Circadian and homeostatic regulation of annelid 'sleep-like' inactivity

M. J. Zoran

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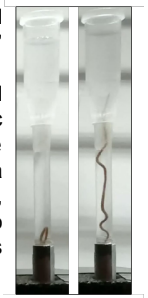
Center for Biological Clocks Research

Abstract

Many invertebrate animals undergo periods of rest or 'sleep-like' inactivity. The aquatic annelid worm, *Lumbriculus variegatus*, is nocturnal in its activity and exhibits a daytime inactive state, which involves a ventilatory posture that facilitates gas exchange. *Lumbriculus* 'sleep-like' inactivity is circadian and expressed during the day, as well as the subjective day in constant conditions (n=31). To determine if worm inactivity is also under homeostatic regulation, both physical disruption (agitation; n=25 per group) and pharmacological perturbation (caffeine; n=5 per group) were used to deprive animals of resting states. Only 24h of sleep-state deprivation with agitation (n=25 per group) or caffeine (n=5 per group) was necessary to cause a rebound period of inactivity during the subsequent dark phase. Chronic rest deprivation (for 3 days) caused prolonged rebound inactivity and increased ventilatory behavior. The life history of *L. variegatus* includes the regeneration of head segments following injury or asexual fragmentation. Thus, we tested if headless animals possess entrainable circadian rhythms in physiology and behavior in the absence of anterior brain ganglia. Although locomotor activity is decreased in headless worms, detectable circadian rhythms in inactivity persist for many days following head segment amputation. Our results demonstrate that *L. variegatus* possesses a 'sleep-like' resting state that when disrupted leads to homeostatic rebound inactivity. Although circadian rhythms in some behaviors persist in the absence of brain ganglia, ongoing studies are addressing what aspects of this circadian 'sleep-like' state require brain function.

Introduction

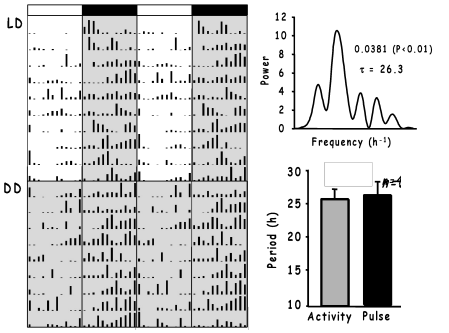
Whether or not annelid worms possess 'sleep-like' behavior remains unclear. Here, we have studied rhythmic inactivity in aquatic worms, *L. variegatus,* where an inactive states involve a species-specific tail posture, where body extension into the water column facilitates gas exchange.



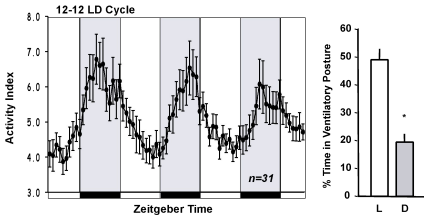
Here, the hypothesis that deprivation of inactivity, using agitation or caffeine, causes rebound inactivity and associated deficits in neural plasticity was tested.

Results

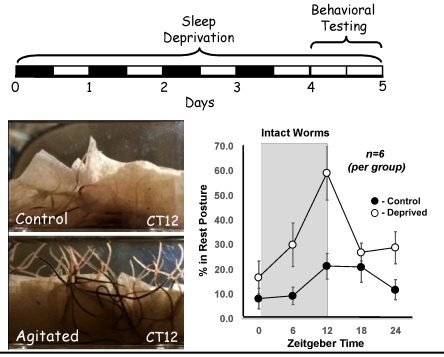
Circadian rhythms in worm activity persist in constant conditions with a period of ~25h



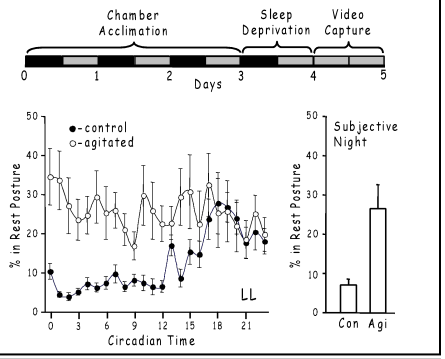
Lumbriculid worms are nocturnal with rhythms in locomotor activity



