Octopus Hypnosis: An Alternative to Global Anesthesia

by

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Short title: Octopus Hypnosis

Keywords: octopus, hypnosis, anesthesia,

ABSTRACT:

Effective methods of anesthesia for octopuses are important for physiological studies as well as for their welfare in scientific research. Neurophysiological studies of octopus usually use global anesthesia for invasive procedures like electrode implantation. However, commonly used forms of global anesthesia using ethanol, magnesium chloride, and similar agents raise certain concerns for levels of stress, recovery, and tissue viability in octopuses much as in humans. We explore an old, rarely used method of octopus "hypnosis", in which a still reaction is induced so that communication between central and peripheral nervous systems is seemingly decreased. We modify the procedure for better handling, continuous respiration, and isolation of peripheral nervous system of the arm cords from the central nervous system (CNS), providing alternative to global anesthesia for studies of arm-sucker coordination and electrode placements. The modified procedure limits negative side-effects, markedly reduces animals' stress levels, and offers new avenues for octopus neurobehavioral research.

INTRODUCTION:

Effective methods of anesthesia are critical to physiological studies of octopus in scientific research as well as to their welfare. Anesthesia, defined as loss of sensation to the body, is indicated by lack of response to noxious stimuli as well as a state of neural depression [1,2]. Here we describe "octopus hypnosis", a still reaction induced by mild restraint in a mouth-side up position and involving significantly reduced stress and rapid recovery. As an anesthetic method, it allows minor invasive manipulations like electrode implants and prolonged measures of peripheral behavior in the arms and suckers.

Global Anesthesia

Ethanol mixed in sea water at 0.5% - 2% is commonly used global anesthetic for octopuses [3]. However, some concerning negative effects of ethanol anesthesia have been observed. In particular, ethanol anesthesia sometimes causes some stress in the animal. Even with very slow introduction of the ethanol solution, the animal may become noticeably agitated (increased ventilation, jetting water, and escape reactions), and as the anesthesia takes effect the octopus may also ink. We have also noticed a recurring problem with experiments done during ethanol anesthesia: in several instances, an arm implanted with electrodes or isolated seems to show decreased spontaneous behavior and sensitivity even after recovery from ethanol anesthesia. For instance, the arms of an octopus implanted with electrodes under ethanol anesthesia appear to show increased injury responses even after recovery from the anesthesia, including having less fine motor control over the implanted arms with significantly diminished neuromuscular responses to stimuli in the arm, increased curling, and tucking of the implanted arm underneath its web. This apparent increased injury response could possibly be due to activation of sensitized nociceptive afferents in the arm following ethanol washout [3-5]. Further, arms amputated under ethanol anesthesia and moved to fresh seawater show severely diminished responses to sensory stimuli and even electrical stimulation, suggesting that the ethanol solution may also produce cytotoxic damage in exposed tissue.

Magnesium chloride solution ($MgCl_2$; 330 mM, iso-osmotic to sea water and the animal's blood, pH 7.6) is also commonly used for octopus global anesthesia and seems to lack some of the

negative effects of ethanol anesthesia, such as behavioral stress responses. However, the divalent magnesium ion is sticky and typically more difficult to wash out from exposed tissues than ethanol , and recovery from global $MgCl_2$ anesthesia is quite slower. Moreover, recovery from both $MgCl_2$ and ethanol anesthesia can produce behavioral changes in the octopus. Typically, several minutes after an octopus recovers from ethanol anesthesia back in its home tank, it will rarely take food when offered. In contrast, recovery from hypnosis usually takes under a minute, and often the octopus will readily take food when offered in its home tank. For these reasons, both ethanol and $MgCl_2$ global anesthesia pose certain concerns for neurobehavioral studies of the intact or isolated octopus arm. Here we introduce a modified procedure of octopus hypnosis that serve well as an alternative to global anesthesia for certain experiments.

