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# Flash-visual evoked responses in male mice are faster than in female mice.

**Stefano Loizzo[1], Irene Guarino[1], Alfredo Martinangeli[1], Alberto Loizzo[1], Anna Capasso[2]**

[1]Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy; [2]Department of Pharmacy, University of Salerno, Italy.

## Abstract

**Visual evoked potentials (VEPs) and rapid oscillatory potentials (OPs) of male and female DBA/2 mice were studied. Gender confrontations of the OPs spectral content and effects induced by a single low dose of physostigmine (0.05 mg/kg) were also evaluated. VEPs and OPs responses to luminous stimuli in male mice had a consistently lower latency than in females, but no gender differences in OPs spectral content (60-300 Hz) were evidenced. Physostigmine induced a further significant decrease in latency of evoked responses only in male mice and a significant decrease of amplitude in females. Spectral information contained in rapid OPs was not affected. Gender differences did not appear to be dependent barely on hormonal conditions, but on a more complex structural background.**

**Keywords:** visual evoked potentials (VEPs), rapid oscillatory potentials (Ops), physostigmine, OPs spectral content, gender differences.

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## Introduction

Visual evoked potentials (VEPs) and oscillatory potentials (OPs) are well-established means of investigation of the optic system in humans and in animals because they provide an objective functional measurement of the visual pathway and related neural systems. In particular, flash stimulation has been used in both clinical and experimental research, because of its feasibility in non-cooperative, non-sedated subjects [1,2,3]. Scalp recorded oscillatory potentials (OPs) in response to luminous stimuli, which reflect short-term synchronization of neuronal electrical activity: oscillations around the gamma range (40-100 Hz) reportedly play a role in attention-related mechanisms and synaptic transmission and are necessary events in stimulus processing [4,5].

Recently, we studied a new method for recording VEPs and OPs in freely-moving, unanaesthetized rats, with the aim of proposing a tool useful for pharmacological investigations in visual diseases [3]. However, this method was set on male animals only.

Gender pharmacology is now taking an important place among specialists, and data on visual evoked responses, considering the gender differences in laboratory animals, are strongly needed.

Therefore, we started an investigation in order to study:

1) latency and amplitude of visual evoked potentials (VEPs) and rapid oscillatory potentials (OPs) in male versus female mice
2) OPs power spectral frequency content, to examine whether gender differences can be found in the ‘content’ of message, not only in the time course from stimulus to cortex.
3) Whether difference of message transmission depends on speed of stimulus genesis, or on stimulus conduction. To ascertain this topic, we administered a low dose of the cholinomymetic drug, physostigmine, which in previous publications was able to reduce latency of VEPs responses in male mice [6], presumably through a direct action on retinal cholinergic structures.

## Methods

*Animals*

DBA/2 young adult (aged 70-80 days) male and female mice (Harlan- Italy) (n=12 per group) weighing 23.3 ±0.3 g (males) and 21.1 ±0.7 g (females) were housed in transparent plastic cages under standard conditions,

with free access to food and water, 12 hrs light/dark cycle, ambient temperature of 21 ±2 °C, and relative humidity of 55 ±5%. The animal care and use followed the directives of the Council of the European Communities. The Bioethical Committee of the Italian National Institute of Health approved the experimental protocol.

***Surgery***

Animals were anaesthetized with xylazine (Rompun, Bayer AG, Leverkusen-Germany, 20 mg/kg i.p.) and ketamine (Ketavet 100, Gellini Farmaceutici Spa, Peschiera Borromea-MI, 32 mg/kg i.p.) and locally injected lidocaine (Lidocaina 2%, Azienda Terapeutica Italiana A.T.I. srl, Ozzano Emilia-BO, 0.1 ml s.c.), and were implanted with chronic electrodes. The electrode positions were the same for both male and female animals, being determined with reference to the sagittal suture and bregma. For each animal, four L-shaped monopolar epidural stainless-steel electrodes (0.8 mm diam/10 mm length) were implanted on sensorimotor cortex (left and right posterior: 2 mm posterior to bregma and 2 mm lateral to sagittal suture, left and right anterior: 1 mm anterior to bregma and 1 mm lateral to sagittal suture) and fixed with dental cement; distance of the electrodes was the same in all animals [2]. Posterior electrodes' position corresponded to the position that allowed the maximal VEP responses [7]. During surgery, a heated table maintained the body temperature at 37 °C. A recovery period of 1 week was allowed before the EEG recording.

