Heliyon 7 (2021) e08360

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Non-invasive visual evoked potentials under sevoflurane *versus* ketamine-xylazine in rats

Valerio Castoldi [a,b], Raffaele d'Isa [a], Silvia Marenna [a], Giancarlo Comi [b,c], Letizia Leocani [a,b,*]

[a] Experimental Neurophysiology Unit, INSPE - Institute of Experimental Neurology, San Raffaele Scientific Institute, Milan, Italy
[b] Vita-Salute San Raffaele University, Milan, Italy
[c] Casa di Cura del Policlinico, Milan, Italy

ARTICLE INFO

Keywords:
Visual evoked potential
Sevoflurane
Ketamine-xylazine
Repeatability indices
Signal-to-noise ratio

ABSTRACT

Background: Visual Evoked Potential (VEP) quantifies electrical signals produced in visual cortex in response to visual stimuli. VEP elicited by light flashes is a useful biomarker to evaluate visual function in preclinical models and it can be recorded in awake or anaesthetised state. Different types of anaesthesia influence VEP properties, such as latency, which measures the propagation speed along nerve fibers, and amplitude that quantifies the power of electrical signal.

Aim: The goal of this work is to compare VEPs elicited in Dark Agouti rats under two types of anaesthesia: volatile sevoflurane or injectable ketamine-xylazine.

Methods: VEP latency, amplitude, signal-to-noise ratio and recording duration were measured in Dark Agouti rats randomly assigned to two groups, the first subjected to volatile sevoflurane and the second to injectable ketamine-xylazine. Taking advantage of non-invasive flash-VEP recording through epidermal cup electrodes, three time points of VEP recordings were assessed in two weeks intervals.

Results: VEP recorded under ketamine-xylazine showed longer latency and higher amplitude compared with sevoflurane, with analogous repeatability over time. However, sevoflurane tended to suppress electrical signals from visual cortex, resulting in a lower signal-to-noise ratio. Moreover, VEP procedure duration lasted longer in rats anaesthetised with sevoflurane than ketamine-xylazine.

Conclusions: In Dark Agouti rats, the use of different anaesthesia can influence VEP components in terms of latency and amplitude. Notably, sevoflurane and ketamine-xylazine revealed satisfying repeatability over time, which is critical to perform reliable follow-up studies. Ketamine-xylazine allowed to obtain more clearly discernible VEP components and less background noise, together with a quicker recording procedure and a consequently improved animal safety and welfare.

1. Introduction

In preclinical research involving animal models of certain diseases, anaesthesia during experimental procedures is routinely applied for both practical handling and ethical reasons. In the field of neuroscience, animal models are widely used to elucidate the biological mechanisms of physiological and pathological conditions, with the aim of discovering innovative therapeutic strategies. For this purpose, the evaluation of brain function through neurophysiological measurements, such as electroencephalogram (EEG) and evoked potentials (EPs), can help medical researchers in understanding the worsening of neuronal activity in pathological conditions, together with the possible recovery during a treatment period (Leocani and Comi, 2000). In particular, to ensure a correct interpretation of EPs, it is important to distinguish the evoked responses (represented by waveform peaks) from the background EEG.

Some neurophysiological tests, such as visual (Tomiyama et al., 2016) and auditory (Schuelert et al., 2018) evoked potentials, can be performed in conscious, freely moving animals, thus requiring surgery and electrode implantation. These procedures may cause inflammation, gliosis (Szarowski et al., 2003; Griffith and Humphrey, 2006) and stress, which could interfere with autoimmune responses (Karagkouni et al., 2013; Nisipeanu and Korczyn, 2003), altering several experimental outcomes. On the other hand, less invasive approaches maintain animal integrity and do not interfere with physiopathological processes. In particular, visual

* Corresponding author.
E-mail address: letizia.leocani@hsr.it (L. Leocani).

