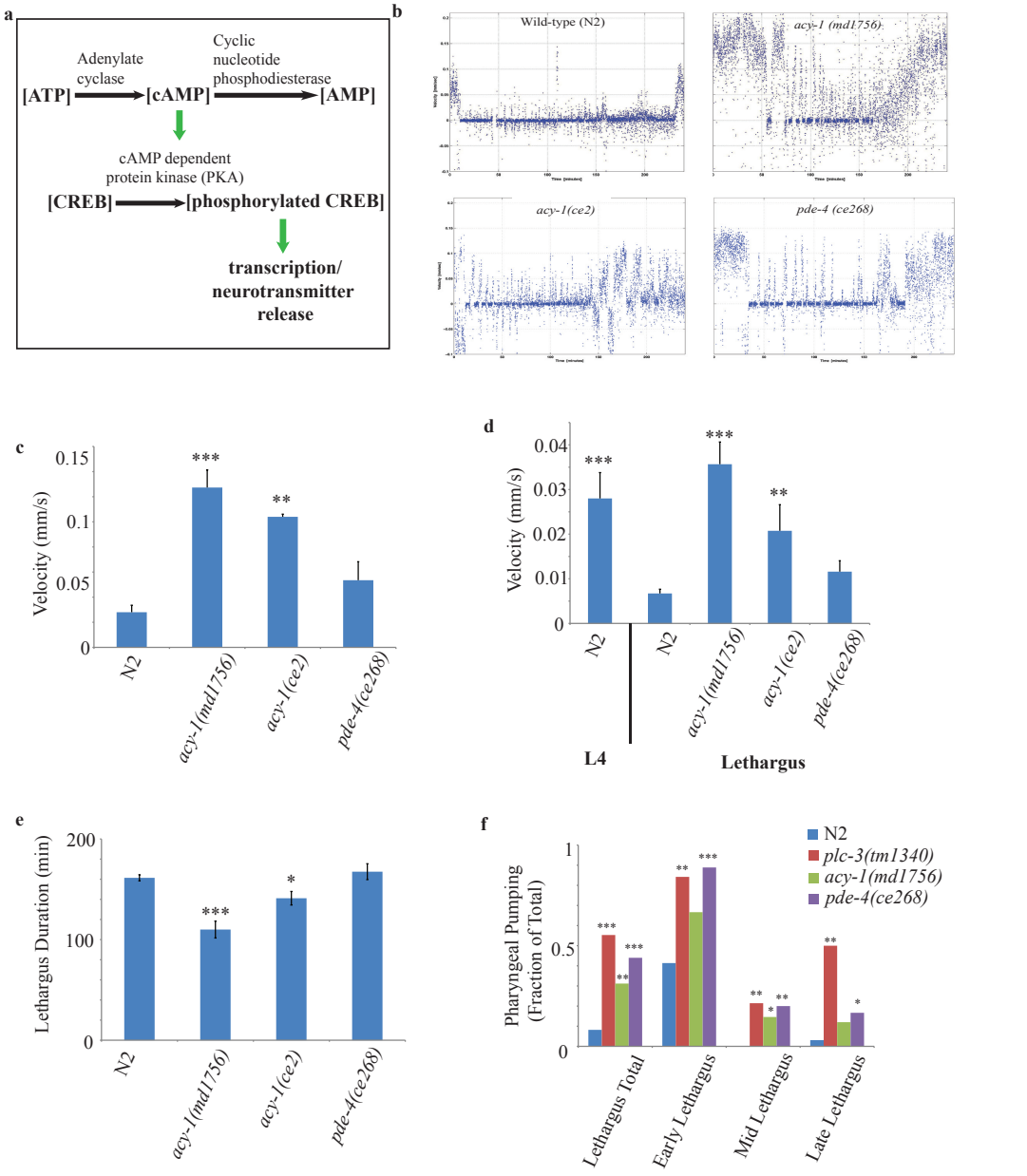


Figure 3. cAMP increase general locomotor and feeding activity during lethargus.
(a) Schematic of candidate *C. elegans* genes in the adenosine signaling pathway. (b) Representative activity traces during lethargus in both wildtype N2, *acy-1(gf)* and *pde-4(lf)* animals. **(c)** Velocity measurements of wildtype N2 (n=25), *acy-1(md1756)* (n=9), *acy-1(ce2)* (n=10), and *pde-4(ce268)* (n=9) animals. ANOVA **p<0.001, ***p<0.0001. (d) Velocity measurements during lethargus of wildtype N2 (n=25), *acy-1(md1756)* (n=9), *acy-1(ce2)* (n=10), and *pde-4(ce268)* (n=9) animals. ANOVA **p<0.001, ***p<0.0001. (e) Lethargus duration in wildtype N2 (n=25), 1-octanol response assays in adult and lethargus animals: wildtype N2 (n=25), *acy-1(md1756)* (n=9), *acy-1(ce2)* (n=10), and *pde-4(ce268)* (n=9) animals. ANOVA *p<0.01, ***p<0.0001. (f) Pharyngeal contractions during lethargus in wildtype N2 (n=171), *plc-3(tm1340)* (n=47), *acy-1(md1756)* (n=109), and *pde-4(ce268)* (n=25) animals. Fisher's exact test *p<0.01, **p<0.001,***p<0.0001.

Figure 4