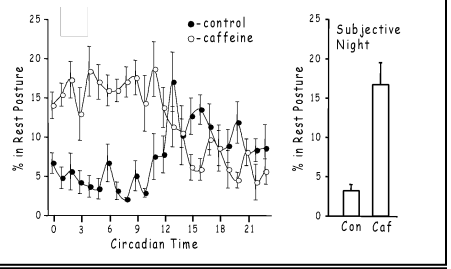
Prolonged deprivation of rest state induces rebound sleep-like inactivity



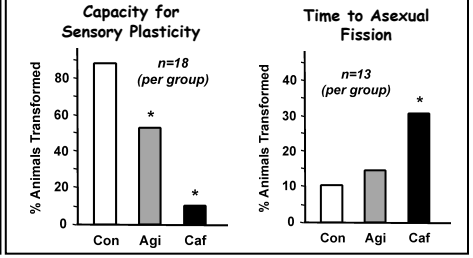
24h rest deprivation with constant agitation induces rebound inactivity



Caffeine-induced rest state deprivation also causes rebound inactivity



Sleep-like state deprivation produces multiple neurobehavioral deficits



Conclusions

- Lumbriculid worms have a circadian rhythm in activity, where daytime inactivity involves a posture that facilitates ventilatory gas exchange.
- Physical agitation and exposure to caffeine both deprived animals of rest-state inactivity and produced a period of rebound inactivity.
- Inactivity-state deprivation disrupted neural plasticity in sensory to interneuron neural circuits and promoted the early onset of neural-regulated asexual fission.
- Lumbriculid worms, therefore, have a sleep-like behavior that involves most core sleep characteristics, including prolonged inactivity, a stereotyped posture, and rebound inactivity following deprivation of rest.
- Similar to sleep disturbances in other animals, rest-state deprivation in lumbriculid worms is accompanied by neural and behavioral deficits, suggesting a role for the central nervous system in inactive, sleep-like states of this annelid species.

The author acknowledges the contributions that several undergraduate student researchers toward the conduct of these studies, including Alan Romero, Anna Chainaranont, Robert Vaughan, Brett Vernier and David Villareal.

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Active Inactive

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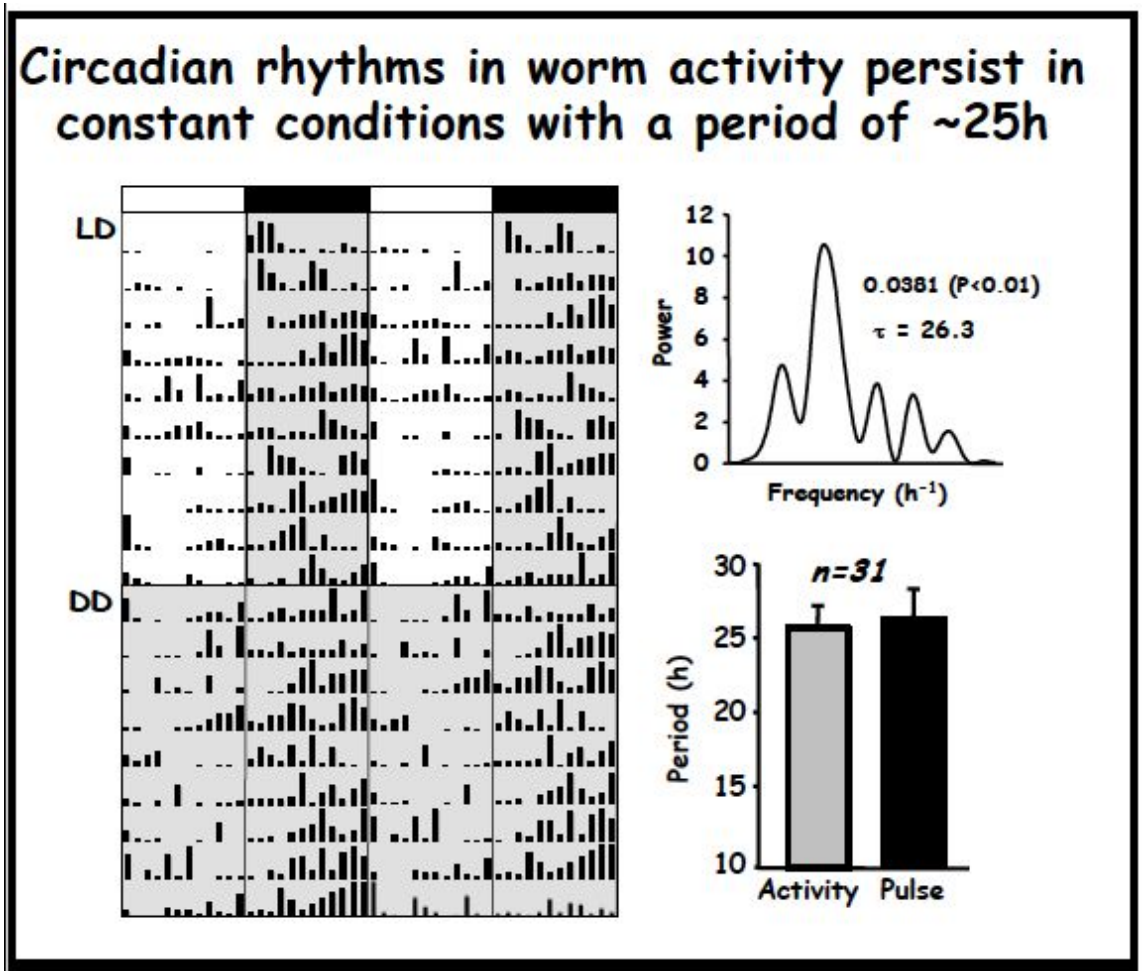
Results



