Flash visual evoked potentials in diurnal birds of prey

Maurizio Dondi, Fabio Biaggi, Francesco Di Ianni, Pier Luigi Dodi, Fausto Quintavalla

The objective of this pilot study was to evaluate the feasibility of Flash Visual Evoked Potentials (FVEPs) testing in birds of prey in a clinical setting and to describe the protocol and the baseline data for normal vision in this species. FVEP recordings were obtained from 6 normal adult birds of prey: n. 2 Harris's Hawks (Parabuteo unicinctus), n. 1 Lanner Falcon (Falco biarmicus), n. 2 Gyrfalcons (Falco rusticolus) and n. 1 Saker Falcon (Falco cherrug). Before carrying out VEP tests, all animals underwent neurologic and ophthalmic routine examination. Waveforms were analysed to identify reproducible peaks from random variation of baseline. At least three positive and negative peaks were highlighted in all tracks with elevated repeatability. Measurements consisted of the absolute and relative latencies of these peaks (P1, N1, P2, N2, P3, and N3) and their peak-to-peak amplitudes. Both the peak latency and wave morphology achieved from normal animals were similar to those obtained previously in other animal species. This test can be easily and safely performed in a clinical setting in birds of prey and could be useful for an objective assessment of visual function.

1   **Author Cover Page**
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3   **FLASH VISUAL EVOKED POTENTIALS IN DIURNAL BIRDS OF PREY**

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19 ABSTRACT

20 The objective of this pilot study was to evaluate the feasibility of Flash Visual Evoked Potentials
21 (FVEPs) testing in birds of prey in a clinical setting and to describe the protocol and the baseline
22 data for normal vision in this species. FVEP recordings were obtained from 6 normal adult birds
23 of prey: n. 2 Harris's Hawks (*Parabuteo unicinctus*), n. 1 Lanner Falcon (*Falco biarmicus*), n. 2
24 Gyrfalcons (*Falco rusticolus*) and n. 1 Saker Falcon (*Falco cherrug*). Before carrying out VEP tests,
25 all animals underwent neurologic and ophthalmic routine examination. Waveforms were
26 analysed to identify reproducible peaks from random variation of baseline. At least three positive
27 and negative peaks were highlighted in all tracks with elevated repeatability. Measurements
28 consisted of the absolute and relative latencies of these peaks (P1, N1, P2, N2, P3, and N3) and
29 their peak-to-peak amplitudes. Both the peak latency and wave morphology achieved from
30 normal animals were similar to those obtained previously in other animal species. This test can
31 be easily and safely performed in a clinical setting in birds of prey and could be useful for an
32 objective assessment of visual function.

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35 INTRODUCTION
36 The relationship between man and birds of prey has been known since ancient times. In fact, it
37 is believed that falconry originated from a hunting technique used on the Mongolian plateau
38 around 6000 B.C. Over the millennia, the art of falconry spread all around the world, becoming a
39 well-known form of huntsmanship practised by the noble classes (Frederik II 1260). Although
40 certain pathologies affecting birds of prey have been known for centuries, anatomical and clinical
41 data available in the literature remain scarce, even though over the past years veterinary interest
42 towards this species has significantly grown (Redig 1993, Zucca 2004, Cooper 2004).
43 In recent years, an increase in public awareness on environmental protection and integrated
44 catchment management has led to a high demand in specialized diagnostic services with the
45 creation of veterinary centres and rehabilitation facilities also dedicated to birds of prey (Tristan
46 2010). In these facilities, the most commonly encountered medical conditions are eye
47 pathologies with a prevalence between 28% (Murphy 1987) and 48% (Labelle 2012). The
48 consequence of irreversible damage to the sight of these animals is extremely relevant with
49 regards to survival. In fact, in these centres, only 12% of animals can be freed, whilst 43% must
50 undergo euthanasia. The remaining 45% of animals is sent to prolonged recovery centres. This is
51 due to the fact that in birds of prey, sight is of fundamental importance in order to maintain
52 predatory skills. A reduction in visual acuity or loss of stereoscopic sight following partial bilateral
53 or complete unilateral visual lesions can determine reduced survival in nature or a reduction of
54 their use in falconry (Labelle 2012).
55 Optimal sight is determined by the correct functioning of all the anatomical structures that
56 constitute the visual pathways, from the eye to the Wustl and Entopallium, and its assessment in
57 birds of prey requires an articulate clinical and instrumental approach. The first approach, which

