RESEARCH ARTICLE

Visual Evoked Potentials to Light Flashes in Captive Rhesus Monkeys: A Study Reflecting Cerebral Cortical Activity and Brain Maturation

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ABSTRACT

Visual evoked potentials (VEPs) are useful electrophysiological diagnostic tools for evaluating retinal response of the visual cortex and detecting its functional integrity in humans and animals. To analyze the VEPs and physiologic response of the visual pathway of a random population of captive-bred monkeys of the *Macaca mulatta* species throughout different physiologic stages after stimulation with stroboscopic light flashes. In this study we used 20 non-human primates (*M. mulatta*), 10 males and 10 females, divided into five age-dependant cohorts of 2 males and 2 females. Two replicable negative waveforms and one positive were recorded, as reliable indicators of electrical conductivity at specific anatomical nuclei of the visual pathways. Statistically significant differences were primarily observed in group 1 when compared against the remaining groups for the three evaluated waveforms. Waveform morphology characteristically presented steady deviations related to ontogenetic development of the studied population.

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INTRODUCTION

Non-human primates (NHP) possess a highly developed nervous system acquired through the same physiological and psychological developmental stages as humans but in a more compressed time span, characteristic making them highly suitable for the study of visual function and different neurophysiologic and neurobiology research, including infant development and animal cognition among various disciplines (Gómez, 2005; Hauser *et al.,* 2000). 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 (Merigan and Maunsell, 1993; Schmolesky *et al.,* 1998). Visual Evoked Potentials (VEP's) are electrophysiological techniques used to evaluate retinal response of the visual cortex and its functional integrity.

In animals and humans electric activity at specific sites of the visual pathway is characterized by at least 2 steady negative and one positive waveforms (Maurer *et al.,* 1996), where LGN is the lateral geniculate nucleus, V_1: primary visual cortex and V_2: extrastriate visual cortex representing the second major area of the visual cortex and the first region within the visual association area. VEP's responses are dependent on the functional integrity of the central visual field at any level of the visual pathway including the eye, retina, the optic nerve, optic radiations and occipital cortex (Odom *et al.,* 2010); however, since no assessment of VEP's has ever been reported considering the influence of developmental ontogeny in monkeys, the objective of this study was to determine the responses of the central visual fields of small groups of rhesus macaques (*Macaca mulatta*) keep

in captivity in ages ranging from neonate to senile using a series of hand-held stroboscopic light flashes stimuli.

MATERIALS AND METHODS

This research was undertaken in adherence of the NOM-062-ZOO-1999 (Anonymous, 1999) and was approved by our local IACUC and by the Ethics and Research Commission of the Centro de Investigación Proyecto CAMINA para Curar la Parálisis A.C. This study was performed using 20 NHP of the *Macaca mulatta* species, 10 males and 10 females, divided in five age-dependant groups of 2 males and 2 females as follows: Group 1 (0-1 year-old), Group 2 (2- 3 year-old); Group 3 (4 -9 year-old); Group 4 (10-15 year-old), Group 5 (15 years and older) (Hernández-Godínez *et al.,* 2011; Ibáñez-Contreras *et al.,* 2011). All the research animals were donated by the Centro de Investigación Proyecto CAMINA para Curar la Parálisis A.C. (Mexico City, México).

Housing characteristics: The monkey colony maintained in group-housing conditions. Building materials consists of seamless concrete flooring and all interior surfaces are covered with high-impact ceramic tiles. External walls are made of cyclone-fence mesh firmly anchored to walls and floors to allow the macaque's access to a natural photoperiod. Roofs provided a safe waterproof covering along the corrals, and steel doors are equipped with security latches. Daily animal care includes restricted (4% per body weight) three-daily feeding of pelleted diet (Monkey Diet 5038, PMI Nutrition International, St Louis, Mo, USA) containing 25% protein, provided through food dispensers designed to avoid physical contact of pellets with feces and urine; fresh water was available *ad libitum* by means of an automatic watering system. Medical veterinary care is provided regularly in adherence to the Mexican NOM-062-ZOO (Anonymous, 1999)**.** Tuberculosis testing is performed twice annually, as it is routine parasite monitoring with negative results. Before the study began, all monkeys were assessed as clinically healthy and showed no nervous or behavioral dysfunctions.