***Stimuli***

A Grass Instrument PS-22 photic stimulator supplied the flash stimulation. During the recording, 90 successive light flashes (stimulus rate: 1.01 flash per second, duration: 10 μs) were presented. One intensity of luminous stimulation, corresponding to 236.4 mcd/m$^2$*s, was used, referring to the results obtained in preliminary sessions published elsewhere [2]. Care was taken to ensure uniform light conditions with both a diffusing filter and diffusing coatings on the surfaces surrounding the mouse, within the testing room. As a result, in the worst condition, our measurements with the photometer showed only a 10% variation related to different mouse positions and gaze direction within the testing room.

***Experimental protocol***

Recording sessions took place at the same time each morning to avoid interferences due to circadian rhythm. Each awake, free moving mouse was recorded alone while housed in an anechoic cage with white walls, with the ceiling corresponding to the flash stimulator positioned outside the sound-proof cage to avoid acoustic interferences by flash. Female animals were recorded in another identical cage, to avoid olfactory interferences, in random time, independent from their hormonal cycle. In each animal we recorded antero-posterior sensorimotor EEG during administration of flash sessions, 2-3 sessions/animal. Then we administered physostigmine salycilate (Sigma Chemical Co, Germany, 0.05 mg/kg as base weight; IP) and VEPs were recorded two more times (10 and 25 minutes after drug). The EEG signal was amplified through a DC powered preamplifier, with a gain of 1000X and a 1-500 Hz band-pass analogic filter (6dB/octave).

Acquisition was performed at 2.5 kHz sampling rate. An InstruNet A/D 16-bit conversion board delivered the signals to the acquisition system (Superscope-GW Instruments, Somerville, Massachusetts, USA) on a personal computer (Macintosh G4-PC), adapted and set by Analysa (Cuneo, Italy). Brain activity and trigger were continuously acquired and saved as raw data to be analyzed off-line. Animals were not scotopically adapted. Amplitude and latency of VEPs responses were measured after baseline normalization. Latency was calculated as the absolute value between stimulus onset and the peak of the first negative response (N1), whereas amplitude was taken as an absolute value of the first negative peak amplitude. Oscillatory potentials were extracted through the application of a digital 60 Hz HP filter (linear phase FIR, 6 dB/octave) to the raw data. The latency of the oscillation was measured in ms from the trigger to the response peak. The amplitude was calculated as the baseline-to-peak value ($ON_1$). Moreover the raw EEG signal underwent 60-300 Hz digital band-pass filtering, and after averaging, OPs spectral content was computed [2,3]. The frequency bands considered were: 60-100 Hz, 100-200 Hz and 200-300 Hz. Total power and mean absolute power content of the three frequency bands were compared before and after physostigmine administration.

***Statistical analysis***

The latencies and amplitudes of the major peaks of the VEPs and of the OPs were measured, and differences between before and after physostigmine administration, and between male and female mice were evaluated. Differences in latency and amplitude between groups were evaluated using one-way ANOVA followed by Duncan test. Differences of latency and amplitude between males and females were evaluated using Student's *t* test. Differences were considered to be significant when $P<0.05$.

The spectral statistical analysis on all bands power was computed using one-way ANOVA followed by Student t-test for single band power.

## Results

VEPs and OPs morphology was quite similar to the ones published in previous papers [2,3].