Octopus Hypnosis

Octopus "hypnosis" was first described by Vasily Danilewsky in 1890 in a description of handling-induced still responses in various animal species [6], and then reproduced by J. Ten Cate in 1928 [7]. In such responses in our hands, octopuses assume a calm, relatively stationary state, with largely only local responses to sensory stimuli or even local $MgCl_2$ injections. To our knowledge, this method has not been revisited until the present study. In the original studies, hypnosis was induced by holding the octopus out of water for an extended time, mouth-side up and making sure that the arm suckers did not come into contact with anything to receive stimulation [7]. We have modified the hypnosis procedure to further minimize stress: instead of holding the octopus out of water, we make sure it is nearly fully immersed to maintain its respiration and we use a soft net or mesh-material to gently hold the animal mouth-side up close to the surface of the water.

Octopus hypnosis is not an actual global anesthesia. However, the procedure is preferable to ethanol and $MgCl_2$ anesthesia in many instances. Recovery from hypnosis is much quicker and more complete, with the animal often taking food right after recovery. It avoids the sluggish and aversive behavior often accompanying ethanol anesthesia and may also prevent potential ethanol-induced, cytotoxic tissue damage. We find that hypnosis combined with local $MgCl_2$ injection is better than ethanol anesthesia for arm nerve cord isolation from the brain and electrode implantation . Ethanol seems to negatively affect the quality of electrophysiological activity as well as arm behavior. Moreover, the hypnosis procedure has so far never suppressed the animals' respiration, as sometimes happens with ethanol.

While octopus hypnosis can take more time and effort to induce than ethanol or $MgCl_2$ anesthesia and appears ineffective for senescent animals, our findings suggest that this method can often serve as an alternative to the most common global anesthesia methods, that it has significant potential for promoting octopus welfare, and that it may facilitate novel neurobehavioral studies.

METHODS AND RESULTS:

Animals

Specimens of *Octopus rubescens* trapped in Monterey Bay, CA were obtained from Monterey Abalone Co. (Monterey, CA) and housed separately in artificial seawater (ASW) at 11-12°C. Specimens of *Octopus bimaculoides* were obtained from Chuck Winkler of Aquatic Research

Consultants (Long Beach, CA), him, and housed separately in ASW at 18-22°C. 17 female octopus (2 *O. bimaculoides* and 15 *O. rubescens*, ranging from 20 to 200 grams) were used in these experiments. Animals were fed pieces of shrimp or squid flesh every 1-2 days. All experiments were carried out in accordance with protocol #23015 approved by the University of Illinois Urbana-Champaign (UIUC) Institutional Animal Care and Use Committee (IACUC).