https://doi.org/10.1016/j.heliyon.2021.e08360
Received 20 January 2021; Received in revised form 19 March 2021; Accepted 5 November 2021

evoked potential can be reliably recorded with epidermal electrodes in both rats (Santangelo et al., 2018; d'Isa et al., 2020) and mice (Marenna et al., 2019). This procedure needs anaesthesia to avoid animal movements that could detach the recording electrodes. The correct interpretation of neurophysiological tests requires knowledge of the effects caused by different types of anaesthesia on the central nervous system. Therefore, comparing different anaesthesia can considerably help neurophysiologists in testing animal models of neurological diseases, primarily when recording motor, somatosensory or visual evoked potentials.

Visual evoked potentials (VEPs) are employed for the functional evaluation of visual pathways. In particular, VEP latency (the time between the presentation of the visual stimulus and its detection in the visual cortex) measures the propagation speed along nerve fibers and it is a marker of demyelination and remyelination, both in humans (Halliday, 1993; Brusa et al., 2001) and preclinical models of demyelinating disorders (You et al., 2011b; Castoldi et al., 2018). VEP amplitude (the potential difference generated between the recording electrode placed over the visual cortex and the reference electrode after the presentation of the visual stimulus) quantifies the power of the electrical signal produced along the nerve and it can monitor the integrity of optic nerve fibers (Graham and Klistorner, 2017; You et al., 2011b). Human subjects can undergo VEP recording in awake state (Holder et al., 2010), whereas small animals, such as rodents, generally need anaesthesia to better control stimuli presentation, avoid movements and minimize external noise sources. Different types of anaesthetics or sedatives have been used to perform VEPs in animals, such as halothane (Imas et al., 2004), sevoflurane (Castoldi et al., 2020), isoflurane (Aggarwal et al., 2019), urethane (Porciatti et al., 1999), ketamine-xylazine (Roth et al., 2018; Land et al., 2019), pentobarbital (Maertz et al., 2006), fentanyl (Kuroda et al., 2009), detomidine (Ström and Ekesten, 2016), chloral hydrate (Siegel et al., 1993), and morphine (Kuroda et al., 2009). The focus of this work is the examination of VEP property changes depending on two types of anaesthesia. In particular, VEPs obtained in Dark Agouti rats under volatile (sevoflurane) or injectable (ketamine-xylazine) anaesthesia were compared through the analysis of latency, amplitude, inter-session repeatability, signal-to-noise ratio (SNR) and recording procedure duration.

2. Materials and methods

2.1. Animals

This study was designed in compliance with the ARRIVE guidelines and the European Community Directive (2010/63/EU), with the approval of the San Raffaele Institutional Animal Care and Use Committee (IACUC). Eighteen ($n = 18$) female Dark Agouti rats aged eight weeks with a body weight of 110–130 g (Janvier Labs-Saint-Berthevin, France) were included for this experiment. All animals were housed under temperature-controlled conditions (21 ± 1 °C) with *ad libitum* availability of chow pellets (VRF 1 (P), SDS - Special Diets Services) and tap water. Rats were kept on a 12:12 h dark/light cycle with lights on at 9:00 am in order to guarantee constant circadian rhythms.

2.2. Experimental protocol

VEP recording sessions were conducted during the light phase. After shipment (at 7 weeks of age), rats underwent 1 week of acclimatisation, then were randomly assigned to one of two equally sized groups: rats recorded under sevoflurane-based volatile anaesthesia mixture ($n = 9$) or ketamine-xylazine anaesthesia ($n = 9$).

Animals were randomised through their identification number to avoid cage effects (even numbers were assigned to the sevoflurane group, while odd numbers were allocated to the ketamine-xylazine group). In particular, randomisation prevented the bias of having all the rats of one cage treated with the same type of anaesthesia. The administration of the same anaesthetic on the same rat across three timepoints permitted the calculation of the within-subject repeatability. Switching the anaesthetic in the same animal at the end of the three consecutive timepoints was avoided because ageing could become a confounding factor for VEP properties.

