

Original Research Article

The neurobiology of splitbrain crayfish

**Baltazar Barrera-Mera¹, Juan José Granados-Romero², José Juan Vargas-Morales¹,
Elvira Barrera-Calva¹, Rodrigo Banegas-Ruiz³, Emilio Pérez-Ortega¹,
José Ángel Barajas Colón⁴, Francisco Fabian Gómez-Mendoza⁵,
Carlos Rubén Baca-Domínguez⁶, Yuri Jiménez Caprielova⁷, Román Alberto de la Rosa Llergo¹,
Rodrigo Mendoza-Aceves⁸, Ana Lilia Abundez-Pliego⁹, Karen Uriarte-Ruiz¹,
Mariana del Carmen Radilla-Flores¹⁰, Nelson Niels Espinosa Queb¹⁰,
Alejandro Ángel Corona Figueroa¹⁰, Bruno Andrés Salazar Trujillo¹⁰,
Alan I. Valderrama-Treviño^{11*}**

¹Department of Physiology, ²Department of Surgery, Faculty of Medicine, UNAM, CDM, Mexico

³Department of Traumatology, Rehabilitation Hospital, Luis Guillermo Ibarra, Ibarra

⁴Department of Angiology and Vascular Surgery, CMNSXXI, CDMX, Mexico

⁵Department of Hand Surgery, Central Military Hospital, CDMX, Mexico

⁶Department of Hand Surgery and Microsurgery, Rehabilitation Hospital, Luis Guillermo Ibarra Ibarra, CDMX, Mexico

⁷Department of Surgery, Angeles Villahermosa Hospital, Mexico

⁸Department of Anesthesiology, Angeles del Pedregal Hospital, CDMX, Mexico

⁹Department of Surgery, PEMEX General Hospital, Ciudad del Carmen, Mexico

¹⁰Department of Plastic Surgery, CENIAQ, CDMX, Mexico

¹¹Department of Surgery, Central North Hospital PEMEX, CDMX, Mexico

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*Correspondence:

Dr. Alan I. Valderrama-Treviño,

E-mail: alan_valderrama@hotmail.com

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ABSTRACT

Background: They crayfish brain is segmented into two symmetrical hemiganglia. Normally both hemiganglia are in direct communication through a series of well-defined neural bridges that cross de midline to form reciprocal cross connections.

Methods: An original study was carried out in the Department of Physiology of the Faculty of Medicine, UNAM during the period from August 2019 to August 2020. 13 Crayfish *Procambarus clarkii* weighing between 1g to 30g were used. Each eyestalk of the animals was tied to a displacement transducer coupled with a polygraph so that optomotor or electrical activity was bilaterally recorded. The separation of the right from the left hemiganglia from the cerebral or supraesophageal ganglion was performed with a sagittal section, splitbrain (SB).

Results: The normal photo motor reflex in crayfish eye is measured as a gradual decrease in the ERG amplitude. During tactile stimulation, the visual activation of both eyestalks in normal crayfish leads to a highly regular bilateral activity. The regular activity can only be altered by disturbing the mechanoreceptors located in the shell surrounding the eyestalks.

Conclusions: The procedure presented in this article provides unique characteristics for the study of the nervous system such as a detailed response of the bilateral optomotor reflex.

Keywords: Circadian rhythm, Electroretinography, Ganglia / physiology, Crayfish brain, Splitbrain crayfish, Circadian pacemakers

INTRODUCTION

They crayfish brain is segmented into two symmetrical hemiganglia.¹ Both of them containing a set of neurons and neuropil structures with the corresponding counterpart in each brain hemisphere. Normally right and left defined hemiganglia are in direct communication through a series of well-defined neural bridges that connect through the midline and form reciprocal cross connections between the corresponding structures of the left and right sides. For vertebrates and crustaceans, sensory information coming from photosensitive, chemical, and gravity motion, is processed by the cerebral and thoracoabdominal hemiganglia. This information is then organized and determines the endocrine and behavior response of the subject.²

In this way, it is clear the importance of symmetry in visual, caudal photosensitivity and locomotive functions, and so this method offers a most suitable model to explore the significant role of bilateral integration upon corporeal activity in these crustaceans. For this purpose, we will explore the effects that occur when severing the midline neural bridges of cerebral hemiganglia upon visual, locomotion, feeding and mood in splitbrain (SB) crayfish.

METHODS

An original study was carried out in the Department of Physiology of the Faculty of Medicine, UNAM during the period from August 2019 to August 2020.

13 Crayfish *Procambarus clarkii* weighing between 1g to 30g were used. All of them were brought from their natural habitat.

During the experiments, the animals were: Kept in very similar conditions to their natural environment to measure the magnitude of escape reflex. Kept under controlled laboratory conditions; into medium size tanks of water 100- 60- 8 cm. Fastened by means of a cork glued with dental acrylic to the back of their shells.