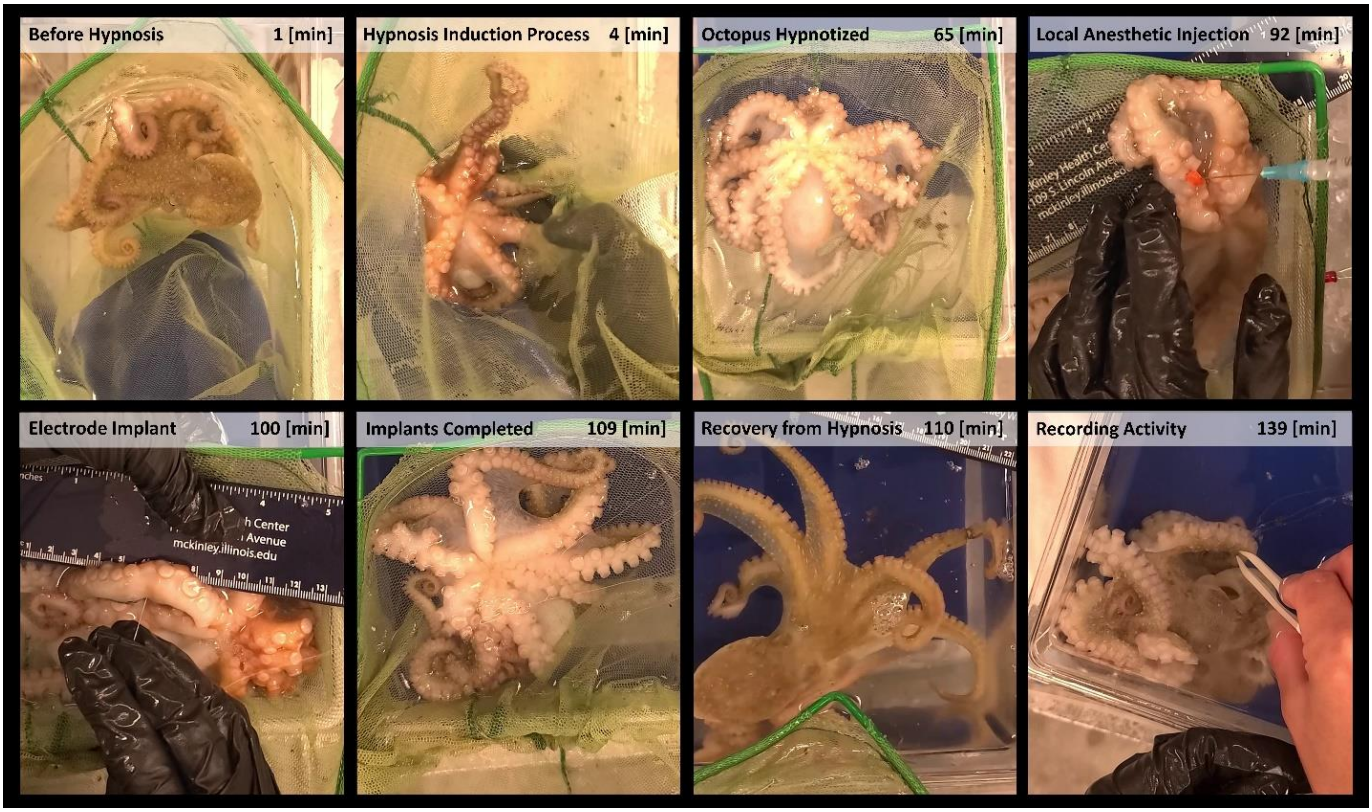

Figure 1. Still images from **Supplementary Video S1**, showing the octopus hypnosis procedure and electrode implantation in a *O. rubescens*: induction of hypnosis (t = 1 to 65 min), followed by local anesthesia with $MgCl_2$ injection (t = 92 min), electrode implantation (100-109 min), recovery from hypnosis (t = 109-110 min), and recording of neuromuscular activity from implanted electrodes (t = 110-139 min).

Octopus Hypnosis Protocol

Our modified protocol for octopus hypnosis is demonstrated in **Supplementary Video S1** and is shown in **Figure 1**. The octopus is first placed in a large hand-net and/or soft mesh material (such as pantyhose) inside a container with aerated ASW maintained at appropriate temperature for the species (11-13°C for *O. rubescens* and 16-22°C for *O. bimaculoides*). The octopus is gently turned mouth-side up and kept close to the surface of the water by adjusting the net mesh, ensuring that the head and mantle are always submerged and that the octopus breathes. Maintaining an optimal water level is critical, such that the submersion is sufficient for breathing but not enough to fully cover their arms or mouth. If, during hypnosis induction, the octopus attempts to flip back over and move to the edge of the net, we gently move it back to the center of the net to maintain its mouth-side-up position by adjusting the net. This is repeated as needed. Typically, as the hypnosis induction process continues, the octopus stays still for longer times and attempts to flip over are less frequent. Positioning the octopus in a cradle-like manner, keeping the arms and suckers at or

above the surface of the water, covering their eyes and generally limiting exposure to visual stimuli appears to help the hypnosis induction process. The hypnosis induction process is continued until the octopus remains mouth-side up with its arms and body still except for breathing for at least 5 minutes. At this stage we define the hypnosis as successful if we can lightly touch and squeeze one of the octopus' arms and only observe local responses, such that only the stimulated arm moves without recruiting activity in the other arms and the animal shows no other changes in its behavior. Notably, the responses of a hypnotized octopus are markedly similar to those of a sleeping octopus.

Local Anesthesia

Hypnosis facilitates administration of local anesthetics, such as $MgCl_2$ injection in an arm. Local arm anesthetization was done by injecting the base of an octopus arm with iso-osmotic 330 mM $MgCl_2$ solution with 10 mM HEPES buffer, pH = 7.6 [5]. This temporarily disrupts communication between the octopus's arm peripheral nervous system (PNS) and its central nervous system (CNS). This has been tested in both hypnotized and awake animals by strong tactile stimulation of the injected arm without other recruited responses in the octopus itself or its other arms. Successful anesthetization may also commonly result in a blanching of the octopus arm distal to the injection site. Typically, $MgCl_2$ injection using thinner needles (30 ga), more precise depth control (aided with rubber stopper on needle) to target the arm central nerve cord, and relatively small volumes (0.1 ml for the arm sizes of the small animals we worked with here) yielded more successful arm anesthetization.

