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Short Communication

Morphology of Brain Waveforms and Electrical Activity of Visual Evoked Potentials (VEP) of *Iguana iguana* Species under Captivity Conditions in Mexico

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Abstract

Reptiles have been characterized by relevant number of papers as well-known animal models in biomedical research and neuroethology due to their ancestral evolutionary relationship with birds and mammals, leading to further hypothesis that the dorsal ventricular ridge (DVR), the dorsal pallia of amphibians and the isocortex of mammalian brains share a common anatomical origin. The objective of this study was to assess the morphologic features and measurement of brain waveforms of *Iguana iguana* through Visual Evoked Potentials (VEP). This research employed six healthy and ophthalmologically-suited iguanas (*Iguana iguana*), with computerized tomography brain scans followed by VEP under simple temporary physical restrain. The systematic study of VEP individually analyzed both eyes of the *Iguana iguana* species displaying a response that consisted of five steady waveforms during the first 200 msec, further characterized as P1, N1, N2, P2 and P3. Comparison among these waveforms revealed no significant differences.

Keywords: dorsal ventricular ridge, *Iguana iguana*, reptilian brain, visual evoked potentials, waveforms

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บทคัดย่อ

ลักษณะของคลื่นสมองและคลื่นไฟฟ้าของการกระตุ้นระบบการมองเห็นด้วยเครื่อง (VEP) ของอีกัวน่าเลี้ยงในประเทศเม็กซิโก

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สัตว์เลื้อยคลานเป็นสัตว์ที่ใช้อย่างแพร่หลาย เพื่อเป็นต้นแบบการศึกษาวิจัยทางชีวเวชและพฤติกรรมที่สัมพันธ์กับการทำงานของสมองจากการที่มีวิวัฒนาการสัมพันธ์กับนกและสัตว์เลี้ยงลูกด้วยนม อันนำไปสู่การตั้งสมมติฐานว่า dorsal ventricular ridge (DVR) ในสัตว์เลื้อยคลาน dorsal pallia ของสัตว์ครึ่งบกครึ่งน้ำ และ isocortex ของสมองของสัตว์เลี้ยงลูกด้วยนมมีต้นกำเนิดทางกายวิภาคใกล้เคียงกัน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินคุณลักษณะรูปร่างและขนาดของคลื่นสมองของอีกัวน่าผ่านทางการกระตุ้นระบบการมองเห็นด้วยเครื่อง (VEP) การวิจัยนี้ใช้อีกัวน่า (*Iguana iguana*) สุขภาพแข็งแรงและไม่บกพร่องด้านการมองเห็นจำนวน 6 ตัว ทำการเอกซ์เรย์สแกนสมองด้วยคอมพิวเตอร์และตามด้วย VEP โดยอาศัยการจับบังคับควบคุมระยะสั้น การวิเคราะห์ VEP กระทำที่ดวงตาทั้งสองข้างของอีกัวน่า และพบว่ามีการตอบสนอง โดยพบคลื่นสมองจำนวนคงที่ 5 คลื่นในช่วง 200 มิลลิวินาทีแรก จำแนกเป็น P1 N1 N2 P2 และ P3 เมื่อทำการเปรียบเทียบระหว่างคลื่นสมองเหล่านี้ไม่พบความแตกต่างที่มีนัยสำคัญ

คำสำคัญ: dorsal ventricular ridge *Iguana iguana* สมองสัตว์เลื้อยคลาน visual evoked potentials คลื่นสมอง