***VEPs latency and amplitude before and after physostigmine.***

*Latency:* in male mice N1 was recorded at a mean latency of 32.9 ±0.4 ms, in females at a latency of 35.5 ±0.4 ms.

(Fig.1). This difference was significant at P<0.01 level. Fig.2 represents the frequency distribution of VEP latencies observed in male and female mice, considering all control recordings. After administration of physostigmine we observed a significant decrease in latency 10 min after drug administration, only in the male mice (30,7 ±0.6 ms; P<0.01), but not in the females (34.3 ±0.7 ms) (Fig.1).

***Amplitude***

Amplitude of N1 in male mice was 37.4 ±2.5 μV, and 38.4 ±2.7 μV in females, which was not significantly different. Administration of physostigmine induced no significant changes after 10 min. Twenty five minutes after drug administration a significant decrease in amplitude was observed only in the female mice, (26,3 ±4 μV; P<0.05) but not in the males (31.8 ±2.6 μV).

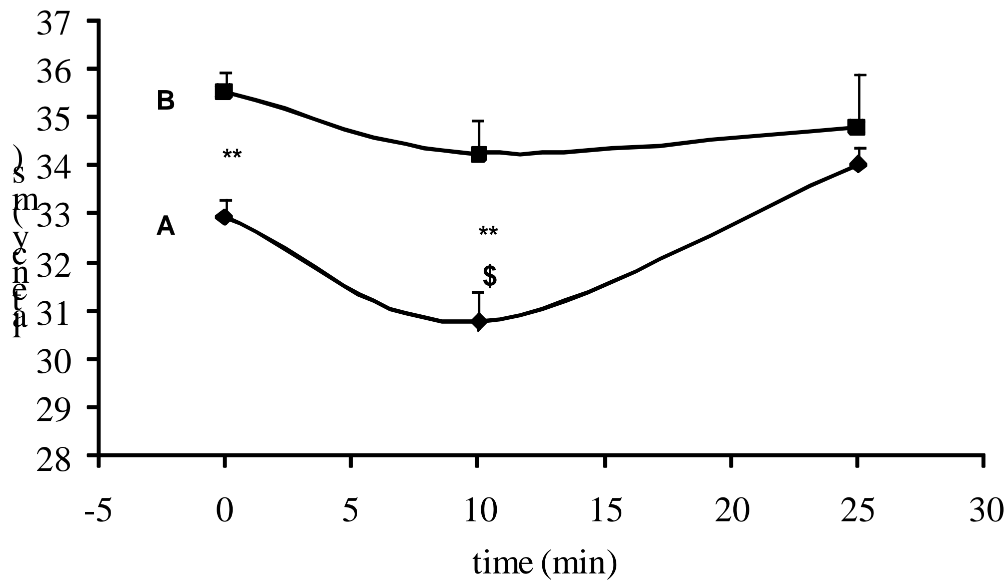


***Figure 1.*** *VEP N1 mean latency in ms ± S.E.M. in control male (A) and female (B) mice, and after treatment with physostigmine. Abscissa: iniection at time 0; latency values at 10 and 25 min after drug. Ordinate: latency in ms; ** = P< 0.01 between genders; $ = P<0.01 between time 0 and 10 min after physostigmine in males.*