Use of Octopus Hypnosis in Broader Experiments

Sucker Stimulation in Hypnotized Octopus

A marked advantage of octopus hypnosis in behavioral studies is that local responses of the arms are preserved, unlike for global anesthesia. In a successful hypnosis the octopus is quite still in its mouth-side-up position with suckers easily accessible and where moderate tactile stimulation produces only local arm responses that have little or no effect on all other arms or the octopus' body. Thus, octopus hypnosis is particularly suitable for characterizing local responses in the arm, as seen in our experiments with sucker orienting responses.

Following hypnosis induction, sensory stimulation of suckers was done in locally anesthetized and non-anesthetized arms. A blunt-end syringe was used to precisely deliver chemotactile stimuli to octopus suckers, such as either an ASW jet or squid extract, both mixed with red food dye for tracking of chemical plumes. Plumes of squid extract observed to contact the arm and suckers at specific points elicited approach or avoidance responses in which the suckers extended towards or retracted away from the plume, recruited neighboring suckers, and even produced arm retraction and reaching behaviors (**Fig. 2A, left**). While a similar range of behaviors was observed in both anesthetized and non-anesthetized arms, it remains to be explored whether there are differences in approach/avoidance behaviors dependent on CNS-PNS communication, internal appetitive state of the octopus in terms of hunger and stress, as well as in distal/proximal sucker stimulation. We expect the hypnosis procedure to facilitate further relevant experiments.

A sucker responds to a single point stimulus (tactile, chemical, or electrical) on its outer rim with a very precise movement directly towards or away from the stimulus. It appeared to us that the sucker could estimate the bearing to a single point stimulus and use it to generate very accurate motor output for approach/avoidance. Previously we and others have observed that responses in arms isolated by amputation rapidly decline, and therefore we tested arms in intact animals for two-

point stimulation. $MgCl_2$ was injected under hypnosis in a proximal arm site. Keeping the octopus in an convenient position for extended time periods, we conducted experiments with two-point stimulation with two bipolar concentric electrodes and a Grass S-5 stimulator to deliver simultaneous shocks (bipolar pulses of 1.5 ms duration, ranging 1-6 V in amplitude, in single-pulse regime or 10 Hz train of pulses ranging in duration from 0.5-3 sec) at two different sites on a single sucker's outer rim (**Fig. 2A, right**). While data in these experiments are still being gathered, early results suggest the sucker produces a winner-take-all response [8,9] to only one of the two loci stimulated, rather than averaging the two response sites to move at an angle between them as has been observed in turn responses of the sea slug *Pleurobranchaea californica* [10,11]). These results are consistent with circuit architectures in the sucker like lateral inhibition [12,13] and are useful in modeling work. In future, we hope to elucidate the mechanisms behind sensory processing in the local circuitry of the sucker, as well as recruitment of neighboring suckers.