58   consists in observing the animal in the aviary, allows to assess the bird's ability to avoid objects,
59   as well as its predatory technique. This approach is limited as it does not allow to identify mild
60   visual impairment that though compensated in captivity, would not allow the animals to survive
61   in their natural habitat (Pauli 2007).
62   The second approach is represented by ophthalmic examination. On the one hand, this
63   examination allows us to accurately identify alterations of the ocular structures. However, on the
64   other, it does not provide functional data especially on post-retinal visual pathways. In today's
65   clinical practice, the functional assessment of the post-retinal visual pathways is based
66   exclusively on Cranial Nerve Examination. Whilst providing functional data, this examination is
67   not particularly sensitive or objective. In contrast, instrumental tests, such as electroretinography
68   (ERG) and Visual Evoked Potentials (VEP), provide objective and quantitative data on the
69   functionality of the retina and of the post-retinal visual pathways (Roze et al. 1990, Willis 1999,
70   Clippinger 2007, Labelle et al. 2012).
71   ERG provides objective functional data on the retina and is widely used in most animal species of
72   veterinary interest. Over the past years, this test has been recommended as a routine test to be
73   carried out on birds of prey before releasing them back into their natural environment (Narfström
74   et al. 2002, Labell et al. 2012).
75   The functional assessment of the post-retinal visual pathways has long been carried out in
76   humans and in dogs using Visual Evoked Potential Testing. Unlike ERG, VEP testing provides
77   functional information mainly with regards to lesions of the optic nerve and of the central visual
78   pathways. Visual evoked potentials (VEPs) are electro-diagnostic tests, which allow us to study
79   the activation of visual pathways, from the retina to cortical areas, as a result of light stimulation.
80   The activations of these neuro-anatomic structures are represented, on the recorded tracks, as
81   a series of waves characterized by positive and negative peaks representing the variation of the
82   electric field over time (Bichsel et al. 1988, Sims et al. 1989, Strain et al. 1990, Kimotsuki et al.
83   2005a, Kimotsuki et al. 2005b, Itoh et al. 2010).
84   Searching the veterinary literature, it emerges that VEP testing is still not used in birds of prey
85   and that protocols and normal reference values are lacking for this species.
86   The objective of this pilot study (Thabane et al., 2010) was to evaluate the feasibility of VEP
87   testing in birds of prey in a clinical setting and to describe a routine method to define baseline
88   data for normal vision in diurnal birds of prey.
89
90   METHODS
91   *Animals*
92   FVEP recordings (n=11) were obtained from the right and left eyes of 6 normal adult birds of prey:
93   n. 2 Harris's Hawks (*Parabuteo unicinctus*), n. 1 Lanner Falcon (*Falco biarmicus*), n. 2 Gyrfalcons
94   (*Falco rusticolus*) and n. 1 Saker Falcon (*Falco cherrug*). The data on VEP responses were collected
95   during regular routine check-ups carried out to assess health and hunting predisposition in a
96   population of client-owned birds of prey used in falconry at the Veterinary Hospital of the
97   University of Parma (Italy) in the year 2013. Owner consent was obtained from all the participants
98   of the study after having thoroughly informed the owner about the procedure.
99   *Procedure*
100 Before carrying out VEP testing, all animals underwent routine neurologic and ophthalmic
101 examination as part of a general health check. Ophthalmic examination included slit-lamp