Visual Evoked Potentials (VEP's) were performed under slight sedation using Tiletamine+zolazepam (Zoletil® Virbac Laboratories, Carros, France) at a dose of 4 mg/kg body weight injected intramuscularly (Galván-Montaño *et al.,* 2010; Hernández-Godínez *et al.,* 2011; Ibáñez-Contreras *et al.,* 2011).

All VEP's studies were undertaken according to the Standard of the International Society for Clinical Electrophysiology of Vision (ISCEV) and conducted at the Neuroprotection Laboratory located at the Instituto Nacional de Rehabilitación (INR) SSa in Mexico City. A specialized computer Viasys Healthcare Niccolet® (Viasys Healthcare, Conshohocken, PA, USA) was used for this study and filter settings bandpass were set up between 10 and 100 Hz, impedance was lower than 5 kΩ. Recordings of electrical brain responses were acquired by using skin disc electrodes placed on according to the International 10/20 system.

Monkey preparations prior to the recordings included shaving and cleaning the skull skin with tap water and neutral pH liquid soap to reduce impedance and to ensure good and stable electrical conductivity. According to data of the International Society for Clinical Electrophysiology of Vision (ISCEV) time analysis was set up as follows: Groups 3, 4 and 5 were established at 250 ms, recording times of Groups 1 and 2 were determined as 500 ms at an average of 150 stimuli. Standard VEP´s stimulation was performed by binocular stimulation to assess the visual pathway functionality of both eyes, applying 250 light flash sweeps using a hand-held stroboscopic lamp (Odom *et al.,* 2010). Noteworthy is the fact that in this study we only evaluated response latencies as a total measure of conduction time, since this constitutes a specific indicator of visual function and its potential pathology or maturation degree (Shkurovich, 1997; Hernández-Godínez *et al.,* 2011; Ibáñez-Contreras *et al.,* 2011).

Statistical analyses: Differences between experimental groups were tested for statistical significance using a Student's t test for independent samples of equal size in two consecutive moments. The first moment compared the sex variable within each group, while the second served for comparative assessment of the right and left afferencies within each group for N_1, P_1 and N_2. Lastly, for comparison of age-related groups an analysis of variance (ANOVA) followed by a Post-Hoc Tukey and Dunnett's test were used through the homogeneity of variances calculation to assess pertinent differences.

RESULTS

Fig. 1 shows the placement of one active electrode along the midline over the visual cortex at Oz, one reference electrode at the vertex Cz location and one referred to Fz as ground electrode. Three sets of constant, steady and well defined waveforms were observed during the first 250 ms, in all groups at pre-determined age for the left and right eye. Figure 2 shows the morphology of the VEP recording, observing the morphology changes according to the age group. Measures of central tendency were obtained, arithmetic mean and standard deviation for all groups of the same age considering the three waveforms recorded, observed in the groups 1 and 5 absolute latencies are more elongated than the other groups. Data within each individual group was further analyzed using a Student's t-test comparing right and left afferencies and sex influence for N_1, P_1, and N_2 without finding significant differences in all groups. Finally, data collected for each factor was analyzed using Analysis of Variance (ANOVA), significant difference was observed in the tree waves (N_1, P_1, and N_2) in the group 1 in relation to other groups (Table 2).

DISCUSSION

To understand visual pathway development several studies have focused on evaluating the ontogeny of visual capacity during different physiological stages to determine early visual impairment, and identify factors affecting the visual pathway conductivity and performance in response to visual stimulation. In

Table 1: Mean VEP Latencies (Mean±SD) in Rhesus Macaques

Groups	Right Eye			Left Eye		
	N1	P1	N2	N1	P1	N2
Group 1	71.61±0.24	87.61±3.94	138.88±9.73	71.83±2.34	86.93±3.61	139.24±8.46
Group 2	66.04±3.38	84.76±3.38	129.44±6.19	65.71±2.76	78.29±2.07	129.59±3.73
Group 3	60.51±4.60	78.74±3.25	128.65±5.08	59.37±4.59	76.40±1.35	127.63±2.02
Group 4	67.03±3.25	77.91±5.35	129.94±4.64	64.90±3.49	78.63±4.23	127.73±3.34
Group 5	71.17±6.12	85.82±7.44	139.17±5.94	70.50±7.06	82.29±2.09	131.94±4.96

Twenty non-human primates were allotted into one group divided in five age-dependant subgroups as follows: Group 1, 0-1 year-old, Group 2, 2- 3 year-old; Group 3, 4 -9 year-old; Group 4, 10-15 year-old, Group 5, 15 years and older.