During the recording time of their optomotor activity (9 to 12 am) or electroretinogram, each animal was kept in a 3.5 liters water container at 21° C under a continuous light environment. Each eyestalk of the animals was tied to a displacement transducer coupled with a polygraph so that optomotor or electrical activity was bilaterally recorded.

Tactile stimulation was applied, using a thin brush, to the eyestalk posterior receptor field in the crayfish carapace. The intensity and frequency of tactile stimuli were automatically controlled through an electronic device. The light stimuli were given periodically from six to fourteen days. Stimuli were applied with a small 222 Philips flashing bulb (0.24 A, 2.2 V) to the eyes. Separation of right and left hemiganglia from the cerebral or supraesophageal ganglion was successfully achieved in 8

animals using a sagittal cut (splitbrain). The first step was to deeply anesthetize the subjects by dropping them from 1.5m into ice cold water, forcing a complete arresting state in the crayfish ranging from 10 to 15 minutes during they were completely immobilized. The supraesophageal ganglion was exposed through a small hole (2.5 mm in diameter) in the anteromedial region of cephalotorax, in the basal portion of the rostrum. The stomach gastric camera was pushed back using a small piece of sterile cotton and a homemade stainless-steel knife (2.5mm length, 3.0 mm width and 100 um thick) was used to separate the hemiganglia. Then a small piece of chitin (2.5 mm) was removed and kept in a Van Harreveld solution at 0°C until it was reimplanted and glued on with acrylic cement to avoid any loss of haemolymph. The entire operation was performed under direct illumination of a Zeiss stereomicroscope. During the postoperative period, all animals accepted food and were cleaned from any residual preparation. Their opto and photomotor responses were examined. Only Animals with clear facets at the corneal surface, compound eye and strong optomotor responses were selected. This procedure demonstrated that when dividing the crayfish brain down the middle into its right and left halves, each cerebral hemi ganglion controls retinal sensitivity and the eyestalk movements in an independent manner from the contra lateral one.

RESULTS

The locomotive capacity was recorded by measuring the magnitude and displacement of the escape reflex (in centimeters), the amount of food consumed and grip strength were also recorded variants. The escape reflex was measured using both visual and tactile stimulation.

Total suppression of visual induction of the escape reflex was observed in splitbrain preparations while tactile-induced escape reflex was progressively recovered.

Optomotor activity

The structures that control eye movement are powerful elements, which stabilize significant features of the visual environment in the retina. For vertebrates and crustacean's sensory information is bilaterally working as the afferent portion of visual stabilization reflexes (Figure 1). Selected stimuli of the external world triggers interneurons that intentionally bring into focus certain specific areas of both compound eyes of the crayfish's eye movement. The importance of moto neural control that is responsible for movement of the crayfish eye is revealed when in the splitbrain crayfish the uncoupling of the hemispheres provokes incessant spontaneous and evoked optomotor activity. Normally during the resting phase of the locomotor and visual activity, the longer axis of both eyestalks of the crayfish maintains a 120° angle between each other (Figure 1). Both eyes always show a simultaneous and equal amount of spontaneous movements from 3 to 10 sec duration. When walking and after the splitbrain surgery, that interocular angle usually

diminishes. In splitbrain crayfish the eyestalk preparation followed their own optomotor movement pattern without contra lateral influence. In comparison, during tactile

stimulation, the visual activation of both eyestalks in normal crayfish leads to a highly regular bilateral activity.