102 biomicroscopy, ophthalmoscopy and Schirmer Tear Test type I (STT I). Patients with neurologic
103 abnormalities were excluded from the study. Therefore, statistical analysis was carried out
104 exclusively on patients who did not present with ocular abnormalities upon ophthalmic
105 examination.
106 VEP tests were carried out on anesthetized animals. General anaesthesia was induced and
107 maintained by administration of isoflurane (induction was carried out by inhalation (mask) and
108 maintained through endotracheal intubation with Isoflurane 3%). Body temperature was
109 maintained within the normal ranges thanks to a heating pad. Tests were carried out using an
110 Electromyography and Evoked Potentials Systems (MyoHandy, Micromed, Treviso, Italy).
111 Animals were positioned in sternal recumbency with their heads raised by a support in order to
112 allow correct luminous stimulation. VEPs were recorded using needle-electrodes (stainless-steel
113 EEG needles) applied subcutaneously to the midline of the forehead between the eyes (Fpz,
114 negative electrode), on the nuchal crest in the occipital region (Oz, positive electrode), and on
115 the vertex (Cz, ground electrode). Prior to recording, no mydriatic drugs were instilled in the eyes
116 because sufficient mydriasis was obtained under the anaesthetic plan. All recordings were made
117 with prior adaptation to the light in a quiet and floodlit room.
118 Stimuli consisted in a flash of white light (approximately 800,000 candlepower) generated by a
119 photostimulator (Flash Stimulator, Micromed, Treviso, Italy). The xenon lamp unit was located at
120 15 cm in front of the eye under examination and the eyelid was gently opened, while the
121 contralateral eye was covered with a black eye patch. Two series of consecutive stimulations
122 were carried out on each eye: the first series at a frequency of 1 Hz and the second at a frequency
123 of 6 Hz with a 3-minute interval between the two series.
124 Two tracks were recorded from each eye with an average of at least 200 flash responses at both
125 frequencies of stimulation. A double-track recording is commonly used to study all evoked
126 potentials and helps to define track repeatability and highlight possible random peaks due to
127 muscle artifacts. The final measurement was carried out only on the first of the two tracks. Low
128 and high filter settings were at 1 Hz and 100 Hz, respectively; 50 Hz filtering was not required.
129 *Data analysis*
130 Waveforms were analysed to identify reproducible peaks from random variation of the baseline.
131 Measurements consisted of the absolute latencies, expressed in milliseconds (ms), each of the
132 six peaks, identified as P1, N1, P2, N2, P3 and N3 and the peak-to-peak amplitudes expressed in
133 microvolt (μV). The measured relative latencies (interpeak) were P1-P2, P2-P3, P1-P3, P1-N2 and
134 N2-P3, whilst the relative amplitudes of the potential considered were P1-N1, N1-P2, P2-N2, N2-
135 P3 and P3-N3. Absolute latency was defined as the time from stimulus onset to the peak of a
136 wave. Relative latency or interpeak was defined as the interval between two peaks. The relative
137 amplitude was calculated as the mathematical difference between the absolute values of
138 electrical potential between two peaks.
139 Positive and negative peaks latencies and potentials were measured using a cursor on the
140 computer monitor and were recorded to the nearest 0.1 ms value of latency. With this type of
141 electrode configuration, the positive peaks point upwards and are indicated with the letter P
142 followed by a number. Whilst the negative peaks point downwards and are indicated with the
143 letter N followed by a number.
144 Descriptive statistics consisting of mean (M), variance (Var), standard deviation (SD) and standard
145 error (SE) for each absolute and relative latency and amplitude measurements were calculated.

146   Qualitative analysis of waveform morphology was carried out in order to assess possible
147   similarities or differences between species.

148

149   RESULTS
150   The results of the VEP recordings are summarized in tables 1 to 6. A maximum of six peaks were
151   identifiable in the recordings, consisting in three positive peaks (P1, P2, and P3) and three
152   intervening negative peaks (N1, N2, N3). As regards track morphology, a few differences were
153   observed between species (Fig. 1). It must be pointed out that in all subjects that underwent
154   testing, lower stimulation frequency waves (1 Hz) were more evident than higher stimulation
155   frequency waves (6 Hz).
156   The statistical analysis of the results obtained showed that P1, N2 and N3 peaks are present on
157   all recordings, whilst the remaining peaks are not always measurable. N1 was present in 73% of
158   recordings at 1 Hz and in 55% of those at 6 Hz; P2 was present in 91% of recordings at 1 Hz and
159   in 73% of recordings at 6 Hz and N3 in around 91% of recordings at 1 Hz and in 82 % at 6 Hz. The
160   variability of the absolute latencies of all peaks within the group was rather limited despite having
161   considered different species of birds of prey even if of similar structure and size.
162   However, from the analysis of the interpeak latencies, it emerges that at both stimulation
163   frequencies the values that are always measurable in all tracks are P1-P3, P1-N2 and N2-P3. These
164   values are also those that highlight a lower variability than compared to SD and Var. The low
165   variability of the absolute and relative peak latency values may be of clinical relevance. On the
166   other hand, the relative amplitude of the interpeak potentials, calculated in intervals P1-N1, N1-
167   P2, P2-N2, N2-P3 and P3-N3 is extremely variable and at present does not allow to hypothesize
168   its use in the clinical practice.
169   From a visual analysis of the recordings, in some cases there is a tendency of the first (P1) and
170   second (P2) positive wave to overlap. These overlapping waves can be defined in practice by
171   observing the change in steepness of the interpeak segment and form the presence of additional
172   peaks that are often confused with the dominant potential. The frequency of overlap between
173   P1 and P2 was of 9% with a stimulation at 1 Hz and of 27% with a stimulation at 6 Hz. Under these
174   conditions, the N1 and P2 values were not considered in statistical analysis due to their difficult
175   localization. Finally, it was not possible to define all N3 values due to reduced amplitudes and
176   lack of repeatability in the control recordings.
177   As well as the previously described peaks, on the recordings of all the birds of prey studied, as
178   regards to amplitude of potentials, delayed, unrepeatable and less evident peaks were also
179   highlighted between P1 and N3. Not much importance was given to these potentials but they
180   should be considered as in the future, they may prove to be interesting.