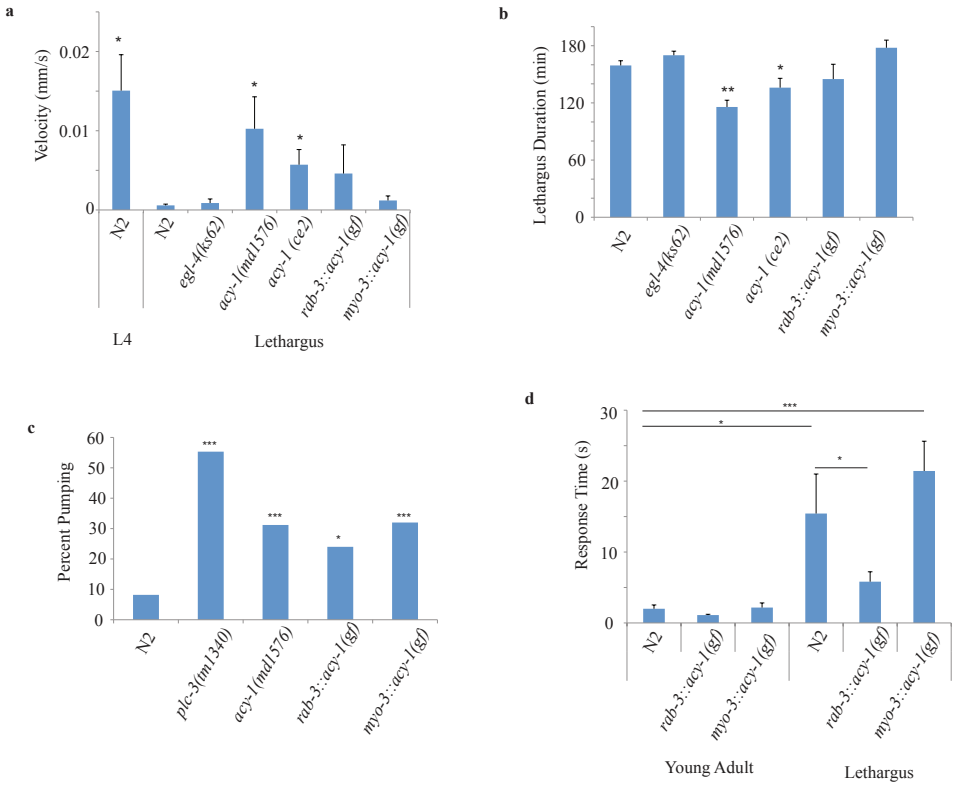


Figure 4. cAMP driven muscle excitability increase hyperactivity but not arousal.
(a) Velocity measurements during lethargus of wildtype N2 (n=10), *egl-4(ks62)* (n=7), *acy-1(md1756)* (n=9), *acy-1(ce2)* (n=10), neuronal expressing *rab-3::acy-1(gf)* (n=6) and muscle expressing *myo-3::acy-1(gf)* (n=8) animals. ANOVA *p<0.01 (b) Lethargus duration in wildtype N2 (n=10), *egl-4(ks62)* (n=7), *acy-1(md1756)* (n=9), *acy-1(ce2)* (n=10), neuronal expressing *rab-3::acy-1(gf)* (n=6) and muscle expressing *myo-3::acy-1(gf)* (n=8) animals. Chi-square test *p<0.01 (c) Pharyngeal contractions during lethargus in wildtype N2 (n=171), *plc-3(tm1340)* (n=47), *acy-1(md1756)* (n=109), neuronal expressing *rab-3::acy-1(gf)* (n=25) and muscle expressing *myo-3::acy-1(gf)* (n=25) animals. Chi-square test *p<0.01, **p<0.001,***p<0.0001. (d) 1-octanol response assays in adult and lethargus animals in wildtype N2 (n=25neuronal expressing *rab-3::acy-1(gf)* (n=25) and muscle expressing *myo-3::acy-1(gf)* (n=25) animals. ANOVA *p<0.01, ***p<0.0001.