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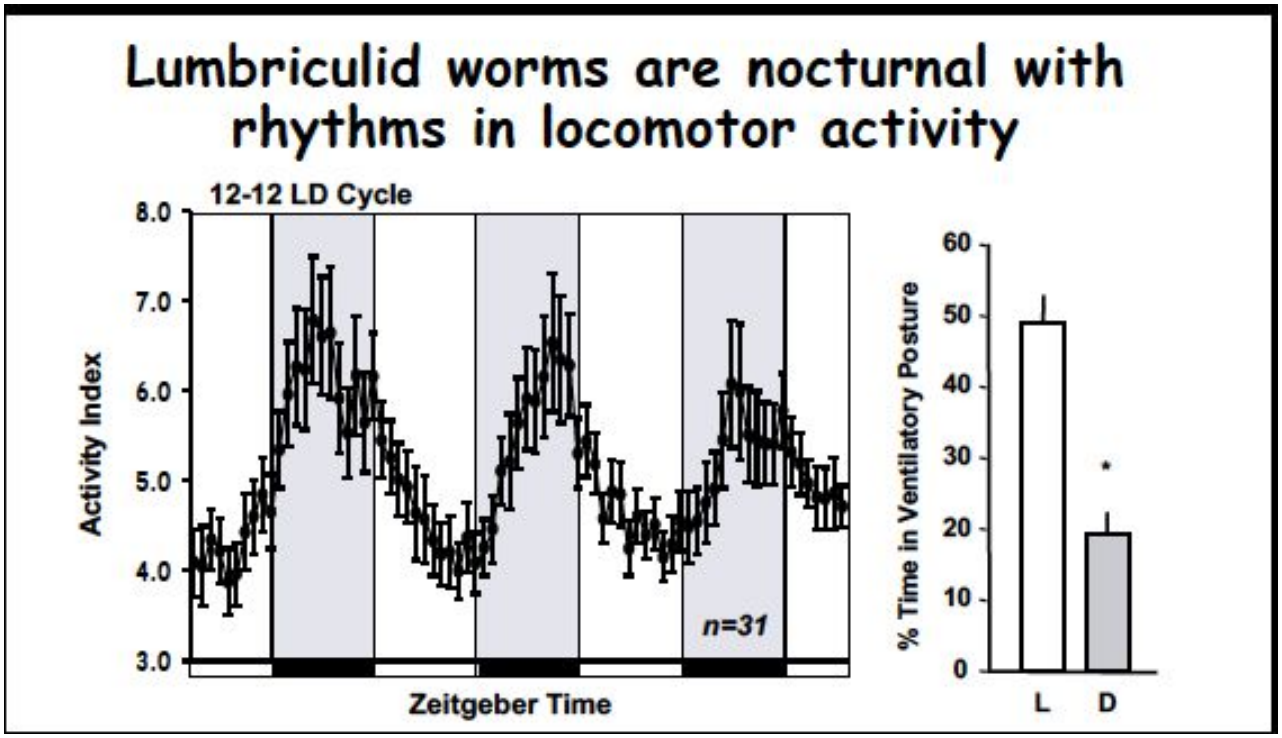