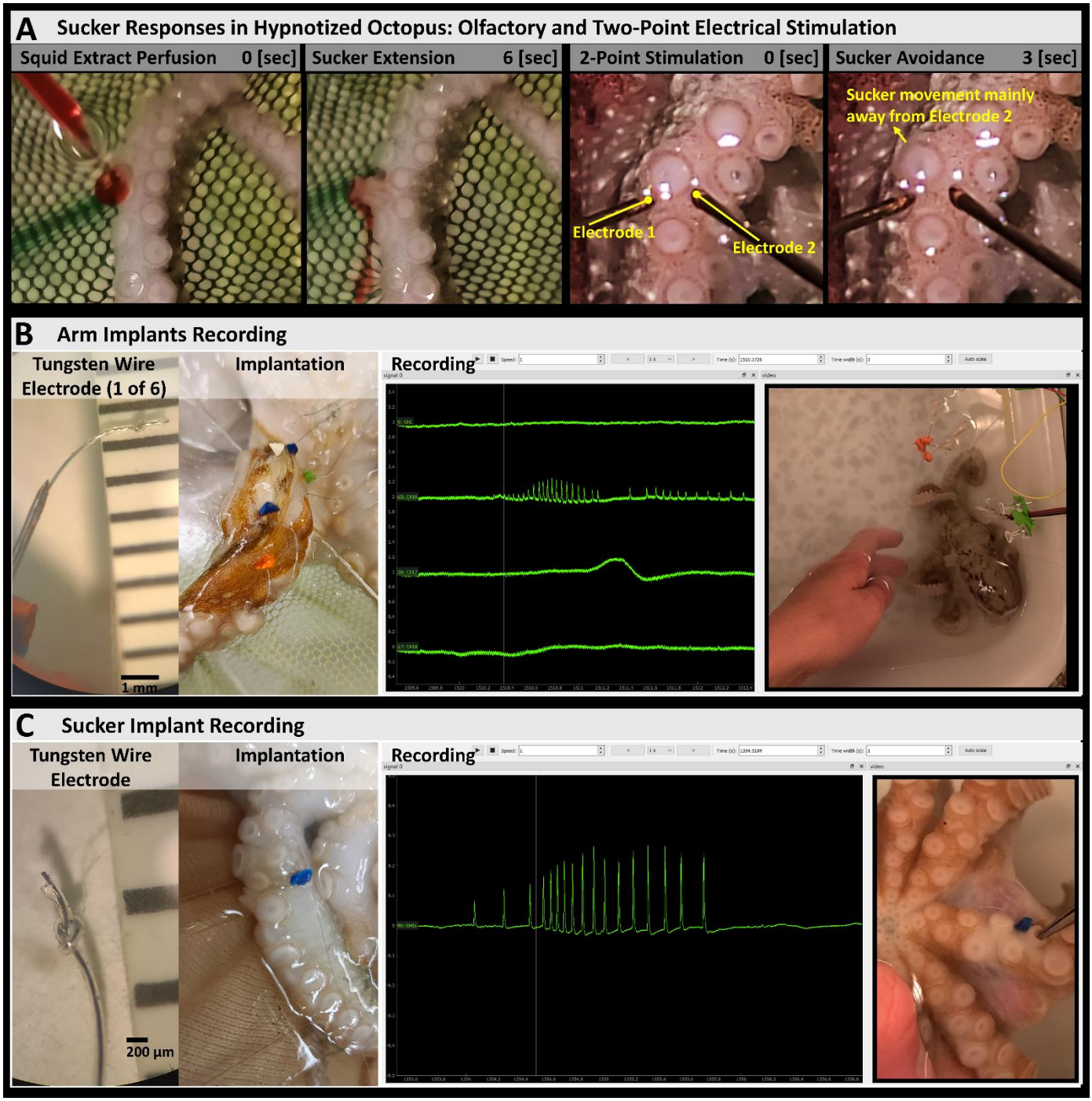

Figure 2. Application of octopus hypnosis in broader experiments. **A)** Hypnosis facilitated the testing of sucker responses to different stimuli. The left panels show a chemical stimulus (squid homogenate, with red food dye) being perfused primarily onto one sucker at t = 0 sec, followed by a precise (appetitive) extension of the sucker towards the chemical stimulus (t = 6 sec), with a few slight movements in neighboring suckers, and no noticeable responses in other arms. The right panels show two bipolar concentric electrodes positioned on the outer rim of a sucker (labels in yellow). A train of electrical pulses (at 10 Hz, each pulse 4 V and 1.5 ms duration) was delivered through both electrodes simultaneously starting at t = 0 sec and lasting for about 1.5 sec. This caused aversive movement of the sucker (t = 3 sec), primarily away from the rightmost electrode. **B)** Octopus hypnosis was combined with local anesthesia ($MgCl_2$ injection) to implant tungsten wire electrodes in the octopus arm and obtain electrophysiological recordings in a freely-moving octopus. **C)** Octopus hypnosis was used to facilitate the implantation of a tungsten wire electrode in an octopus sucker, as well as for testing sucker electrophysiological responses with tactile stimulation.

Electrophysiology in Freely-Moving Octopuses.