181

182   DISCUSSION
183   The results of this study have shown that it is possible to record FVEPs in diurnal birds of prey
184   using the technique that has already been described and used in dogs (Strain et al. 1990).
185   Moreover, the morphology of the tracks achieved and the peaks of the potentials considered (P1,
186   P2 e P3) are the same as those observed in dogs. Therefore, the use of this protocol in the future
187   in clinical practice can be hypothesized. In fact, the absolute and relative latency values of the
188   FVEPs in the birds of prey studied proved to have reduced variability.

189   The clinical usefulness of FVEPs is well known in human medicine, as it allows to objectively assess
190   the functional integrity of the visual pathways, from the retina to the visual cortex, even when
191   the patient is not in a position to collaborate, for example, during general anaesthesia, comatose
192   states or when carried out on neonates (Chiappa and Hill 1997). FVEPs are commonly used in
193   clinical practice also in dogs to study visual function but with some differences compared to man.
194   In fact, in animals, FVEPs require pharmacological containment due to the lack of active
195   collaboration during the test. Compared to what is described in man, where the test is normally
196   carried out on awake individuals and a broad inter-individual variability of potentials exists
197   (Odom and others 2010), in diurnal birds of prey, as is the case in dogs, the use of a general
198   anaesthesia reduces the variability of the evoked visual responses (Khimotsuki et al. 2005b).
199   Two further elements play a role in determining the usefulness of this protocol in birds of prey:
200   the first is adaptation of the eye to light. In fact, the test is carried out in phototopic conditions,
201   which determine a retinal potential that has reduced amplitude and duration, which gives the
202   possibility of better highlighting the potential delay produced by the post-retinal nervous
203   structures. The second element is the positioning of the electrodes that being both active and
204   given the small size of the skull of the birds of prey allow to determine the potentials produced
205   by the eye and by the entire visual path on the same track.
206   In dogs, the usefulness of the FVEP test is related to the correlation between the function of
207   particular structures of the visual path and the presence of precise peaks on recordings. In
208   particular, it is possible to identify the neuro-anatomical location of the visual lesions following a
209   lack of determined potentials or following an increase in their latency times. In fact, Sims
210   demonstrated the post-retinal origin of FVEPs in dogs and also that complete lesions of the optic
211   nerve cause the disappearance of all potentials after N1 (Sims et al. 1989). Then, Kimotsuki, again
212   in dogs, showed that a lesion of the Lateral Geniculate Body causes the immediate disappearance
213   of peaks N2 and P3 (Kimotsuki et al. 2005b). It is therefore possible to state that P1 represents
214   retinal potential and can be identified with wave B of the ERG; the N1-P2 interval is generated by
215   the optic nerve, by the chiasm and the visual pathway; interval N2-P3 is generated by the lateral
216   geniculate body and by optic radiations.
217   In birds of prey, similar conclusions are not possible due to lack of accurate data on their neuro-
218   functional anatomy, even if significant neuroanatomical similarities with the visual pathways of
219   mammals exist and could allow to make parallel hypotheses. In fact, in birds of prey, there are
220   two parallel visual pathways: the tecto-fugal and thalamo-fugal pathways. The first pathway
221   corresponds to the extra-geniculostriate system in mammals and in particular in primates, whilst
222   the second pathway corresponds to the geniculostriate system. The tecto-fugal pathway
223   (collothalamic) is composed of axons of the optic nerve that intersect with different percentages
224   according to the species to form the optic chiasm. Then, these fibres reach the optic tectum and
225   then the round nucleus of the thalamus and finally, the ectostriatum nuclei. The ectostriatum is
226   a wide longitudinal cerebral structure incorporated in the dorsal ventricular ridge (DVR) that is
227   mainly responsible for the elaboration of diurnal sight, whilst the lemnothalamic pathway goes
228   from the retina to the dorsal thalamic nuclei and ends on the visual cortex that in birds is called
229   Wulst. The first pathway (collothalamic) is more developed in species with eyes located laterally
230   (ground-feeding birds), the second one (lemnothalamic) is more developed in owls and hawks
231   for processing the frontal binocular field (Shimizu and Bowers 1999, Husband and Shimizu 2001).