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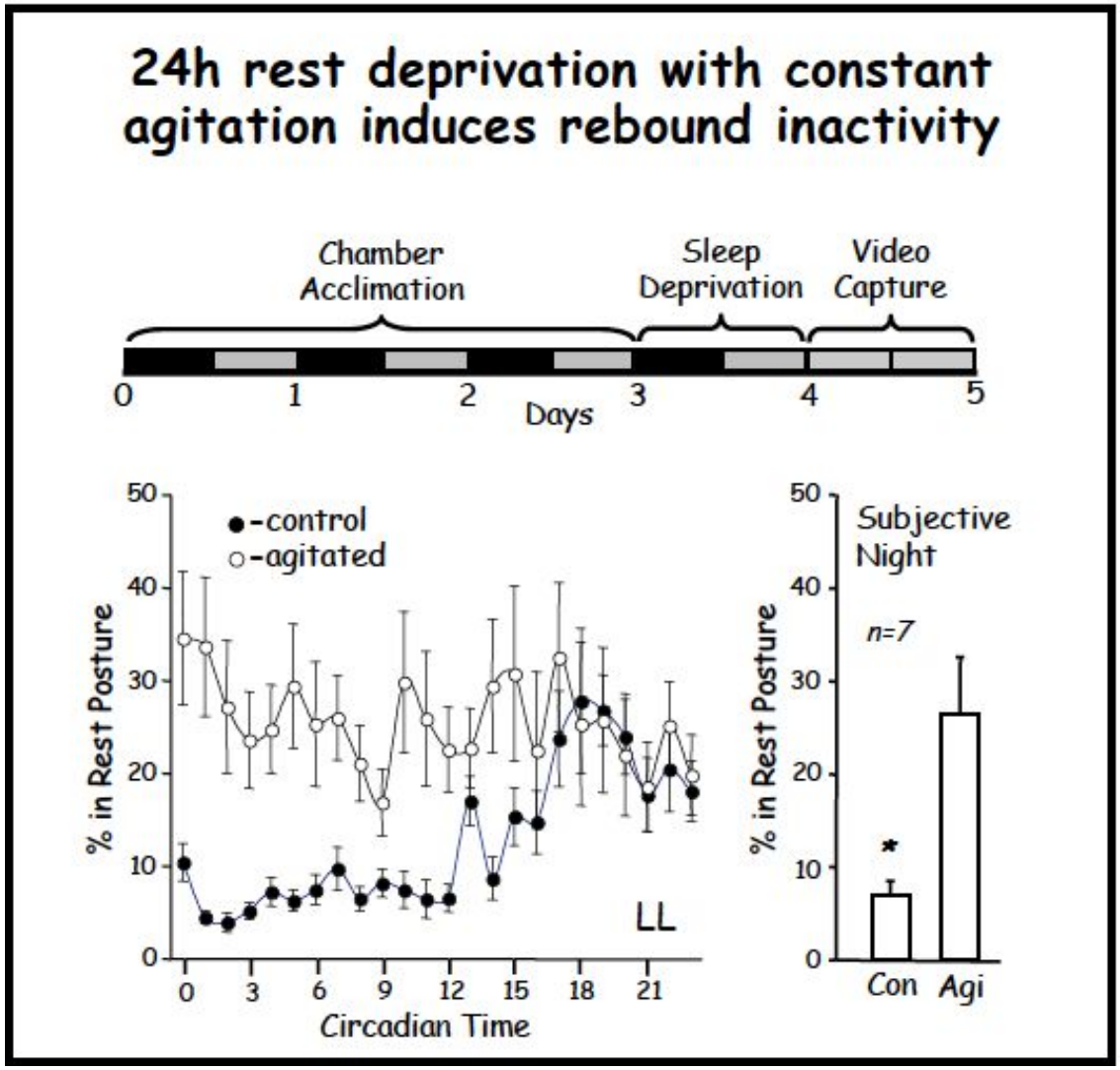