Using two-way ANOVA for repeated measures for the comparison between anaesthetic agents, a total sample of 18 rats (9 rats for each group tested at 3 time-points) enabled to obtain a statistical power >85% (85.7%) to detect an effect size of 0.7 with a significance level of 0.05. Power analysis was performed with G*Power 3.1 (Faul et al., 2009).

VEPs were recorded once every two weeks for three timepoints (t1, t2 and t3) in rats anaesthetised with sevoflurane (sVEP) or ketamine-xylazine (kxVEP). In each session, VEPs in response to monocular flash stimulation of each eye were recorded (stimulation of one eye at a time). Order of first stimulated eye (left or right) was randomised across rats of each group to exclude any bias that could arise from testing the same eye as first. After the last VEP recording session (t3), rats were euthanised through cervical dislocation under sevoflurane or ketamine-xylazine anaesthesia.

2.3. VEP recording

VEP acquisitions were performed after 5 minutes of dark adaptation in a Faraday cage. For one group (sVEP), VEPs were recorded under volatile anaesthesia with sevoflurane (Sevorane™, AbbVie s.r.l., Campoverde di Aprilia, Latina, Italy), vaporised (Abbott Sevorane Vaporiser 19.3, Dräger Medical GmbH, Lübeck, Germany) and delivered through a snout mask with a gas evacuation system (Fluovac, Harvard Apparatus Ltd, Edenbridge, United Kingdom), scavenging excess gas into a dedicated canister containing activated carbon (Fluosorber, Harvard Apparatus Ltd, Edenbridge, United Kingdom). Anaesthesia was induced for 3 minutes with sevoflurane 3.5% and maintained for 2 additional minutes at 2% before starting the VEP recording session. Sevoflurane was mixed in 30% oxygen *plus* 70% nitrogen and the bulk flow of gas per time unit was measured with a flow meter. For the second group (kxVEP), VEPs were recorded 10 minutes after intraperitoneal injection of ketamine (40 mg/kg, Ketavet, Intervet Productions s.r.l., Aprilia-Latina, Italy) *plus* xylazine (5 mg/kg, Rompun, Bayer s.p.a., Milan, Italy). A half-dose of ketamine-xylazine was possibly administered if sedation did not last for the entire VEP recording. During each VEP session, body temperature was maintained at 37 °C with a homeothermic heating pad connected to a flexible rectal probe (Harvard Apparatus, Holliston, Massachusetts, USA). Before experimental tests, rats were allowed to reach a steady-state with the anaesthetic, namely a satisfying level of sedation that was necessary to avoid rat movements and to obtain stable VEP responses. The adequate level of anaesthesia was verified by checking for the absence of both tail-pinching and corneal reflexes (Bolay et al., 2000). In addition, heart rate frequency was continuously monitored from two subcutaneous needle electrodes in right and left forelimbs. This enabled to control closely the depth of anaesthesia, which is crucial to maintain optimum visual responsiveness (Gordon and Stryker, 1996). Pupils were dilated with 1% Tropicamide (Visumidriatic, Visufarma s.p.a., Rome, Italy) and 2% Hydroxypropylmethylcellulose (GEL 4000, Bruschettini s.r.l., Genoa, Italy) was applied to avoid eye drying. For epidermal VEP recording, removable 6 mm Ø Ag/AgCl cup electrodes (SEI EMG s.r.l., Cittadella, Italy) were used, as already reported (d'Isa et al., 2020). The recording electrode was placed on the scalp (previously shaved) in correspondence of the primary visual cortex (V1), contralaterally to the stimulated eye (4 mm lateral to the midline and 3.5 mm anterior to the interaural line; Paxinos and Watson, 2014). To assure a strong adherence to the skin, cup electrodes were fixed with an electro-conductive paste (Elefix EEG paste, Nihon Kohden, Japan) that also improved the electrical signal conduction. A reference cup electrode was positioned over the frontal regions (over the midline and 1