232   In conclusion, the results of this work indicate that it's possible to use FVEPs on birds of prey for
233   clinical purposes, and allows us to hypothesize the future use of FVEPs in the functional
234   assessment of the visual pathways of these species.
235
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Figure 1

FVEP waveforms of a) Saker Falcon (Falco cherrug); b) Gyrfalcons (Falco rusticolus); c) Harris's Hawks (Parabuteo unicinctus); d) Lanner Falcon (Falco biarmicus). Low band pass filter was set to 0.1 Hz and high band pass filter was set to 100 Hz. Sweep speed was 50 msec/Div and the gain at 10 µV/Div. In each part the larger waveform was achieved with a luminous stimulation at a frequency of 1 Hz whilst the smaller one at a frequency of 6 Hz. Only the first 3 positive peaks were identified if evident. All tracks are shown only in the part that follows the light stimulus, which is considered to be 0 msec.
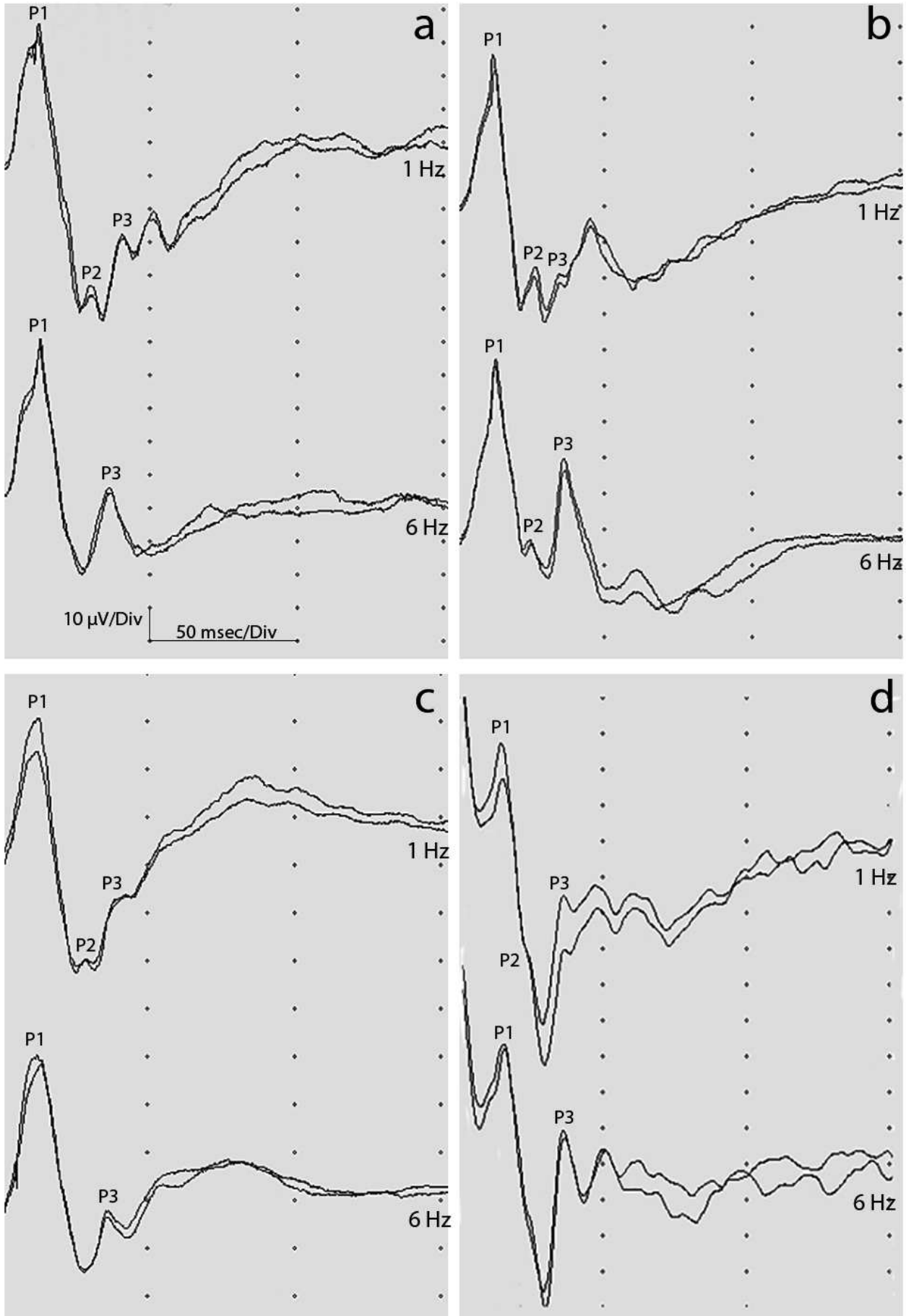
Table 1(on next page)