Table 2: Results among Age-Related Groups of Rhesus Macaques- Statistical Significance

Studied Variable	Post-Hoc	F	ANOVA	Groups	Mean±SD	Groups	Mean±SD	p
N1 Right Eye	Tukey	5.03	0.0089	Group 1	71.61±0.24 VS	Group 3	60.51±4.60	0.010
				Group 3	60.51±4.60 VS	Group 5	71.17±6.12	0.014
N1 Left Eye	Dunnett	5.1	0.0084	Group 1	71.83±2.34 VS	Group 3	59.37±4.59	0.040
P1 Left Eye	Tukey	8.39	0.0009	Group 1	86.93±3.61 VS	Group 2	78.29±2.07	0.005
						Group 3	76.40±1.35	0.001
						Group 4	78.63±4.23	0.008
N2 Left Eye	Tukey	3.69	0.027	Group 1	139.24±8.46 VS	Group 3	127.63±2.02	0.035
						Group 4	127.73±3.34	0.037

SD = standard deviation; Differences are less significant at P<0.05, ANOVA, F value with Pshoc Tukey and Dunnet.
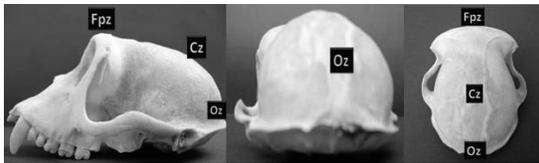

Fig. 1: Topographic Placement of Scalp Electrodes According to the International 10/20 System during VEP's in Rhesus Macaques (*M. mulatta*). Note: Monkey cranium was obtained from one aged Rhesus macaque dying of natural causes.

medicine Visual Evoked Potentials (VEPs) are considered indispensable tools in pediatric ophthalmology and neurology as they can provide solid prognosis for children´s visual disorders (Chayasirisobhon *et al.,* 2012). Further research focusing the field of supplemented nutrition in infant monkeys proved unsubstantiated benefits contributing to visual acuity or retinal function, defining that the rates of acuity development are quite different between humans and monkeys such as the rhesus monkey, species showing remarkable differences regarding their fovea's developmental process (Jeffrey *et al.,* 2002).

In this paper recordings of VEP´s in rhesus macaques (*Macaca mulatta*) at different age-related physiological status were executed using white stroboscopic flashes. The waveforms morphology of the studied NHP groups presented, as expected, age-related statistically significant differences primarily for group 1, contingent on the degree of myelination and body maturation (Shkurovich, 1997).

Diverse studies have showed that during the developmental phases leading to the maturity of the sensory pathways, changes at the inner cerebral electrical conductivity may arise as a result of the different physiologic stages encountered primarily during early stages (Tsuneishi *et al.,* 1995; Shkurovich, 1997).

Results obtained in this study have shown statistically significant differences at N_1 for both afferencies between groups 1 and 3 (Table 2). We must stress that waveform N_1 stands as the first synaptic site starting at the primary photoreceptor cells located at the retina considered as the output neurons of the retina ending at the lateral geniculate body. It has been also described that in the case

of humans and NHP there is an adequate optic quality along with a normal pupil response at birth (Boothe *et al.,* 1985; Jacobs and Blakemore, 1988), but regardless of this relative good quality optic capacity it has been observed that new-born monkeys present poor visual acuity under monocular vision, deficit derived from a naso-temporal asymmetry (Jacobs and Blakemore, 1988; Kiorpes and Kiper 1996). Such deficiency has been observed in 8 week old monkeys where immature development of the retina´s large number of photoreceptor cells prevails (Jacobs and Blakemore, 1988; Forte *et al.,* 2006; Qiao-Grider *et al.,* 2007). It is remarkable that in order to attain full stereoscopic vision, NHP depend on complete frontalization of both ocular orbits leading to a high orbit convergence and broad binocular visual field, which contribute to the tridimensional visual perception characterized by enhanced light sensitivity and contrast discrimination (Heesy, 2004).