Tungsten wire electrodes were implanted into octopus arms for recording electrical activity. Electrodes were PFA-coated tungsten wire (0.1 mm diameter, A-M Systems) with a terminal half-hitch knot to anchor in the tissue (**Fig. 2B-C**). For implantation (**Supplementary Video S1**, **Fig. 1**), after hypnosis chilled 330 mM $MgCl_2$ solution was injected at the base of the arm to disrupt arm-brain communication. The wire electrodes were carefully inserted into the tissue using a 30 ga needle (with an optional rubber stopper on the needle for additional control over implantation depth) [14-16]. Data were recorded with a headstage (Intan RHD 32-channel) and an acquisition board (Open Ephys) with a sampling rate of 20 kS/s and amplifier hardware bandwidth from 0.1 to 7600 Hz. Videos were recorded with a phone camera at 30 fps, and synchronized with electrophysiological recordings using a simultaneous visual and electrical signal: the flashing of a light-emitting diode (LED) coupled with a 5 V stimulator. Synchronized recordings were visualized and reviewed using the Python library ephyviewer [17,18] (ver. 1.5.1, Python v. 3.9.7) , with the standalone viewer slightly modified to display both electrophysiological recordings and video. Modified code is available at https://github.com/KatyaGribkova/ephyviewer.

The arm implant recordings showed spiking activity during arm movements in a freely moving octopus, while the implant by the sucker base (expected near the sucker ganglion) recorded the most activity with inner-central tactile stimulation of the sucker (**Fig. 2B-C**). To our knowledge, this may be a first successful sucker electrode implantation and recording.

Variability in Octopus Hypnosis Onset and Duration

There was appreciable variability in latency to hypnosis onset as well as hypnosis duration across the animals we worked with. We define “latency to hypnosis onset” as the time taken for the octopus to achieve an uninterrupted still state of at least 5 minutes, and with only local responses to stimuli as described earlier in the Octopus Hypnosis Protocol. "Hypnosis duration” is the length of time that the hypnosis was maintained in any particular experiment, whether spontaneously recovered or interrupted by the experimenter to test procedures (electrode implantation, sucker stimulation, etc.).

Multiple factors may affect latency of hypnosis onset, including octopus species, age, size, circadian rhythm, senescence, hunger, and other characteristics and dynamic internal states of the octopus. Notably, we approximated the masses of the octopuses used in hypnosis experiments, based on previous mass measurements and still images of the hypnosis procedures, and compared them against the latencies (**Fig. 3A**). There is a significant positive correlation between octopus mass and latency of hypnosis onset (Pearson correlation test: $r(19) = 0.714$, $p < .001$). This is explainable in a number of ways, including difficulty in handling larger octopuses as well as secondary factors that also correlate with size, like age and senescence.