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Introduction

Within the Neurobiology field the study of reptiles has generated a broad interest due to their ancestral evolutionary relationship with birds and mammals; therefore reptiles have also been developed as a well-known animal model in biomedical research (Bautista et al., 1995; O´Rourke, 2002). However, intrinsically while reptiles possess a richer and more complex brain and cerebellum than those of amphibians and fish, and comprises also the first group of vertebrates presenting twelve pairs of cranial nerves as well as highly developed connections to the Central Nervous System (CNS) than the more primitive phylogenetic groups of their line, their brains consist of a very small organ not exceeding 1% of total body mass (Mader, 2006). Considering such innate distinctiveness, different hypotheses have been postulated at the present time, regarding phylogenetic relationships among different intrinsic anatomical areas between reptiles, birds and mammals. It is thought that due to the size and position regarding ascending sensory conductivity and the flow of nervous information, the dorsal ventricular ridge (DVR) could be considered one of the high-level sensory and cognitive activity association site in both reptiles and birds (Kardong, 1999) as a visual, auditory and somatosensory pathway generating nerve projections into the same receptor kernel, which is the DVR where the locations of the visual (Vtt) and auditory (A1) zones are very similar among reptilian groups (Reinera and Northcuttb, 2000).

The DVR can be further divided into anterior (ADVR) and basal (BDVR) portions and is referred to archistriatum in birds, which is composed of segments receiving visual, auditory and somatosensory inputs through several nuclei of the thalamus and cortex as well as from several structures of the brain stem (Schaeffer and Waters, 1996). This has generated the hypothesis that the DVR of lacertillians, the dorsal pallium of amphibians and the distinctive laminar isocortex of mammals share a common anatomical origin (Karten, 1967; Butler, 1994; Northeutt and Kaas, 1995; Striedter, 1997).

Apart from the unique neuroanatomical characteristics of the lacertillians, along with its unparalleled ability to neuronal regeneration and the capacity of primary vision of a rather highly organized photoreceptive organ with relation to environment, it prompted our group to further learn about the neurophysiologic representations of the visual pathway of these animals since relatively little is known about the morphology and behavior of the visual pathway of the *Iguana iguana* (Crocco, 2005). Moreover, the ultimate goal of this work was to further measure the electrophysiological activity of the Visual Evoked Potentials (VEP) in this species.

Materials and Methods

This research was undertaken in adherence to the Official Guidelines of the Mexican Guide for the Care and Use of Experimental Animals (NOM-062-ZOO- 1999), as well as the OMS's International Zoosanitary Code and was approved by our local IACUC and the Ethics and Research Commission of the Animal Research Unit of BIOINVERT®. (Biología Integral para Vertebrados: Unidad de experimentación animal).

The study used a total of 6 iguanas (*Iguana iguana*) about 0-6 years old weighing between 200-2000 g, stressing the fact that none of the animals had any ophthalmolgic findings capable of compromising this study. All subjects were maintained under natural semi-freedom status within stone-walled pens or gardens provided with natural vegetation; daily diet was based on fresh greens offered at free will (mostly fruits and vegetables) and routinely dusted off with powdered calcium and multivitamins.

The brain images were obtained using a Computerized axial tomography scans (CAT) Siemmens® tomography model Somaton Sensation Simens 3D, thus attesting to the appropriate positioning of electrodes during the neurophysiological evaluations (Fig 1). The protocol for the imageneologic studies was based on NOM-157-SSA1-1996 and carried out at the Imageneology Unit, Hospital General Dr. Gea González S.S., México.

The VEP studies were conducted and studied at the Centro de Atención Médica y Educación Especial (CAMES), using Akonic® Bio PC multifunctional equipment through the standard stimulation of one eye at each time, by using a hand-held stroboscopic lamp at a distance of 10 cm and of 1.5 msec in duration. Filter settings for bandpass were set up between 300-1.50 Hz range and impedance lower than 5 kΩ. VEP time analysis was set up to 200 msec and 50 stimuli. All electrical responses were replicated at least once to ensure sound reproducibility of events. In order to attain best conductivity, the acquisition and recording of VEP electrical brain responses were conducted using needle electrodes placed according to the International 10/20 system, having one reference electrode placed at the parietal vertex (Cz+)*, a second at the supraoccipital location (Oz-)* and a third grounded at Fpz (Fig 1). In order to avoid any morphologic variation of waveforms, only physical restrain of the iguanas was used during both imageneologic and neurophysiologic studies.