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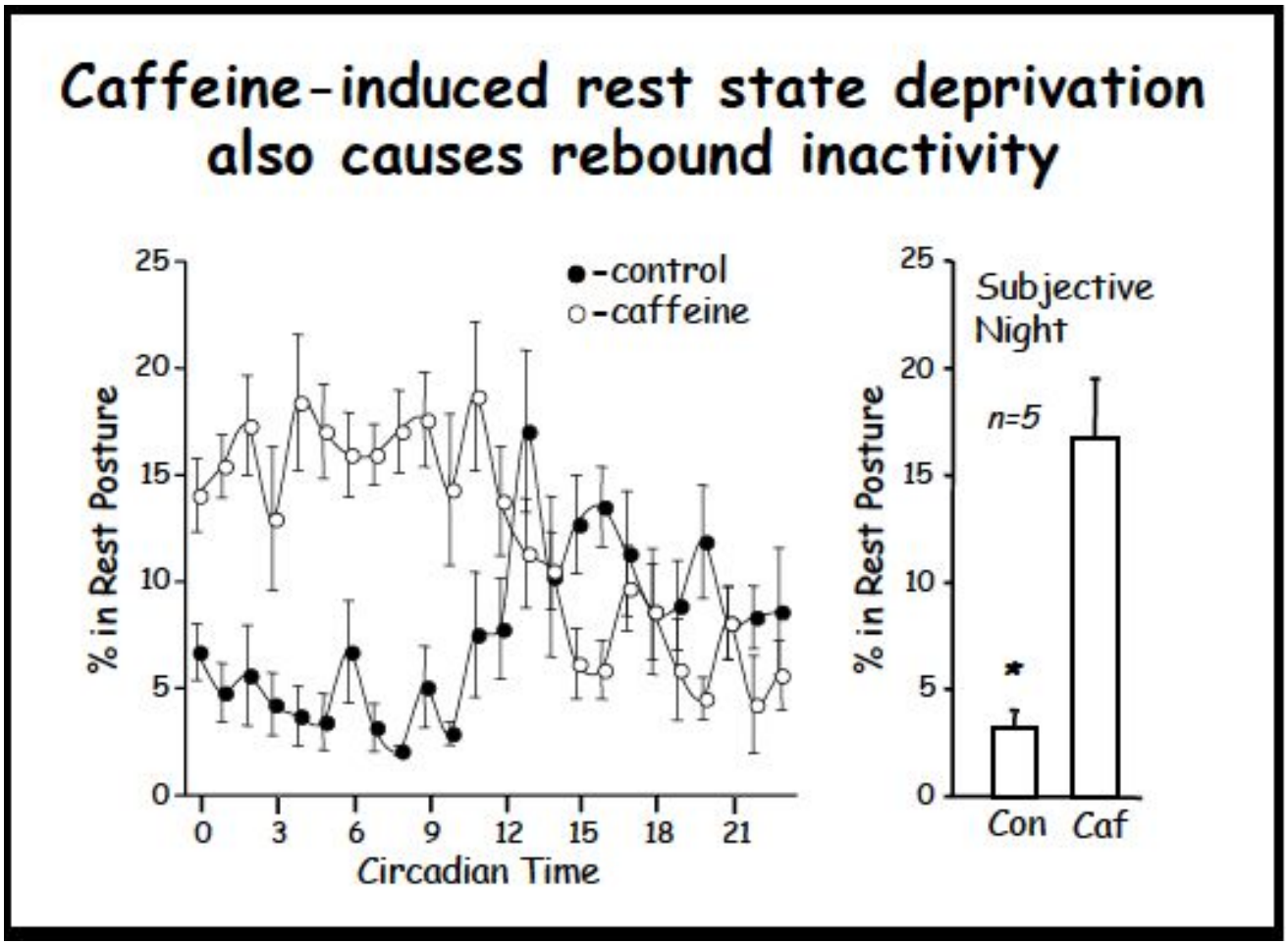