Figure 5

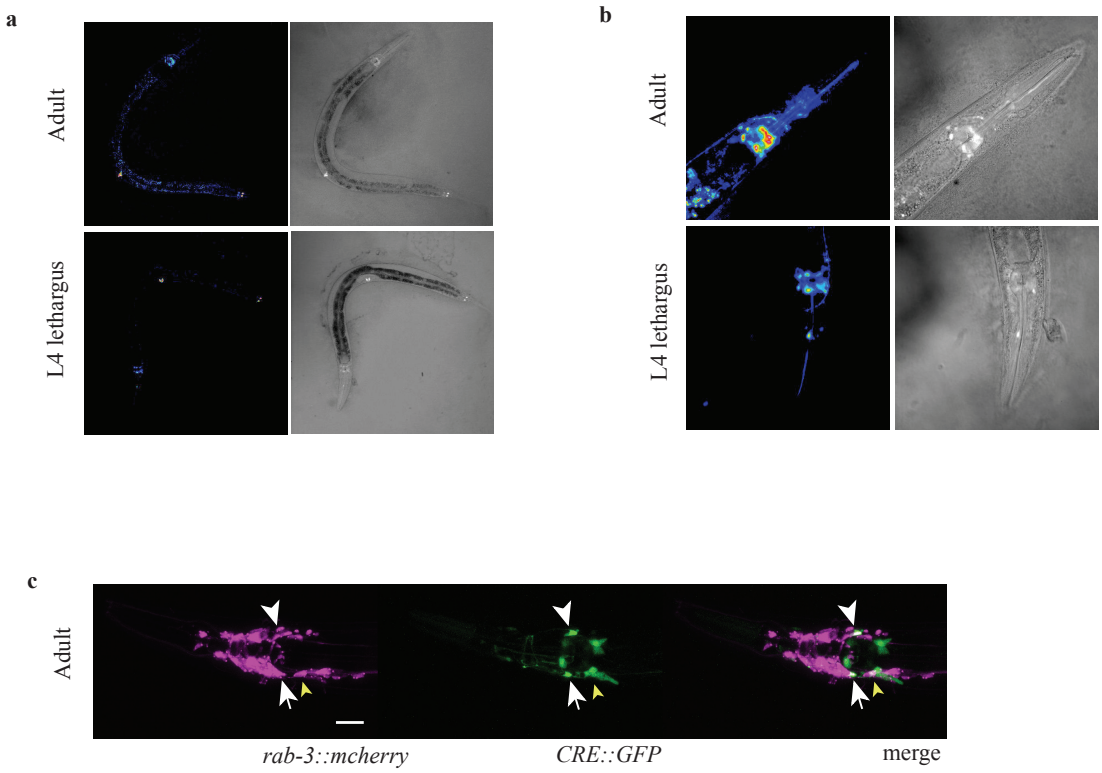
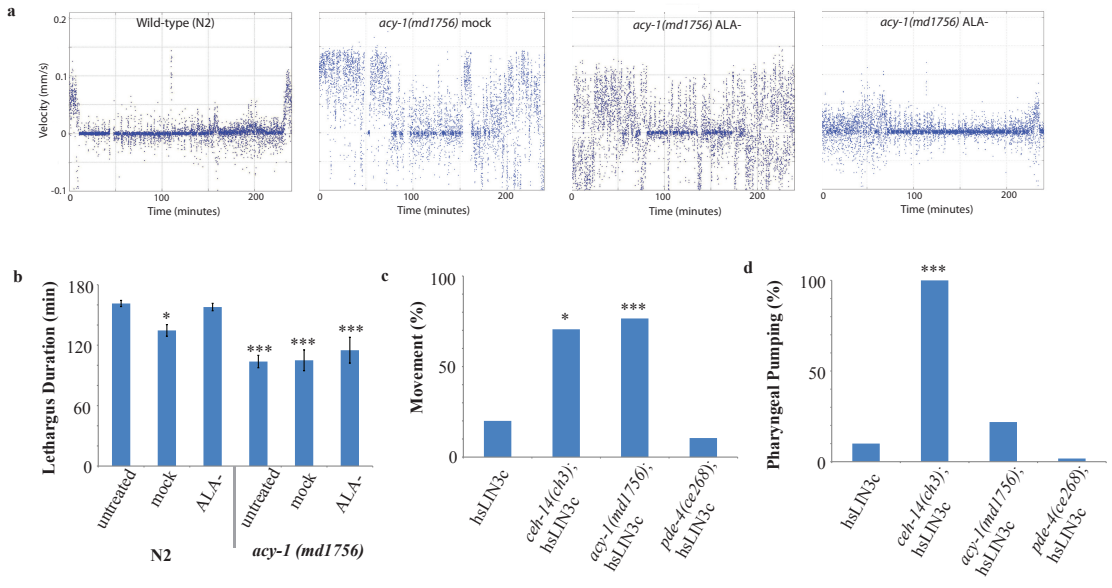


