Original Research Article

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20201068

Decerebration induced by surgical transection of cerebral ganglion of crayfish

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Received: 01 March 2020 Accepted: 06 March 2020

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ABSTRACT

Background: Since the neural structures of the crayfish brain closely resemble their equivalent in the mammals. This can be suggested by observing the similarity that exists in the brain divided by the surgical transection of the crayfish brain in which the protocerebrum remains attached to the first two cranial nerves, findings also described by Frederic Bremer in 1935 in cats with cerebral transection.

Methods: Total 11 Adult male crayfish were trained to respond with defense reflex, the animals were placed in water at 0°C, remained without any movement, and subsequently through a small incision of 3 mm in diameter in the medial antero region and dorsal cephalothorax region, a surgical section of the cerebral ganglion was performed. Immediately after surgery, metal microelectrodes were implanted to collect the activity of the photoreceptors and visual fibers.

Results: Once the defense reflex begins to recover in previously decerebrated crayfish, it means that it shows signs of reconnection. The isolated protocerebrum with the deutocerebrum olfactory lobe remain alive for several days and the neuronal connections were reestablished, as measured throughout the bilateral defense activity. The defense reflex was observed in all animals and then recovered after surgery.

Conclusions: The crayfish is an excellent model to work the visual activity, all coding of visual information was suppressed in de-cerebrated crayfish. The recovery of the neural disconnection is observed from 40 days, where the defence reflex appears again before visual stimuli.

Keywords: Crayfish, Crayfish brain, Decerebration, Defence reflex, Neuronal reconnection, Protocerebral

INTRODUCTION

Direct visualization of the dorsal plane of the crayfish brain offers the opportunity to obtain surgically through fine manipulations, the brain neuronal elements necessary to understand the coherence of neural mechanisms dedicated to organizing the most basic vital processes such as heart, respiratory and circadian rhythm. More generally, these processes increase in complexity due to the size and sexual age of more developed animals, which favors the availability of the necessary resources, such as food and shelter. The action of integrating information in the crayfish depends exclusively on the nervous system and the elements of the brain, so that multiple stimuli from the outside world, as well as the internal ones, travel infinite synaptic pathways to regulate behavior.¹⁻⁴

In general, it is possible to isolate many of the crayfish brain components in vivo, experimentally the protocerebrum has been neurally disconnected and for this reason isolated from immediately nearby structures, under these conditions the protocerebrum remains functioning independently of the afferent innervation by the thoracoabdominal nerve. The protocerebrum is therefore revealed as an autonomic neuroendocrine structure of a higher order.^{5,6} As in many other metazoans, the brain of these crustaceans processes high-order information, coordinating multiple and successive primary sensory signals, which can be classified and stored or trigger the execution of a specific behavior, secondary to the activation of peripheral neuronal structures, in other words, the effects of feedback reach a relative degree of importance for the animal's biological plan.

Regarding visual function, we have achieved its disconnection from the rest of the nervous system by means of a brain section surgery in the crayfish as the procedure performed by Frederic Bremer 85 years ago, to create an autonomous functional structure isolated from retina, iris and fibers visual, which contains a robust circadian pacemaker.⁷ Figure 1 Given its symmetry and few neuropils and nerve tracts, this preparation could be suitable for studying some of the most basic integrative properties of visual functions in these crustaceans.⁴

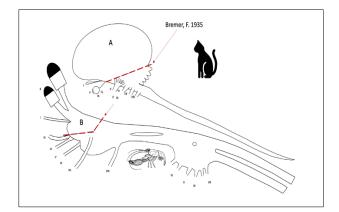


Figure 1: (A) Cerebral transection by Frederic Bremer in 1935, (B) Crayfish cerebral transection by Baltazar Barrera.

METHODS

An experimental study was conducted from January 2019 to January 2020, 11 crayfish Procambarus clarckii were included. The 11 adult male crayfish (body weight 15 grams) were trained to respond with defense reflex induced by the placement of a small rubber toy dinosaur (20 cm

long) in front of their visual field. The duration of this reflex time was measured in seconds. The animals were placed in water at 0°C, remained without any movement, and subsequently through a small incision of 3 mm in diameter in the medial antero region and dorsal cephalothorax region, the cerebral ganglion was exposed. The extracted 3mm diameter chitin was kept in Hartmann solution at 0°C until it was re-implanted. A surgical section of the cerebral ganglion was performed according to previously described techniques, immediately after surgery, metal microelectrodes were implanted to collect the activity of the photoreceptors and visual fibers. The size of the eve glow area (EGA) was measured directly from the corneal surface inside the water, with direct light stimulation by means of a Zeiss stereoscopic microscope (Figure 2).

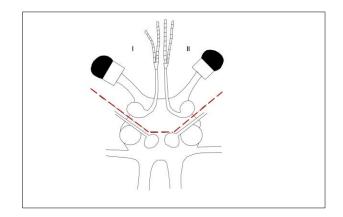


Figure 2: Anatomical representation of decerebrated crayfish, separating cranial nerves I and II from the rest.

The spike potentials were recorded from the axon's bundles of the visual interneurons by means of metal microelectrodes implanted in the left or right protocerebral tract of the disconnected protodeutocerebral preparation. The neural signals were pre applied and recorded in a moving film camera (Grass) from a Tektronix 502 two-beam oscilloscope. The electroretinogram (ERG) that in the crayfish is fully generated by photoreceptors of the retina was recorded extracellularly by metal microelectrodes through the cornea and changes in amplitude were recorded with a Grass Polygraph model 79D. The white light stimuli were automatically applied every 2.5 minutes using a Grass photo stimulator.