According to the above we can expect to observe significant differences in group 1 with regard to the rest of the cohorts. It is important to mention the fact that delayed electrical conductivity was present for both afferencies; such outcome supports the assumption that an ongoing development and maturation process is present and arises from normal anatomo-functional changes, which are clearly not related to any pathological process in this group. Differently to findings of group 1 where significant differences were seen for both afferencies, group 5 (71.17±6.12 ms) showed a significant difference only in the right afferency with comparison to group 3 (60.51±4.60 ms). Tables 1 and 2 shows that latency in group 5 was higher than that of group 3; the reason for such increase is primarily related to dysfunctional regeneration of the optic nerve's myelination process with probable degeneration of the retina's ganglion cells due to natural age-degenerative developments associated to group 5.

In the case of P_1 (primary visual cortex), significant differences were noted for the left afferency between group 1 (86.93±3.61 ms) versus group 2 (78.29±2.07 ms), group 3 (76.40±1.35 ms) and against group 4 (78.63±4.23 ms). Absolute latency values of group 1 were remarkable larger than in the rest of the groups and consequently, delayed electrical conductivity resulted from incomplete
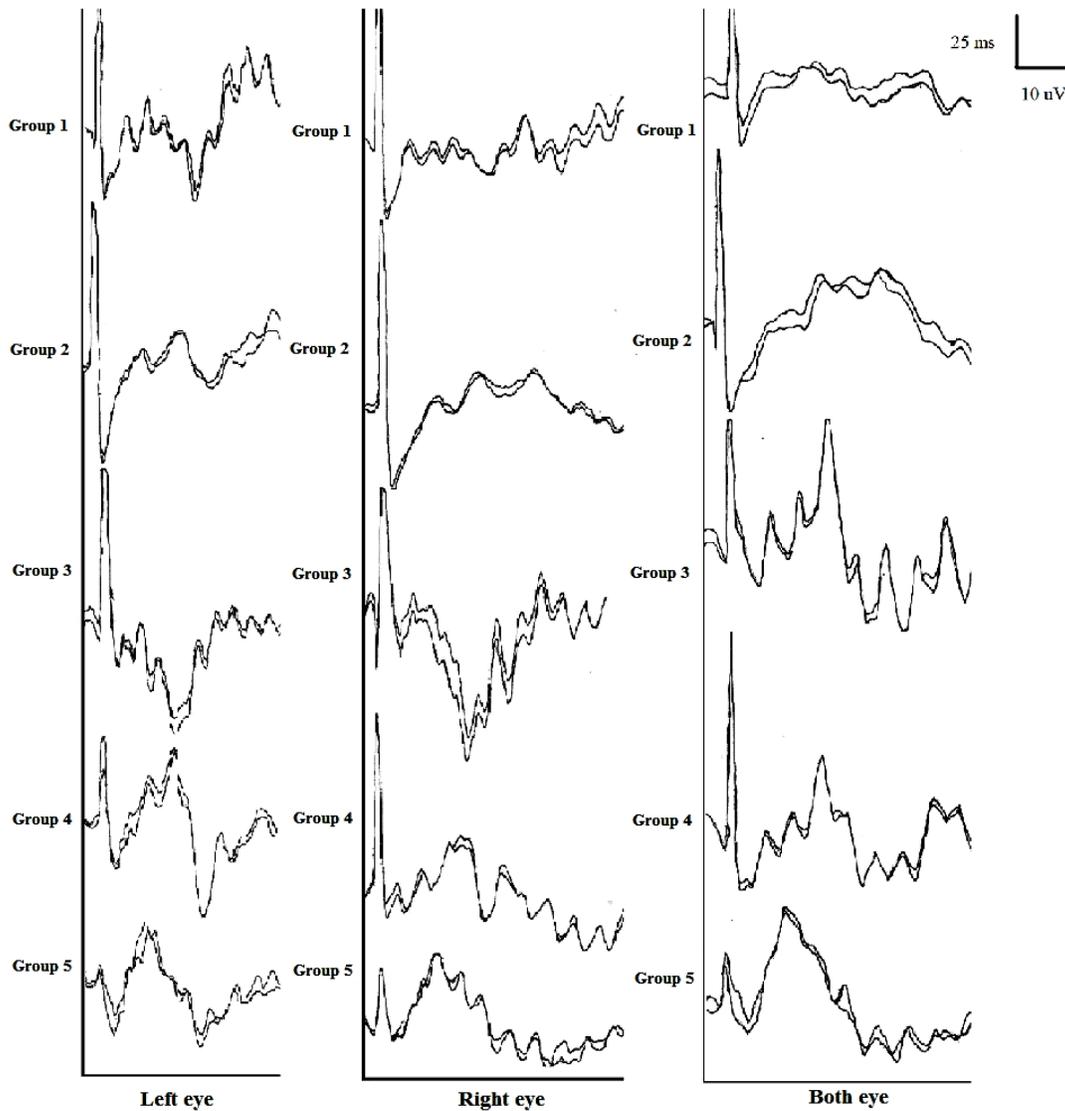
Fig. 2: Morphology of the visual waves in the different physiological stages from a group of monkeys rhesus (*Macaca mulatta)*, in left, right eye and both eyes.

