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科研費 KAKENHI

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Brain activity of cephalopods during active and rest states.

研究課題名（英文）Brain activity of cephalopods during active and rest states.

研究代表者

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研究成果の概要（和文）：頭足類に関する体系的な科学研究は１世紀以上前に始まった。頭足類は数多くの学習実験に用いられ、それらの結果から、遊び行動から複雑な学習まで幅広い認知能力を保有していることが明らかになってきた。頭足類の体勢、動き、皮膚の色や構造は、彼らの繊細な行動や学習プロセスを調べるための指標になる。また、これらのプロセスの根底には、巨大で複雑な神経システムが存在し、他の軟体動物とは異なり、巨大脳に制御されている。過去百年にわたって、幅広く様々な麻酔技術が、侵略的または終末的実験に使用されてきた。

研究成果の学術的意義や社会的意義
First unrestrained recording of cephalopod EEG activity. We were also able, for the first time, to connect behaviours and brain activity!
Our study will provide the starting point for more further studies.

研究成果の概要（英文）：Our study aims to establish the first-ever electro potential recordings from the brains of behaving unrestrained octopuses, and the first-ever multi-channel recordings from a cephalopod. Previous studies were limited to animals with implanted wired single-channel electrodes directly connected to an amplifier and recording device outside of the water.
The neurologger is fully contained within the animal, and does not rely on protruding cables, so that the animal can’t tamper with wires or corrupt the recording by altering the location of the electrodes. The octopus is an ideal animal for studying the capabilities of the advanced invertebrate brain because of its complex vertebrate-like behaviors. Octopuses are extensively used for training experiments because they learn rapidly. Their body posture, motion, skin color and texture, eyes and iris permutation indicate subtleties of the learning process.

研究分野：Neuro behaviour

キーワード：Octopus　EEG　Behaviour

１．研究開始当初の背景

Modern cephalopods have large and complex brains. The usage and function of this brain is only partially known compared to other vertebrates and invertebrates.
In mammals, EEG is the accepted method for monitoring brain activity, anesthesia and wake-to-death transition states. It is well known to check activities of certain sections of the brain during different situations and behaviors.
Unlike other animals, cephalopods possess an active camouflage system: they can rapidly change both the texture and pattern of their skin in response to stimuli. The unique anatomy of octopuses, and the incredible flexibility of their arms has made wired recordings of brain activity from freely behaving animals technically unfeasible. In the past year we have developed a novel method for recording from awake behaving cephalopods using implantable small sized neuro-loggers (TSE). We have been able to collect the first multi-channel EEG recordings from the brains of behaving unrestrained octopuses. Our current research has found differences in the EEG power spectrum during different activity states. This advance allows us to record EEG through the transition from awake to anesthetized and death. In cuttlefish studies on the brain and its function have been far more limited (Nixon & Young 2003) as surgery on cuttlefish is very difficult. This is due to a set of blood vessels that run on top of the brain right beneath the central cartilage, which are easily damaged during surgery or by penetrating electrodes. Using the neuro-loggers together with standard electrodes embedded trough the cartilaginous capsule into the brain, we can EEG activity with electrodes either remaining above the brain or inside the brain. This and future recordings will help us to better understand the evolutionary origins of sleep behavior and chronobiology. Additionally, knowing the activity cycle of these animals will be important for any attempts regarding sustainable marine culturing of this highly important species.

２．研究の目的

Our study aims to establish the first-ever electro potential recordings from the brains of behaving *unrestrained* octopuses, and the first-ever *multi-channel* recordings from a cephalopod. Previous studies were limited to animals with implanted wired single-channel electrodes directly connected to an amplifier and recording device outside of the water. An octopus has eight sensitive and maneuverable arms, which can grab and extract an electrode even if just a fragment protrudes from the skin, yielding displacement of the probe, unstable recordings, electrode damage, and abortion of the experiment.

３．研究の方法

The experiments are divided into two categories, behavioral experiments and neurophysiological recordings of brain activity during different activity states. The first set of behavioral studies will lay the foundation for the later neurophysiological recording experiments by establishing diurnal activity and rest/sleep states in *Octopus cyanea*. In the successive neurophysiological recording f experiments these behaviors will be linked to the recorded EEG activity of the central brain of the octopus. Due to the descriptive nature of the initial experiments and the complete lack of any data for this species no problems are expected during the first experiments. The second more challenging experiments will always be discussed with a group of outside experts to make sure problems are detected early and counteracted. However, like in the first study due to the complete lack of any data on the EEG activity of these octopuses all findings are novel and exiting.