mm posterior to the interorbital line). A needle electrode was inserted into the hind limb as the ground. Data were acquired at a sampling frequency of 4096 Hz, coded with 16 bits and filtered between 5-70 Hz. Flash stimuli with 522 mJ intensity, 35 cd s/m^2 illuminance and 10 μs duration were delivered at a frequency of 1 Hz by a Flash10S xenon photostimulator (Micromed s.p.a., Mogliano Veneto, Italy) placed 15 cm from the stimulated eye (Cambiaghi et al., 2011), while the non-stimulated eye was covered with a black patch. For each session involving both sVEP and kxVEP, 3 averages of 20 stimuli were used for measuring the latency of N1 and P2, together with peak-to-peak amplitude of N1-P2 complex of flash-VEPs (Onofrj et al., 1985). Each flash train was followed by a 5-minute break. For each rat, all the VEP sessions were timed (from anaesthesia induction to the end of recording) for a total of 27 recordings/group to assess possible differences of procedure duration between sVEP and kxVEP. After the last recording session, a blinded experimenter (unaware of the type of anaesthesia used on each rat) analysed VEP waveforms, assessing latency, amplitude, repeatability indices, SNR and VEP recording duration.

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics software (version 23.0). Since the left and right eyes were recorded from the same subject, "eye" was considered as a "within-subjects" factor to account for autocorrelation between eyes of the same animal (Armstrong, 2013). To test for possible differences in VEP latencies and amplitudes obtained in sVEP or kxVEP, firstly N1 and P2 components from the right and left eyes were compared, running a two-way ANOVA with "time" (3 levels: t1, t2, t3) and "eye" (2 levels: right, left) as "within-subjects" factors. Since no significant effect of the factor "eye" was found, N1-P2 latencies and amplitudes from the right and left eyes were averaged to obtain a single value for each animal.

For N1-P2 latency and amplitude, two-way ANOVA for repeated measures was used, entering "time" as "within-subjects" main factor (3 levels: t1, t2, t3) and "anaesthesia" as "between subjects" main factor (2 levels: sevoflurane, ketamine-xylazine), followed by post-hoc protected t-tests to compare the two different anaesthesia at every time point. Referring to Levene's test for equality of variances, Student's or Welch's t-tests were used in case of homoscedastic or heteroscedastic samples, respectively.

The inter-session coefficient of variation (CoVw), the inter-session relative standard error (RSEw) and the inter-session intra-class correlation coefficient (ICC) were used to measure the repeatability across the three VEP recording sessions under the two anaesthetic regimens. CoVw was calculated normalizing the "within-subject" standard deviation (SDw) by the "within-subject" N1/P2 average value across sessions (AVGw) and expressed as a percentage ([SDw/AVGw] × 100). Similarly, the RSEw was obtained normalizing the SEMw by the "within-subject" N1/P2 average value across sessions and expressed as a percentage ([SEMw/AVGw] × 100). Finally, inter-session ICC was calculated according to the two-way mixed effects model (absolute agreement, single measures; Koo and Li, 2016).

For each anaesthesia, all VEP traces from the first time point (t1) were used to calculate the SNR. In particular, a 50 ms window (from 25 ms to 75 ms post-stimulus) was considered for the signal, compared with a 50 ms window of basal EEG without light stimulation for the noise (from 450 to 500 ms post-stimulus). Amplitude values of all sampling points were measured in the "signal" and "noise" windows, then from each sampling point, the mean value of its corresponding window was subtracted and subsequently, all the values were squared. Finally, SNR was obtained from the ratio between the sum of the squares of the "signal" and the "noise". Both SNR and VEP procedure duration were compared using Student's t-test in case of homoscedastic samples or Welch's t-test in case of heteroscedastic samples, after testing for the equality of variances by Levene's test. Data in the present work are expressed as mean ± SEM and all statistical analyses were considered significant at $p < 0.05$.