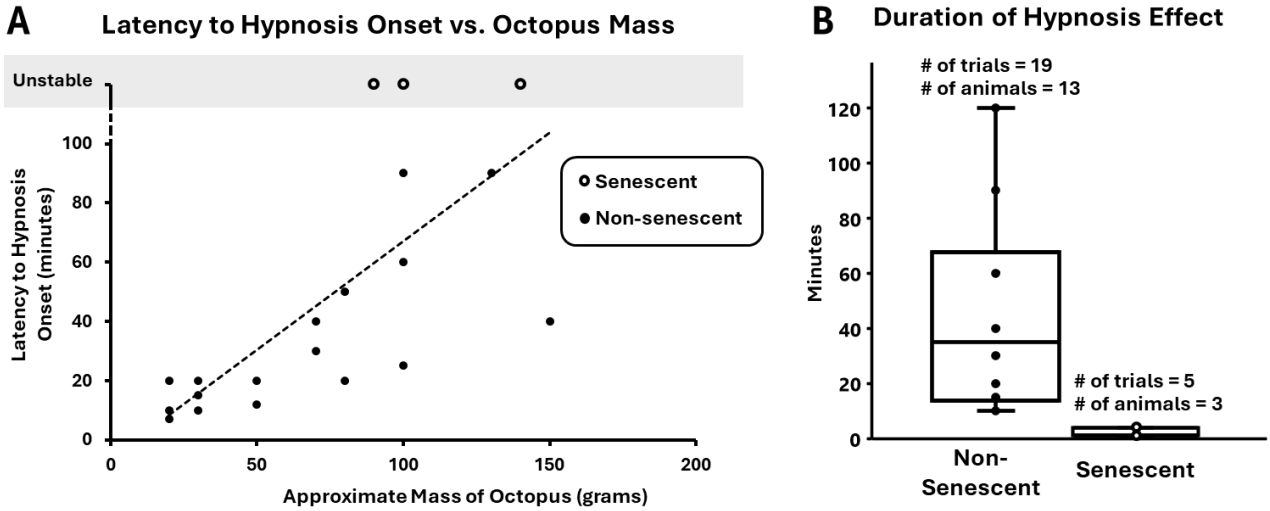

Figure 3. Exploring the variability of octopus hypnosis onset and duration. **A)** Latency to hypnosis onset has a strong positive correlation with approximate octopus mass (Pearson correlation test: $r(19) = 0.714$, $p = 0.000116$). **B)** While the number of observations for clearly senescent octopuses is presently small, hypnosis for these animals was quite unstable: still states were maintained for less than five minutes, whereas in non-senescent animals still states were maintained for at least 5 minutes, and up to 2 hrs, with a significant difference (Mann-Whitney U test: $U = 80$, $p = 0.00112$).

In particular, while our number of observations for clearly senescent octopuses (1 *O. bimaculoides*, and 2 *O. rubescens*, all which had laid eggs) was quite limited, we observed striking differences when attempting to induce hypnosis with these animals. In 5 trials with these senescent octopuses, we were unable to maintain a still state for more than 5 minutes (**Fig. 3B**), even though most of our attempts lasted more than 2 hrs (**Fig. 3A, unstable**). While senescent animals could be sufficiently calmed to conduct certain experiments, such as testing of sucker orienting responses, they seemed much more likely than non-senescent animals to respond to light arm touches or squeezes with more global responses, involving recruitment of other arms. This may be due to neurobehavioral changes that may occur with onset of senescence in octopuses, particularly those associated with the care and guarding of octopus eggs.

CONCLUSIONS:

In summary, the still reaction induced by the hypnosis procedure as we describe it has distinct advantages over global anesthesia where strongly invasive surgical procedures are not necessary.

While global anesthesia with ethanol and $MgCl_2$ is sometimes essential in octopus research, there are certain negative effects of these most commonly used methods that can be avoided with octopus hypnosis. Notably, global anesthesia affects both CNS and PNS function and impedes testing of local sensorimotor responses. To our knowledge, our modification of octopus hypnosis (**Fig. 1**) may be the only reported method to sufficiently calm non-senescent octopus to test local sensorimotor responses. With hypnosis, even without local anesthesia, there seems to be decreased communication between octopus CNS and PNS, such that lightly squeezing the arm of a sufficiently hypnotized octopus generally produces only local responses, and no responses in other arms or body. This effect of hypnosis facilitates the administration of local anesthetic like injection of $MgCl_2$

solution, and since the octopus lays still in its mouth-side-up position, it offers a unique opportunity for testing local arm and sucker responses (**Fig. 2A**). Thus, isolation of the arm from the brain and the rest of the peripheral nervous system to test autonomous behavior may be easily gotten without amputation in hypnotized animals by local $MgCl_2$ injection. Further, the combination of local anesthesia and octopus hypnosis greatly facilitates implantation of electrodes for recording neuromuscular activity (**Fig. 1**, **Fig. 2B-C**). In our various experiments, we also noted interesting variability in octopus hypnosis onset and duration, closely correlated with both octopus size and senescence (**Fig. 3**). It seems that hypnosis may basically be ineffective for senescent animals; however, further testing is needed to explore this difference.

Our modified octopus hypnosis procedure can therefore provide new opportunities to advance both octopus laboratory welfare and neurobehavioral studies. There yet remains much to be learned about the mechanisms that give rise to the still reaction. As the procedure involves holding the animals mouth-side up, the vestibular system might be involved. Whatever the mechanisms, if ways could be found to manipulate them directly it might further facilitate experiments to further unravel details of the complex brains and behaviors of these charismatic creatures.

ACKNOWLEDGEMENTS:

This study was supported by Navy N00014-19-1-2373. We are grateful to Trevor Fay, Art Seavey, and the staff of Monterey Abalone Co., and Chuck Winkler of Aquatic Research Consultants for their efforts in animal supply.

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