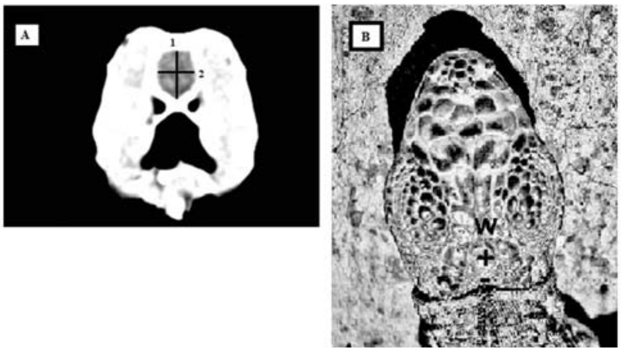

Figure 1A Brain measurement of six years old iguana, 1 – 13.0 mm 2 - 9.7 mm. **1B** Location of electrodes in the parietal leads (+), the supraoccipital (-) and (w) in the Fpz.

The statistical analysis was executed using the Windows SPSS program version 15,5 (SPSS, Chicago, IL) and results were evaluated using common measures of central tendency: mean, minimum, maximum, standard error and standard deviation. These results were additionally analyzed through the non-parametric Mann-Whitney U test for two independent samples. Significant differences were noted when p was < 0.05.

Results and Discussion

In medicine, VEP constitute an indispensable clinical tool in neurology since they provide important diagnostic information regarding the functional integrity of the visual system. Consequently, their use can easily enhance our current basic knowledge to species other than man and mammals (Odom et al., 2004). In this work, as expected, the waveforms obtained during the VEP recordings of *Iguana iguana* demonstrated striking similarities between the left and right eyes. From our results, it was concluded that no significant differences were observed among the right and left afferences, reason for which they were further unified, and following such rationale the results were later compared among the age groups and again no significant differences between the subjects were found (Table 1 and 2).

Table 1 Values obtained from the right eye and left eye of organisms to perform a frequency analysis, measures of central tendency of each wave encountered.

	Afferents	N	Mean±SD (ms)
P1	Right	4	20.82±0.19
	Left	4	30.60±0.77
N1	Right	6	50.07±2.88
	Left	6	40.79±1.31
P2	Right	6	90.24±3.54
	Left	6	70.83±1.31
N2	Right	5	100.41±2.62
	Left	6	110.61±2.93
P3	Right	5	120.64±2.86
	Left	3	120.74±2.38

Table 2 Frequency analyses of the values (ms) of each wave found in all organisms studied.

United Afferents	N	Minimum	Maximum	Mean±SD
P1	8	20.56	40.68	30.21±0.67
N1	12	20.00	80.80	40.93±2.14
P2	12	50.88	140.16	80.53±2.65
N2	11	80.08	160.00	110.06±2.73
P3	8	90.64	160.92	120.68±2.51

During VEP data acquisition five constant waveforms namely P1, N1, P2, N2 and P3 (Fig 2) were recorded for each eye during the first 200 msec. In mammals, visual impulses are sent and travel from the eyes through the dorsal lateral geniculate nuclei (dLGN) of the thalamus to the primary visual cortex (Schmolesky et al., 1998). Therefore, the results obtained through the VEP in mammals show only three waves: P1 corresponds to the Lateral Geniculate Nucleous, N1 to the primary visual cortex, and P2 to the visual association cortex (frontal-temporal) of which the primary function is the perceptual processing and comprehension of visual stimuli acquired binocularly by men and animals (Chiappa, 1997; Hernandez-Godínez 2011; Solís-Chavéz et al 2012). These waves appear in the first 250 ms with latency values of N75 ms, P100 ms, and N145 ms in the case of humans (Shkurovich 1997). In diapsid reptiles such as *Iguana iguana*, five constant waveforms were observed; which can be homologated along the following sites: P1 for the dorsal lateral optic body, N1 for the dorsal pallia cortex and P2 corresponding to the projective-associative areas of the DVR, latency values shown in Table 1. The VEP's wave morphology of the *Iguana iguana* showed similarities with mammalian VEP, however the wave's amplitude was smaller in *Iguana iguana*. This may be due to the small visual cortex presence compared to the more specialized cortex of mammals.