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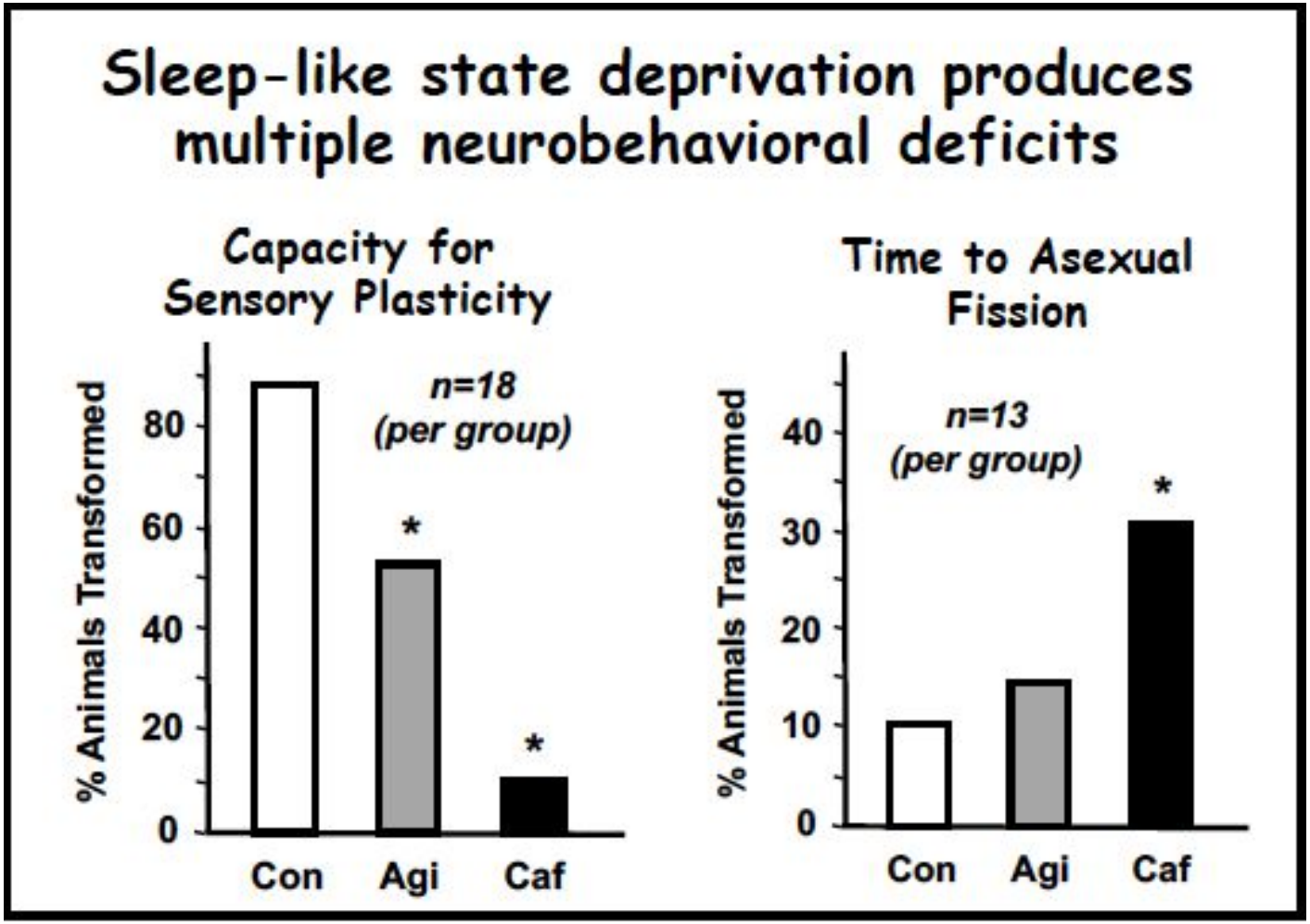

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Conclusions

- **Lumbriculid worms have a sleep-like behavior that involves most core sleep characteristics, including prolonged inactivity, a stereotyped posture, and rebound inactivity following deprivation of rest. It is also regulated in a circadian fashion with a 25 hour period.**

- **Rest-state deprivation is accompanied by neural and behavioral deficits, suggesting a role for this sleep-like state in central nervous system function.**