# Scientists establish leech as model for study of reproductive behavior

March 16 2010

Researchers at the California Institute of Technology (Caltech) and the University of California, San Diego (UCSD) have discovered that injecting a simple hormone into leeches creates a novel way to study how hormones and the nervous system work together to produce species-specific reproductive behavior.

A paper describing the work appears in the March 11 online edition of the journal Current Biology.

Daniel Wagenaar, Broad Senior Research Fellow in Brain Circuitry at Caltech and first author of the paper, found that injecting a particular hormone into a medicinal leech (*Hirudo verbana*) induced a series of movements that closely mimic natural reproductive behavior, including a stereotypical 180-degree twisting of the body. Wagenaar's studies were initiated at UCSD.

The twisting, which occurs with a period of approximately five minutes—making it one of the slowest behavioral rhythms ever discovered, aside from diurnal and annual rhythms—serves to align the reproductive pores on the ventral (under) side of one leech with the complementary pores on the ventral side of a partner, thus facilitating copulation. Without this behavior, copulation would fail.

"In many animal species, sexual reproduction involves highly specific and complex behaviors at all stages from courtship to copulation and beyond," Wagenaar says. "Most animals perform these behaviors

without any learning, which strongly suggests that the behaviors are somehow 'hardwired' in their nervous systems."

The relationship between the activity of nerve cells and leech behavior has been very well studied, and the simplicity of the leech nervous system, which contains only about 15,000 neurons—orders of magnitude fewer than even a mosquito—has greatly facilitated this work.

The studies described in Wagenaar's paper were inspired by the combination of the complex behaviors of leeches breeding in the laboratory and its relatively simple nervous system.

Reproduction is one of the most important activities of all animal species, Wagenaar notes, but in leeches, as in other sexually reproducing species, it has proven difficult to understand how this critical behavior is produced by activity in the nervous system.

"Few animals will execute reproductive behaviors while they are being subjected to neurobiological recording methods," Wagenaar says.

Wagenaar and his colleagues got around the relative reticence of the leeches by injecting them with a type of hormone found in a wide variety of animals. In humans and in other mammals, two versions of this hormone—vasopressin and oxytocin—play a powerful role in reproductive physiology and pair-bonding. Leeches also produce a member of this hormone family, called hirudotocin. The groups at UCSD and Caltech discovered that the hormone plays a role in normal leech mating behavior.

Within minutes after a leech has received an injection of hirudotocin, it displays a variety of courtship behaviors, even if it is alone in a container. During courtship, leeches open their mouths wide and explore the bodies of potential partners by running the mouth along the skin,

while also twisting their bodies like a corkscrew. These behaviors were known to be elicited by hirudotocin and other closely related members of the vasopressin molecular family.

"Hirudotocin is produced by the leech, but under ordinary conditions it may be present in very small quantities," Wagenaar says. "By injecting a relatively large quantity of the hormone, we may, in a sense, overwhelm the system. Whereas small doses only increase the tendency toward the behavior, allowing other cues to override it (as in the natural case), larger doses make this tendency so strong that nothing else can get in the way."

Using progressively more reduced leech preparations—that is, smaller and smaller pieces of a leech—the scientists identified the part of its central nervous system responsible for generating the mating behavior. "One of the attractions of the lower invertebrates is that you can literally cut them in pieces, and each of the pieces will more or less keep performing the function it would have performed in the whole animal," Wagenaar explains.

"We started out studying the behavior of whole animals that we simply injected with the hormone. Then we cut leeches in thirds and injected each part with hormone, and found that the hormone acted only in the central part, which contains the reproductive organs. We then cut open that central part and stretched out the skin so we could study in more detail the muscle contractions underlying the behavior of the whole animal."

"Finally," he says, "we removed the body entirely, keeping just the nervous system, and found that even the disembodied central nervous system"—in particular, the ganglia (clusters of nerve cell bodies) located in the reproductive segments of the leech—"produced the appropriate nerve signals to generate the pattern of muscle activity we had observed."

"Our next project will be to use voltage-sensitive dyes to record signals from a large fraction of all the neurons in the reproductive ganglia, to find which ones contribute to generating and maintaining the behavior," he adds.

Wagenaar and his colleagues believe these studies establish the leech as a new model system for studying how hormones act on the nervous system to produce mating behavior, and for deciphering the specific neural circuits that control the behavior.

"The knowledge gained from these studies," adds study coauthor Kathleen French of UCSD, "is expected to shed new light on the interactions of hormones and neurons in controlling courtship and reproductive behavior in a wide variety of sexually reproducing species, from the lowly leech to humans in a singles bar."

Provided by California Institute of Technology