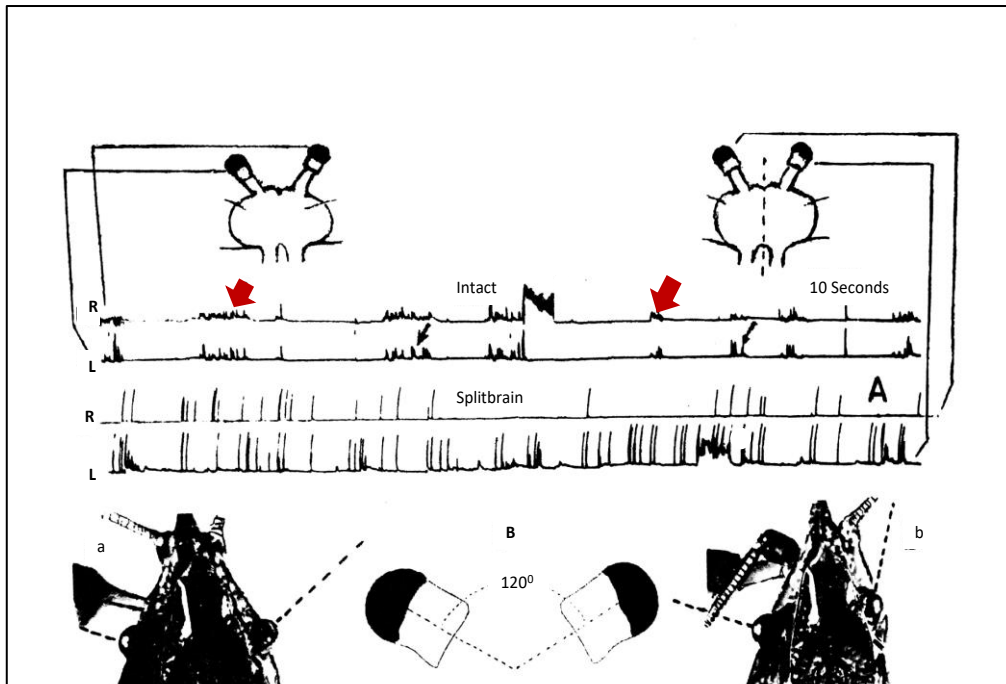


Figure 1: Bilateral response of the optomotor reflex. Effect of tactile stimulation (red arrow) applied to one eye stem in a healthy subject, observing bilateral optomotor response. Effect in bilateral spontaneous activity in a splitbrain subject. B. Normal angle of 120° between eye stalks, a) Normal animal, b) animal with splitbrain.

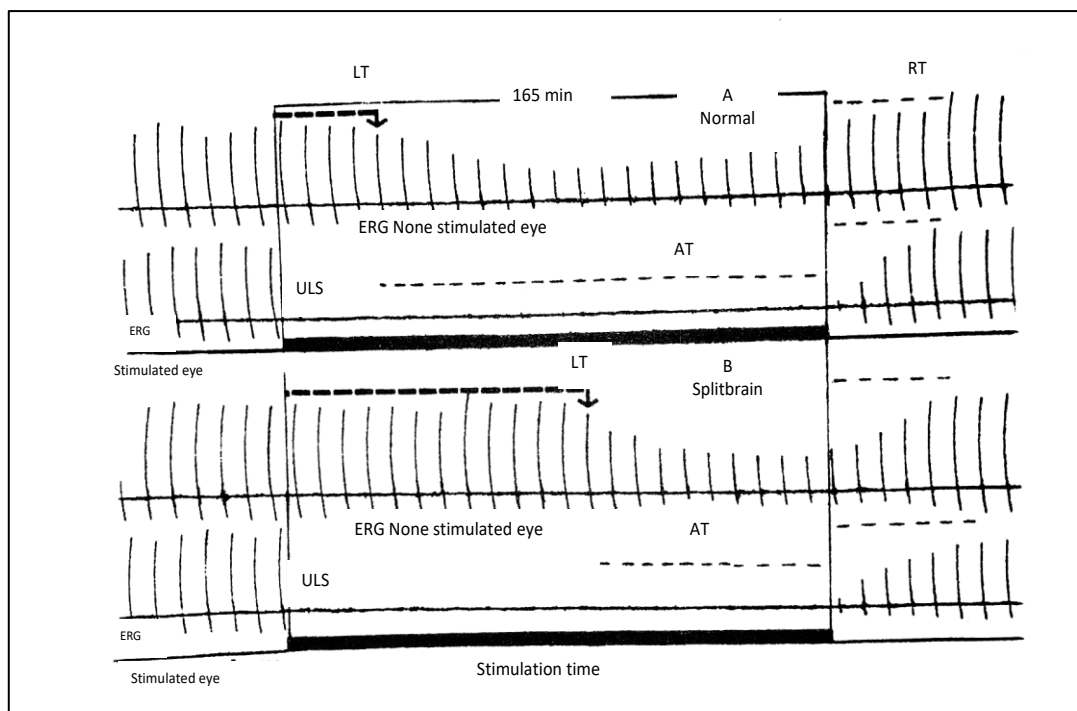


Figure 2: Electoretinogram (ERG). Normal animal (A) with unilateral light stimulation (ULS), note the consensual reflex on the contralateral side, with a short time of action (AT). Short latency time (LT). Prolonged latency time (80 minutes) in a splitbrain subject (B).