As indicated in the central tendency analysis, it can be apparently asserted that the *Iguana iguana* species are born with full-mature visual pathways, and as a result of such developmental feature become fully independent at birth; therefore, they are not subjected to common maternity care as provided by mammals to their offspring. In addition, no decrease in visual capacity was noted among the older iguanas, remarkably different to that of mammals where visual acuity always deteriorates with age. These singular features can be attributed to its unique ability to regenerate tissues, including nerve tissue (Font et al., 2002; Crocco, 2005).

Due to the evolutionary and echo-physiologic system under which the New World lizard *Iguana iguana* has been developed throughout centuries, their visual pathways are highly specialized to detect and process movements around the 30 msec; this represents half the time used for mammals to start processing any signals. Responses to luminance showed no morphologic waveform differences in our results. This may imply that no differences existed regarding the status of maturity or age-related degeneration of the visual pathways occurred in the studied organisms at any given age. Therefore, it is correct to assume that the central visual pathway is mature at birth and retains a life-time optimal performance even in organisms of advanced age, which means that no cellular or myelin degradation takes place as a result of the normal aging process of this species, keeping its perception constant. Such condition enlightens the fact that reptiles differ from men and mammals in the sense that for both of them exists an early maturation process of visual processing occurring outside the brain within the retina of the eye, fact that occurs even in infants born preterm that are subjected to developmental processes inevitably followed by maturation and systematic degeneration when reaching advanced age (Hammarrenger et al., 2007).

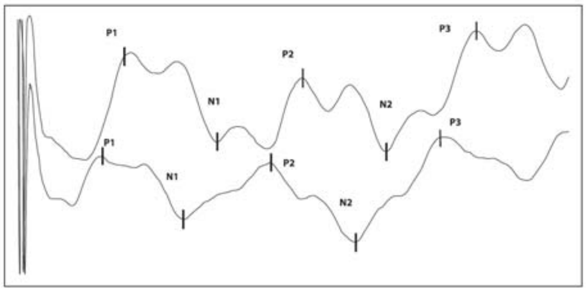

Figure 2 Visual Evoked Potential (VEP) with its replica for the right eye in a specimen of *Iguana iguana*. Waves are observed P1, N1, P2, N2 and P3.

From the evolutionarily standpoint, it is considered an essential advantage that the visual pathway of *Iguana iguana* evolved naturally, providing it quick responses to their nearby environment and that by virtue of such developmental feature its visual system is therefore less analytical within the processing area, given that if it is preyed on, it must possess a visual-sensible perception which allows it to detect any danger in order to escape predators instantly.

Currently, *Iguana iguana* is greatly confined to rainforests and being mainly an arboreal species their predators tend to be fast animals, consequently its vision plays a key role for survival. They also have a monocular vision, a powerful characteristic that favors the detection of gaze contact and delicate movements eliciting quick responses. In this sense it seems possible that a large number of species are able to discriminate gaze contact and act accordingly to escape, simply by differentiating if an approaching treat is looking towards or away from them by perceiving gaze contact and direct approach as threatening, allowing them through this natural mechanism to swiftly escape oncoming danger (Emery, 2000). Some species of iguanas also appear to be sensitive to the size of the approaching eyes, responding quicker when the eyes are larger; fact that enlightens that social interaction among iguanas constitutes another interesting field of study (Burger et al., 1992).

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