O. cyanea is described in the literature as a day-active octopus, but detailed descriptions of activity patterns *in the laboratory* are missing. Filling this gap will be the first task of our study. Subsequently, we will implant into the octopus brain an MCE neurologger recently developed by our collaborator, Prof. H.P. Lipp of ETH, Zurich, which he applied successfully to free-flying birds, to enable multichannel recordings from an unrestrained cephalopod. We have adapted neurologger for cephalopods: (i) the batteries were changed; and (ii) watertight cases for the logger were designed and fabricated to our specification by the mechanical workshop at OIST.

４．研究成果

Behavioural assays

Unlike other animals, cephalopods possess an active camouflage system: they can rapidly change both the texture and pattern of their skin in response to stimuli. Thus, behaviour in response to the three different protocols can be documented in detail. Several behavioural criteria for effectiveness of methods will be documented: Response to stimuli (handling; prick), reflexes (breathing, equilibrium, muscle tone, chromatophore activity). Additionally, to determine basal stress levels of each individual, response to positive and negative stimuli will be evaluated before/during EEG recording experiment (see below).

While for several species (*O. vulgaris, S. officinalis*) there is much previous research to build upon, for the tropical species this information will be new.

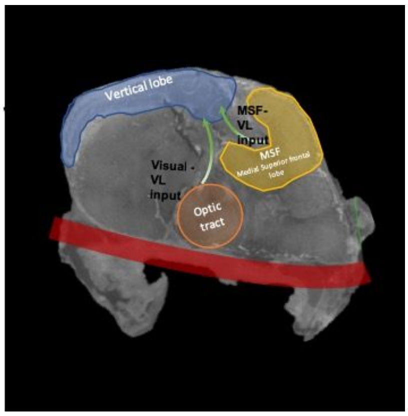
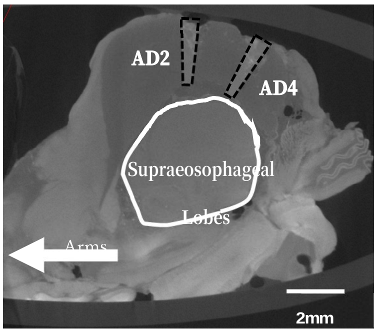


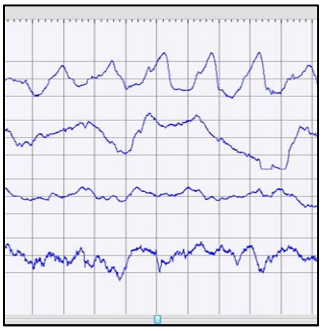
Figure : microCT image of octopus brain (*Octopus cyanea*)

EEG recording

In mammals, EEG is the accepted method for monitoring anesthesia and wake-to-death transition states. It has been shown to be useful in assessing anesthesia and ethical slaughter in fish. One advantage as an addition to behavioral monitoring is revealing differences between anesthesia and paralysis.

The unique anatomy of octopuses, and the incredible flexibility of their arms has made wired recordings of brain activity from freely behaving animals technically unfeasible. In the past year we have developed a novel method for recording from awake behaving cephalopods using implantable small sized neurologgers (TSE). We have been able to collect the first multi-channel EEG recordings from the brains of behaving unrestrained octopuses (Fig. 1). Our current research (Gutnick et al. personal communication) has found differences in the EEG power spectrum during different activity states. This advance allows us to record EEG through the transition from awake to anesthetized and death. In cuttlefish studies on the brain and its function have been far more limited as surgery on cuttlefish is very difficult. This is due to a set of blood vessels that run on top of the brain right beneath the central cartilage, which are easily damaged during surgery or by penetrating electrodes.





2 a. microCT section of cuttlefish brain (supraoesophageal lobe) showing gold screw EEG electrode locations in the cartilage (AD2 and AD4). b: Short section of an EEG trace (10 sec window ; 4 channels), recorded from an cuttlefish implanted with a neurologger.

Using the neurologgers together with standard gold screw electrodes embedded deep in the cartilaginous capsule, we can EEG activity with electrodes remaining above the brain (Fig. 2a, b).

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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