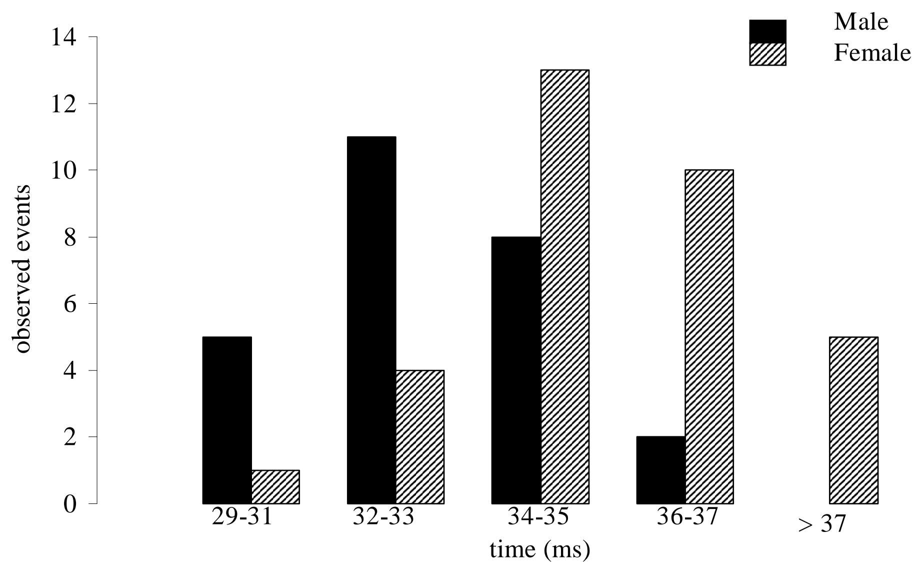


***Figure 2.*** *Frequency distribution of VEP latencies observed in male and in female control mice. Abscissa: latency in ms; Ordinate: number of events in control recordings (2-3 events per mouse).*

***OPs latency, amplitude and spectral content before and after physostigmine administration.***

*Latency:* in male mice $ON_1$ showed a mean latency of 31.4 ±0.4 ms *vs* 34.7±0.2 ms in females (difference significant at P<0.01). After physostigmine no consistent variations were recorded (32.1±0.6 ms and 34.9±0.5 ms, respectively).

*Amplitude:* amplitude was 4.6 ±0.6 μV in males and 5.3 ±0.5 μV in females. After physostigmine no consistent variations in latency and amplitude were observed, both in males and in females.

Analyzing OPs spectral content, at the three-frequency bands considered (60-100, 100-200 and 200-300 Hz) no significant variation between genders (total power: males 0.0168 ±0.0017 $\mu V^2$/Hz *vs* females 0.0149 ±0.0017 $\mu V^2$/-Hz) and after physostigmine treatment (total power: in males 0.0165 ±0.0008 $\mu V^2$/Hz; in females 0.016 ±0.0013 $\mu V^2$/Hz) was observed.

## Discussion

We evidenced that flash-visual evoked potentials showed striking gender differences in DBA/2 mice. In particular, responses to flash-visual stimuli were faster in male mice (average +11%) than in females. Differences could not be related to the brain size of the two genders, or electrodes' position and their distances: the frontal cortex-cerebellar distal portion was 1.16 ±0.03 cm in males *vs* 1.16 ±0.03 mm in females; and the inter electrode distance (anterior dx *vs* posterior dx) was 0.48 ±0.02 cm in males *vs* 0.46 ±0.03 cm in females.

Previous investigations suggested that the latencies of certain components of the pattern reversal potentials were shorter in women than in men [8,9,10]. These differences were attributed to different factors such as, the smaller size of women's brain [9], the absence of the Y chromosome [11] to endocrine factors [12]. However, neurophysiological differences between genders in laboratory animals were found between isolated brain slices in experimental conditions independent from fluctuations in the blood levels of hormones. Bronzino in 1996 [13], showed significant sex differences in input/output response measures of hippocampal excitatory postsynaptic potentials, and slope and population spike amplitude, with males showing consistently higher values than females.

We recorded VEPs in female mice not taking into account their hormonal status. This may be a bias in our investigation. However, hormonal levels in female mice in our opinion do not challenge our data for two reasons. First: data in the literature are not univocal on the possible influence induced by estrogens and progestins on the latency of evoked responses [14,15,16], and moreover, faster VEPs in females with high hormonal levels [10,17] were described, thus enforcing further differences found in our experimental conditions. Second: differences in average latency of VEPs in male mice versus females are far larger than variation found in females : males. Over 60% of responses are in the range of 29 to 33 ms, while only 16% of female responses are in the same range (*$\chi 2$= 11,7,* ***p< 0.01***) although latency times are normally distributed in both genders (Fig. 2). Therefore we suggest that latency gap between genders in VEPs is not due to hormonal artifacts only, if any.