Figure 5. Distribution of neuronal cAMP is altered in lethargus.
(a) Confocal images of worms in adult and lethargus and of the distribution of CRE::GFP expression in the body. Note comparison expression of non-CRE GFP in the vulval cells. (b) Representative images indicating intensity of expression in head neurons of worms in adult and lethargus. (c) cAMP acts through CREB and CRE primarily through a small set of head neurons and pharyngeal muscle cells. Note arrow indicating colocalization of GFP with neuronal mcherry. Scale bar is 20μm.

Figure 6



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Genotype	Pumping (%)	Moving (%)	n	Pumping (p-value)	Moving (p-value)
hsLIN3c	10	20	20		
ceh14(ch3); hsLIN3c	100	70.588	17	<0.0001	0.003
acy-1(md1756)	100	100	16	<0.0001	<0.0001
acy-1(md1756); hsLIN3c	21.875	76.563	64	0.3359	<0.0001
pde-4(ce268)	100	81.25	16	<0.0001	<0.0001
pde-4(ce268) ; hsLIN3c	1.754	10.526	57	0.1636	0.275

Figure 6. Selective suppression of EGF overexpression phenotypes.
(a) Representative activity traces during lethargus in wildtype N2, *acy-1(md1756)* mock ablated, hyperactive *acy-1(md1756)* ALA-ablated and lethargus rescued *acy-1(md1756)* ALA-ablated. (b) Lethargus duration in wildtype N2 (n=20), wildtype N2 mock ablated (n=15), wildtype N2 ALA-ablated (n=15), *acy-1(md1756)* (n=9), *acy-1(md1756)* mock ablated (n=5), and *acy-1(md1756)* ALA-ablated (n=8) animals. Chi-square test *p<0.01 (c) Locomotion phenotype of wildtype N2 and mutant animals with induced EGF overexpression. Percentage of animals exhibiting locomotion after heat shock EGF/LIN-3. Chi-square test *p<0.01, ***p<0.0001. (d) Feeding phenotype of wildtype N2 and mutant animals with induced EGF overexpression. Percentage of animals pharyngeal contractions after heat shock EGF/LIN-3. ***p<0.0001. (e) Phenotype of mutants after heat shock induction.

REFERENCES

1 Saper, C. B., Cano, G. & Scammell, T. E. Homeostatic, circadian, and emotional regulation of sleep. *J. Comp. Neurol.* **493**, 92-98, doi:10.1002/cne.20770 (2005).

2 Cirelli, C., Faraguna, U. & Tononi, G. Changes in brain gene expression after long-term sleep deprivation. *J. Neurochem.* **98**, 1632-1645, doi:JNC4058 [pii] 10.1111/j.1471-4159.2006.04058.x (2006).

3 Borbely, A. A. A two process model of sleep regulation. *Hum. Neurobiol.* **1**, 195-204 (1982).

4 Benington, J. H. & Heller, H. C. Restoration of brain energy metabolism as the function of sleep. *Progress in neurobiology* **45**, 347-360 (1995).

5 Porkka-Heiskanen, T. *et al.* Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science* **276**, 1265-1268 (1997).

6 Arrigoni, E., Rainnie, D. G., McCarley, R. W. & Greene, R. W. Adenosine-mediated presynaptic modulation of glutamatergic transmission in the laterodorsal tegmentum. *J. Neurosci.* **21**, 1076-1085 (2001).

7 Van Dort, C. J., Baghdoyan, H. A. & Lydic, R. Adenosine A(1) and A(2A) receptors in mouse prefrontal cortex modulate acetylcholine release and behavioral arousal. *J. Neurosci.* **29**, 871-881, doi:10.1523/JNEUROSCI.4111-08.2009 (2009).

8 Cape, E. G., Manns, I. D., Alonso, A., Beaudet, A. & Jones, B. E. Neurotensin-induced bursting of cholinergic basal forebrain neurons promotes gamma and theta cortical activity together with waking and paradoxical sleep. *J. Neurosci.* **20**, 8452-8461, doi:20/22/8452 [pii] (2000).