Table 1

Summary of absolute peak latency values expressed in msec for FVEPs at 1 Hz stimulus frequency

1 Hz	P1	N1	P2	N2	P3	N3
N	11	8	10	11	11	10
Mean	12,7	23,0	26,3	30,3	37,2	43,0
Stand. Dev.	0,8	2,6	2,6	2,4	1,7	1,2
Variance	0,7	6,7	6,6	5,9	3,0	1,3
Stand. Error	0,2	0,9	0,8	0,7	0,5	0,4

1

Table 2(on next page)

Table 2

Summary of absolute peak latencies values expressed in msec for FVEPs at 6 Hz stimulus frequency

6 Hz	P1	N1	P2	N2	P3	N3
N	11	6	8	11	11	9
Mean	14,0	23,0	25,3	29,0	35,9	42,3
Stand. Dev.	0,9	1,6	2,1	2,5	1,7	1,7
Variance	0,9	2,5	4,6	6,5	3,0	3,0
Stand. Error	0,3	0,6	0,8	0,8	0,5	0,6

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Table 3(on next page)

Table 3

Summary of interpeak latency values expressed in msec for FVEPs at 1 Hz stimulus

frequency

1 Hz	P1-P2	P2-P3	P1-P3	P1-N2	N2-P3
N	9	9	11	11	11
Mean	13,9	11,6	24,5	17,5	6,9
Stand. Dev.	2,2	1,6	1,8	2,5	1,4
Variance	4,7	2,6	3,4	6,5	2,1
Stand. Error	0,7	0,5	0,6	0,8	0,4

1

Table 4 (on next page)

Table 4

Summary of interpeak latency values expressed in msec for FVEPs at 6 Hz stimulus frequency

6 Hz	P1-P2	P2-P3	P1-P3	P1-N2	N2-P3
N	7	7	11	11	11
Mean	11,6	10,9	22,0	15,10	6,9
Stand. Dev.	1,6	1,4	1,4	2,1	1,1
Variance	2,5	1,8	2,0	4,6	1,2
Stand. Error	0,6	0,5	0,4	0,6	0,3

1

Table 5(on next page)

Table 5

Summary of interpeak amplitudes values expressed in μV for FVEPs at 1 Hz stimulus frequency

1 Hz	P1-N1	N1-P2	P2-N2	N2-P3	P3-N3
Mean	69,2	10,8	14,1	12,9	3,7
Stand. Dev.	25,1	8,0	9,4	10,2	2,5

1

Table 6(on next page)

Table 6

Summary of interpeak amplitudes values expressed in µV for FVEPs at 6 Hz stimulus frequency

6 Hz	P1-N1	N1-P2	P2-N2	N2-P3	P3-N3
Mean	72,2	20,1	8,4	18,7	10,4
Stand. Dev.	30,8	16,3	6,3	8,9	8,7

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