Title: Characterization of the planarian surface electroencephalogram

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Abstract:

Comparative cognitive science currently strives to examine peak performance in different species. To successfully reproduce biological intelligence in artificial intelligence, it might more constructive to examine the cognitive functions of lower animals. A first step into this direction is the characterization of the ongoing electrophysiological activity. This will then allow testing activity patterns related to stimulus processing and cognition, as well as the reproduction of these patterns in artificial neural networks.

Here, we examine the neural activity of the flatworm *Schmidtea mediterranea*, whose nervous system is organized in quantitative dimensions which could be artificially reproduced right now or in the near future. Planarians have successfully survived for 800 million years, and they are the closest living relatives to the original bilateralians, the first animals with two distinct hemispheres and a well-defined movement direction. They are the first animals to develop a head with a central hub of the nervous system in the form of cerebral ganglia and thus a direct precursor of our brain.

A previous study (Aoki et al., 2009) used invasive monopole recordings in cooled planarians, and observed ongoing activity between 0.1 and 5 Hz with a power spectrum characterized by a 1/f relationship. We extend this observation by recording ongoing electrophysiological activity from noninvasive surface electrodes at room temperature. This procedure allows continuous recordings across longer intervals, and repeated recordings from the same animals without harming the animals to study changes in neural activity linked to stimulus processing and cognition.

**1. Introduction**

Comparative cognitive science often strives to examine peak performance in different species (Gómez, 2005). To successfully reproduce biological intelligence in artificial intelligence, it might be more constructive to examine the cognitive functions of lower animals (Fitch, 2014). This will then allow testing activity patterns related to stimulus processing and cognition, as well as the reproduction of these patterns in artificial neural networks. A first step into this direction is the characterization of the ongoing electrophysiological activity of such lower animals. Remarkably, Adrian (1937) already recorded synchronized neural responses in the optic nerve of a water beetle. Despite large morphological differences between the nervous systems of lower animals and humans, striking similarities have since then become evident (van den Heuvel et al., 2016). For example, honeybees show neural oscillations with a similar functional profile as the prominent human 10 Hz alpha oscillation (Popov & Szyszka, 2020). Moreover, Aoki et al. (2009) were able to record neural activity from the flatworm *Schmidtea mediterranea.* Whereas these previous experiments were able to record a rich spectrum of neural activity, they involved decapitating the animals, opening the head capsule, or implanting invasive electrodes into the ganglia. Therefore, to facilitate the recording of ongoing neural activity, ensure animal welfare and enable repeated recordings of the same animals (e.g., before and after learning), noninvasive recordings are necessary. The aim of the current experiment was to demonstrate the possibility to record neural activity safely and quickly from the flatworm *Schmidtea mediterranea* without harming the animals.

The flatworm *Schmidtea mediterranea*, sometimes also called *Dugesia mediterranea* is a small, approximately 20 mm long 2 mm wide freshwater planarian of the order Tricladida, usually found around the Mediterranean Sea (Harrath et al., 2004). In research, it is often used as a model organism due to the ability to regenerate the central nervous system (CNS) even from small pieces of the body within a short period of time (Agata & Yoshihiko, 2008). This planarian is the simplest animal with a bilateral body plan and cephalization (Sarnat & Netsky, 1985), and can therefore be considered a primitive model organism for the human nervous system. It’s CNS itself comprises a cerebral ganglion, which can be considered the most primitive brain in animal evolution. It consists of two lobed cephalic ganglia which are connected by the anterior commissure. In addition, a pair of ventral nervous cords run the length of the animal, and both pairs are connected by transverse commissures (Fraguas et al., 2012). The cephalic ganglia appear to be remarkably complex, and Aoki and colleagues (2009) were able to record ongoing neural activity characterized by a 1/f power spectrum using direct recordings from electrodes implanted in the ganglia.

Whereas the CNS of *Schmidtea mediterranea* is comparatively simple and resembles that of the early developmental stages of the CNS of vertebrates (Agata et al., 1998), the animal is nevertheless capable of complex behavior, learning and memory (Deochand et al., 2018). For example, the animal show long term memory in the form of environmental familiarization, which persists across two weeks, and regeneration of the cephalic ganglia (Shomrat & Levin, 2013). Moreover, the planarians are mobile, avoid open spaces and are negatively phototactic (Talbot & Schötz, 2011), which promised at least basic cognitive capabilities such as sensory discrimination and decision making. While previous research showed ongoing neural activity, the cognitive ability to discriminate stimuli and form long-term memory, it is currently unknown how these cognitive abilities are reflected in neural activity. As a first step towards shedding light on this issue, we here aimed to establish a recording protocol to record ongoing neural activity safely and securely from alive and healthy planarians under different lighting conditions. As a replication of the results by Aoki and colleagues (2009), we hypothesized that the ongoing neural activity is characterized by a 1/f power spectrum, and that changes in lighting will induce changes in neural activity due to the reported photophobia.

**2. Methods**

The aim of the current experiment was to establish a recording protocol to noninvasively measure ongoing neural activity from live planarians without harming the animals. To this end, we developed a recording chamber and used mobile electroencephalography (EEG) device to record the electrical signal from planarians in darkness, under light, and from dead mealworm larvae as a control group.

**2.1 Animal characteristics**

Overall, we collected data from 36 planarian of the species *Schmidtea mediterranea*, of which 17 were recorded during darkness, and 19 were recorded under illumination. All planarians were bred for laboratory use and reared in our dedicated breeding facility at the Christian-Albrechts-University Kiel. As a control group to test how the recording setup responds to environmental noise, we recorded data from six mealworm larvae purchased as food for our reptilians.

**2.2 Recording environment**

All recordings took place in the animal laboratories of the department of psychology at the Christian-Albrechts-University Kiel and were conducted by instructed student assiants supervised by specifically trained PhD students. In a first step, we designed and build a 3D-printed recording chamber to hold the electrodes and the planarians (Figure 1). As electrodes, we used wire electroretinography electrodes. The ongoing electric activity was recorded from a mobile EEG amplifier (mBrainTrain Smarting), and each electrode was referenced to the same reference electrode (bipolar montage). The animals were fixated in agarose gel and placed on top of the wire electrodes, so that the recording electrode was close to the head, and the reference electrode was close to the tail of the animal. Please note that the mealworm larvae covered multiple recording electrodes, and these multiple electrodes were treated as independent recordings in the data analysis. The animals stayed on top of the electrodes for approximately 10 minutes, after which they were rinsed off to free them from the agarose gel.

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Results



Discussion