9 Kalinchuk, A. V., McCarley, R. W., Stenberg, D., Porkka-Heiskanen, T. & Basheer, R. The role of cholinergic basal forebrain neurons in adenosine-mediated homeostatic control of sleep: lessons from 192 IgG-saporin lesions. *Neuroscience* **157**, 238-253, doi:10.1016/j.neuroscience.2008.08.040 (2008).

10 Allada, R. & Siegel, J. M. Unearthing the phylogenetic roots of sleep. *Curr. Biol.* **18**, R670-R679, doi:10.1016/j.cub.2008.06.033 (2008).

11 Siegel, J. M. The REM sleep-memory consolidation hypothesis. *Science* **294**, 1058-1063, doi:10.1126/science.1063049 (2001).

12 Van Buskirk, C. & Sternberg, P. W. Epidermal growth factor signaling induces behavioral quiescence in Caenorhabditis elegans. *Nat. Neurosci.* **10**, 1300-1307, doi:nn1981 [pii] 10.1038/nn1981 (2007).

13 Raizen, D. M. *et al.* Lethargus is a Caenorhabditis elegans sleep-like state. *Nature* **451**, 569-572, doi:nature06535 [pii] 10.1038/nature06535 (2008).

14 Monsalve, G. C., Van Buskirk, C. & Frand, A. R. LIN-42/PERIOD controls cyclical and developmental progression of C. elegans molts. *Curr. Biol.* **21**, 2033-2045, doi:10.1016/j.cub.2011.10.054 (2011).

15 Driver, R. J., Lamb, A. L., Wyner, A. J. & Raizen, D. M. DAF-16/FOXO Regulates Homeostasis of Essential Sleep-like Behavior during Larval Transitions in C. elegans. *Curr. Biol.* **23**, 501-506, doi:S0960-9822(13)00150-4 [pii]10.1016/j.cub.2013.02.009.

16 Zimmerman, J. E., Naidoo, N., Raizen, D. M. & Pack, A. I. Conservation of sleep: insights from non-mammalian model systems. *Trends Neurosci.* **31**, 371-376, doi:S0166-2236(08)00133-1 [pii]10.1016/j.tins.2008.05.001 (2008).

17 Haleen, S. J., Steffen, R. P. & Hamilton, H. W. PD 116,948, a highly selective A1 adenosine receptor antagonist. *Life Sci.* **40**, 555-561 (1987).

[18] Riddle, D. L., Blumenthal, T., Meyer, B. J. & Priess, J. R. Introduction to C. elegans. doi:NBK20183 [bookaccession] (1997).

[19] Mendel, J. E. *et al.* Participation of the protein Go in multiple aspects of behavior in C. elegans. *Science* **267**, 1652-1655 (1995).

[20] Brundage, L. *et al.* Mutations in a C. elegans Gqalpha gene disrupt movement, egg laying, and viability. *Neuron* **16**, 999-1009, doi:S0896-6273(00)80123-3 [pii] (1996).

[21] Wang, L. & Proud, C. G. Ras/Erk signaling is essential for activation of protein synthesis by Gq protein-coupled receptor agonists in adult cardiomyocytes. *Circ. Res.* **91**, 821-829 (2002).

[22] Schade, M. A., Reynolds, N. K., Dollins, C. M. & Miller, K. G. Mutations that rescue the paralysis of Caenorhabditis elegans ric-8 (synembryn) mutants activate the G alpha(s) pathway and define a third major branch of the synaptic signaling network. *Genetics* **169**, 631-649, doi:genetics.104.032334 [pii] 10.1534/genetics.104.032334 (2005).

[23] Kimura, Y. *et al.* A CaMK cascade activates CRE-mediated transcription in neurons of Caenorhabditis elegans. *EMBO reports* **3**, 962-966, doi:10.1093/embo-reports/kvf191 (2002).

[24] Saper, C. B., Scammell, T. E. & Lu, J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* **437**, 1257-1263, doi:nature04284 [pii] 10.1038/nature04284 (2005).

[25] Reiner, D. J. *et al.* Behavioral genetics of caenorhabditis elegans unc-103-encoded erg-like K(+) channel. *J. Neurogenet.* **20**, 41-66, doi:H271777263G80503 [pii] 10.1080/01677060600788826 (2006).

[26] Gruninger, T. R., Gualberto, D. G., LeBoeuf, B. & Garcia, L. R. Integration of male mating and feeding behaviors in Caenorhabditis elegans. *J. Neurosci.* **26**, 169-179, doi:26/1/169 [pii]10.1523/JNEUROSCI.3364-05.2006 (2006).