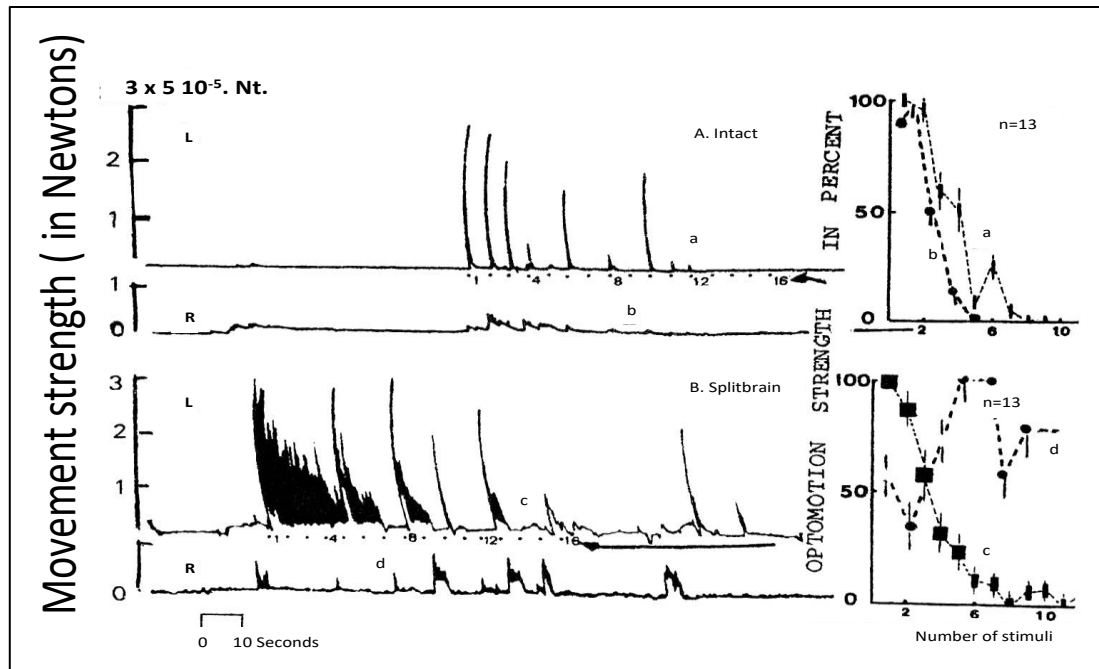


Figure 3: Habituation of the optomotor reaction. Normal animal (A) splitbrain subject (B). In the intact animal, bilateral habituation is observed, a. stimulated eye, b. contralateral eye. In the splitbrain animal, habituation occurs only in the stimulated (c.) eye. and is not seen in the (d.) contralateral eye.

The consensual reflex

The gradual decrease of electroretinogram (ERG) responses as well as the action time (AT) and the RT of retinal sensitivity were diminished compared to normal subjects (Figure 2). In both normal and SB crayfish, the total adaptation to light induced by sustained unilateral light stimulation (ULS) upon one single eye, was followed by a totally different darkness adaptation process in the contra lateral side. Another theory is that the light adapting hormone release of the ipsilateral stimulated ERG, required a typical consensual reflex as the one observed in normal animals (Figure 3).

DISCUSSION

Binocular focusing process makes possible the capturing of visual information accomplished by vestibular, visual, and proprioceptive modulation.^{3,4} The progressive recovery of retinal sensitivity started from zero amplitude of ERG response.⁵ Under the splitbrain (SB) conditions the progressive ERG diminution suggests the existence of a redundant contra lateral modulator influence whose signals travel throughout the circumesophageal connectives and probably also throughout subesophageal ganglion, the sustained ULS could be reaching the contra lateral side by using the sustaining fibers in these crustaceans.^{6,7}

Throughout the specific participation of some protocerebrum interneurons, the redundant afferent signals running from crayfish retina connect ipsilateral optic lobes

with their homologous counterpart.⁸ Besides their key role in visual perception, they revealed the presence of a potent neuroendocrine consensual reflex. This intricate phenomenon can be measure by means of both the light - adapted retinal shielding pigments (RSP) position and also when measuring the diminishing amplitudes of the ERG.⁹ This, help us test the disruption of neuroendocrine bilateral integration in SB crayfish. The regular activity can be bilaterally broken only when disrupting the mechanoreceptors of the areas of the carapace surrounding the eyestalks. In splitbrain conditions we can see the energetic spasmodic movements of one single side. Unfortunately, the violent perpetual corporeal movements these subjects acquire from the active phase of the circadian activity of these animals totally mask the relatively feeble optomotor movements. The rate of the diurnal optomotor response was reduced after repeat tactile stimulation. Note that the non-stimulated or contralateral eyestalk also diminished its activity in a regular manner. The response rate first decreased and was totally absent after the stimuli were successively applied. They were becoming habituated. The identified groups of elements of oculomotor system of crayfish normally coordinating the conjugated movement of eyestalks, remains totally disconnected after the surgical bisection of crayfish brain, and with a large degree of independence.

We strongly consider the possibility that a powerful but reversible inhibitory influence acts simultaneously upon the left and right protocerebral circadian pacemakers while receiving bilateral photic stimulation.¹⁰

CONCLUSION

The model presented has unique characteristics for the study of the nervous system and proving very solid information about the bilateral optomotor reflex.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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