We also found consistent gender differences in OPs latency in basal conditions, but not after physostigmine; no differences, as well, in OPs spectral content (60-300 Hz) between male and female mice in basal conditions and after physostigmine. Thus, we show that the spectral messages contained in the OPs had the same characteristics in both genders and was not influenced by physostigmine. OPs reflect short-term synchronization of neuronal electrical activity: oscillations around the gamma range (40 to 100 Hz) in man, reportedly play a role in attention-related mechanisms and synaptic plasticity in cortical structures, because they promote coherence and possible 'binding' of local and neuronal activity [17,18,19]. Therefore we speculate that rapid oscillations put forward faster messages in male mice, but the content of messages is apparently the same in both genders. This confirms indirectly the previous findings by Roalf D (2006) [20] who recorded event-related brain potentials (ERP) during performance of a global-local reaction time task, and found that women responded more quickly to local targets, while men did not differentially respond to hierarchical stimuli.

The administration of physostigmine induced a decreased N1 latency, significant only in male mice, and a decrease of N1 amplitude significant only in females. In a previous investigation we showed that following low doses of physostigmine a tendency to latency reduction was evident in late components [6]. Present results show that physostigmine anticipates the message transmission, but it does not change the message spectral content. A topic remains to be elucidated, i.e., whether the reduction of VEP latency in male mice is due to a faster energy transduction process in the retina in response to flashes, or to a faster current conduction through the visual pathways to cortex, or both. We suggest that cholinergic retinal structures may be of importance in answering this problem, since in our male mice the cholinomimetic drug physostigmine induced a consistent diminution of VEP latency. In our opinion this is hardly due to an increase in nerve conduction velocity: the mean latency of VEP after physostigmine in males was 30.7±0.6 ms *vs* 34.3 ±0.7 ms. This would mean that physostigmine induces an average stimulus anticipation lag of 10.5%. In alternative, latency diminution may be due to a pharmacodynamic stimulation of retinal (maybe amacrine) cells.

An indirect confirmation of this hypothesis is found in previous papers [21,22]. Both papers suggest that the chemical energy transduction from light to neural impulses takes a greater part of the total potential time than the time required in the case of stimulation produced by electrical stimuli or by accelerated ions stimulation on nervous pathways. Therefore the hypothesis that energy transduction in the retina of males in unanaesthetized conditions is more efficient than in females, can be suggested.

One result remains to be elucidated, i.e., the decrease of VEPs amplitude induced by physostigmine in females only. At present, we have no explanation for this phenomenon. We can only remember that 17β-estradiol was shown to exert inhibiting properties on neuronal firing rate of ovariectomized rats, and an interference was described between estrogen and acetylcholine effects on several brain neurones [23,24], finally resulting in a lower amplitude of VEPs.

## Conclusion

We found a significant difference in VEP latency between male and female DBA/2 mice, and this difference was further enhanced following the administration of the cholinergic agonist physostigmine, whereas no differences were ever found in the spectral content of high OPs frequencies considered.

We suggest that there is a gender difference in the efficiency of nervous structures (perhaps cholinergic receptors), which determine signal production/ransmission along the nervous pathways somewhere between the retina and the somatosensory cortex. Future experiments shall be performed on different mice strains to verify whether the phenomenon is limited to the present experimental conditions (e.g., DBA/2 strain), or, it can be extended to other strains as well.

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**Correspondence to:**

Stefano Loizzo
Istituto Superiore di Sanità
Viale Regina Elena 299
00161 Roma, Italy