myelination of the optic nerve, fact supported by further research describing that new–born monkeys lack full maturation of V_1 site from the axonal arborizations of the lateral geniculate body which are not entirely developed (Chino *et al.,* 1997; Hatta *et al.,* 1998; Zhang *et al.,* 2008). This statement is confirmed by Shkurovich (1997) which indicated that ganglion cells and synaptic unions of the LGN (lateral geniculate nuclei) as well as occipital lobes, suffer specific developmental processes which have not been fully attain at childbirth in human beings. As the purpose of this paper findings encountered at P_1 demonstrated that both humans and NHP are immature creatures at birth and therefore their central nervous system (CNS) are subjected to proliferative timetable maturation.

Regarding visual area N_2 (extrastriate visual association cortex) significant differences were observed for the left afferency between groups 1 (139.24±8.46 ms) and 3 (127.63±2.02 ms) and against group 4 (127.73±3.34 ms). This visual area participates in a complex array of phenomena linked to visual association receiving strong

feed forward from V_1 and sending stimuli to V_3, V_4, and V_5, thus creating a complex repertoire of feed forward and feedback connections between visual cortical areas that also depend upon cell type-specificity. Differences described for group 1 outlined the existence of an immature visual pathway leading to abnormally delayed potentials of stimuli up to the visual association area where a prolonged latency was noted in comparison to the rest of the study groups.

Diverse studies support a theory implying that a rather variable timetable exists for myelination to take place in the human and animal nervous system, due to the fact that this proliferative process initiates at the sensory-motor system and continues at the secondary cortical area and finally at the association cortex, which leads the conclusion that poorly myelinated visual pathways present in neonate monkeys played a decisive role in neural conductivity of the visual pathway.

In the field of neurobiology processes involved in the development, maturation and myelination of the neural pathways are fundamental to interpret research

information gather in animal models. Regarding this paper this phenomenon can be inferred from the study of different age-related groups of monkeys in which we observed a tendency of diminished time-response for the various waveforms recorded (Table 1), this is to say, that prolonged latencies were registered for group 1 when compared to the rest of the cohorts. In group 3 conformed with young, fully-mature, healthy animals short latencies prevailed, but again prolonged latencies were seen in group 5 where inadequate pathway myelin regeneration was present. Such responses have been previously documented in neurophysiologic studies conducted in monkeys (Malkova *et al.,* 2006; Hernández-Godínez *et al.,* 2011), and has also been described for the somatosensorial pathway. Oliver *et al* (1997) indicated the existence of a relationship between corticospinal low speed conductivity in the medulla of neonate monkeys in direct correlation to their myelination degrees. This suggests that complete myelination in rhesus macaque monkeys was attained at 36 months of age.

Based on this information and on well documented statements describing the complex developmental myelination statuses normally occurring in mammals, evidence indicates that the visual association area conforms one of the lasts segments to be entirely myelinated, and throughout this study it has been observed that the visual pathway maturation in monkeys culminates at 6 y of age while a gradual degeneration process starts from year 12 on.

Conclusion: The neurologic development and organization of the primate visual pathways naturally occurring in macaques has demonstrated that projections of the primate visual system at maturity are characterized by a fine degree of precision, that is not seen during the gradual maturation that takes place throughout the complex ocular growth associated with refractive error and delayed electric conductivity, highly dependent on maturity of visual pathways observed in our animal models. The responses seen in our study enlighten the fact that it is reasonable to extrapolate data derived from neonatal VEP's in monkeys to human beings, and that data collected during the maturation of normal animals could be useable as age-matched control in this species.

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