RESULTS

Circadian variations of the size of EGA

Normally the distal retinal protection pigments (DRSP) modulate the entry of light to the photoreceptors of the retina, they appear slow but with periodic movements, they apparently receive the influence of light through the release of the light-adapting hormone (LAH) released from the sinus gland in the eye cleft. During the first hours (four to five hours in the morning) of each day, the

release of LAH induces the dispersion of DRSP and, consequently, the size of EGA is reduced. On the contrary, in the afternoon, LAH is no longer released and EGA moves quickly towards its dark position (adaptation position), the results of the EGA size increased. EGA changed throughout the day due to the movement of DRSP in animals with surgical separation between proto/ deutocerebrum and there was activity of the sinus gland despite its drastic neuronal disconnection. With respect to the activity of the visual interneurons, all possible coding of visual information was suppressed by possible visual stimuli, since in the record of the induced activity it does not show any type of neuronal influence at all, including the defense reflex.

It has been proposed that the defense reflex begins with the stimulation of the crayfish retinas by means of specific stimulation in the visual field such as the presence of figures or by the simple presence of contrasts and shapes at any threshold of light in the environment. The organization of the neuronal codes that trigger the defense reflex, are received mainly by the eyes and their corresponding protocerebral neural tract, by the sustained participation of bilateral fibers of each eye under a level of ambient lighting. Once the defense reflex begins to recover in previously de-brained crayfish, it means that it shows signs of reconnection.

Recordings of neuronal visual activity

To protect the cone the ommatidium lead to the eye the light and EGA decreases or increases its size according to the time of day. Figure 3 shows the size of EGA during the day (10:00) and during the night (22:00 Hrs.) in a debrained crayfish. Note that there were no significant changes in the measurement of circadian EGA before and after surgery (Figure 3). This robust tract only carries visual activity, the simultaneous ERG response and the support fiber observed the successive response of a visual interneuron that only responds to the sudden movement of a target in front of the visual field of the crayfish.

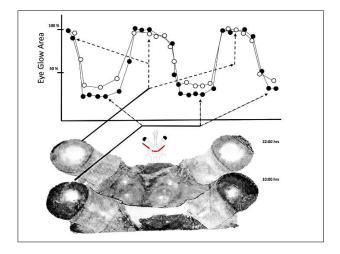


Figure 3: Size of EGA during the day (10:00) and during the night (22:00 Hrs.) in a de-brained crayfish.

The defense reflex

The defense reflex was observed in all animals and recovery of the defense reflex began after 40 days after surgery.

DISCUSSION

Different methods have been developed for the study of the crayfish brain, and due to the anatomicalphysiological similarities, classical techniques were developed in the brain to study the nervous system. ⁶ In this study, symmetric circadian pacemakers can be seen in the de-brained crayfish, most of the anterior portions of the brain remain alive only when the ophthalmic arteries remain intact during surgery, under such conditions the articulated neuropils eyes with the main endocrine apparatus remain in a continuous activity.^{2,6,8}

Normally in intact crayfish the activity of the mechanoreceptive and visual fibers of the crab's protocerebral tract are recorded as a robust afferent activity. Under conditions of decerebration, they remain unchanged, as in normal conditions and are well correlated with the size of EGA.^{8,9} Apparently after surgery the neurohemal and neural systems of each ocular stem maintained their functional properties.

Circadian EGA shows an almost normal behavior, which can be an interesting finding since in centromedial areas of the crayfish brain, where a strong union between the protocerebrum and the circadian pacemakers has been proposed.⁶ This tract consists of a complex assortment of afferent mechanical interneurons, the sensory information of the whole-body surface of the animal comes to complete the integration into the neuropil of both eye stems.

In the opposite direction, this tract also contains visual affinity to the brain, olfactory pathways and retinas from the deutocerebrum to the ellipsoid body and terminal lamina arise. All these neurons are of mechanical, visual and chemical sensitivity included in the protocerebral tract that remain connecting all the protocerebrum components of these preparations. The habituation response is observed as the decrease in the number of action potentials, before and after surgical disconnection due to components of the tritocerebrum in the crayfish. Under these conditions the isolated protocerebrum with the deutocerebrum olfactory lobe remain alive for several days and the neuronal connection was reestablished, with bilateral activity or ERG circadian rhythm.⁶

The crayfish's defense reflex is surprising, wild and highly symmetrical, which is essential for its defense and sexual selection. Although this reflex has been extensively studied, little is known about its underlying neural mechanisms.^{10,11} The present work is not intended to characterize or quantify this reflex, but it is essential to record its recovery-reconnection time after surgical disconnection in the crayfish.

CONCLUSION

The crayfish is an excellent model to work the visual activity, all coding of visual information was suppressed in decerebrated crayfish. The recovery of the neural disconnection is observed from 40 days, where the defense reflex appears again before visual stimuli.

Funding: No funding sources

Conflict of interest: None declared Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Barrera-Mera B, Ortega EP, Ruiz RB, Caprielova YJ, Mendoza FFG, Aceves RAM, et al. Decerebration induced by surgical transection of cerebral ganglion of crayfish. Int J Res Med Sci 2020;8:1217-20.