3. Results

3.1. Study feasibility

During each VEP recording session, clear waveforms were acquired both under sevoflurane and ketamine-xylazine anaesthesia, with N1 and P2 peaks that were markedly distinguishable and measurable (Figure 1). Latency and amplitude values were obtained at t1, t2 and t3 without missing time points. After each session, all the rats recovered from general anaesthesia without sudden deaths.

3.2. VEP latency under sevoflurane and ketamine-xylazine

Comparing N1 latency measured from left and right eyes (Table S1A), no significant effect of the factor "eye" emerged from sVEP ($p = 0.356$) and kxVEP ($p = 0.158$). No significant effect of the factor "time" was detected in sVEP ($p = 0.661$) and kxVEP ($p = 0.255$). Moreover, there was no significant "time*eye" interaction in sVEP ($p = 0.838$) and kxVEP ($p = 0.103$). Since no difference was found between left and right eyes, their values were averaged and all subsequent analyses were performed on the binocular mean of N1 latencies (Table S2A).

Two-way ANOVA for repeated measures, with "anaesthesia" as "between subjects" factor and "time" as "within-subjects" factor, displayed no significant effect of "time" ($p = 0.441$). On the other hand, a significant effect of "anaesthesia" was found ($p = 0.006$). Post-hoc analysis revealed a significantly longer N1 latency for kxVEP compared with sVEP at all time points (t1: $p = 0.044$; t2: $p = 0.032$; t3: $p = 0.002$; Figure 2A). No significant "time*anaesthesia" interaction was detected ($p = 0.242$).

Considering the repeatability of N1 latency over time (Table S3A), sVEP and kxVEP presented no significant differences in CoVw and RSEw (for both, Student's t-test: $p = 0.097$; Figure 3A). A statistically significant ICC between timepoints was found for both sVEP ($p = 0.002$) and kxVEP ($p = 0.0002$; Table 1). Nevertheless, a higher ICC was observed for VEP recordings under ketamine-xylazine (sVEP ICC = 0.606; kxVEP ICC = 0.702).

Regarding P2 latency measured from left and right eyes (Table S1B), no significant effect of the factor "eye" was found in sVEP ($p = 0.568$) and kxVEP ($p = 0.580$). No significant effect of the factor "time" was detected in sVEP ($p = 0.763$) and kxVEP ($p = 0.411$). In addition, there was no significant "time*eye" interaction in sVEP ($p = 0.334$) and kxVEP ($p = 0.248$). Since no difference emerged between left and right eyes, their values were averaged and all subsequent analyses referred to the binocular mean of P2 latencies (Table S2B).

Two-way ANOVA for repeated measures, with "anaesthesia" as "between subjects" factor and "time" as "within-subjects" factor, showed no significant effect of "time" ($p = 0.383$). Notably, a significant effect of "anaesthesia" was found ($p = 0.029$). Post-hoc analysis highlighted a significantly longer P2 latency for kxVEP with respect to sVEP at t1 ($p = 0.047$) and t3 ($p = 0.030$), while the increase was not significant at t2 ($p = 0.084$; Figure 2B). No significant "time*anaesthesia" interaction was detected ($p = 0.519$).

Concerning the repeatability of P2 latency over time (Table S3B), sVEP and kxVEP displayed no significant differences in CoVw and RSEw (for both, Student's t-test: $p = 0.056$; Figure 3B). Inter-session ICC was statistically significant for both sVEP ($p = 0.0003$) and kxVEP ($p < 0.0001$; Table 1). However, a higher ICC was observed for VEPs recorded under ketamine-xylazine (sVEP ICC = 0.711; kxVEP ICC = 0.859).

3.3. VEP amplitude under sevoflurane and ketamine-xylazine

Comparing N1-P2 amplitude recorded from left and right eyes (Table S1C), no significant effect of the factor "eye" was found in sVEP (p

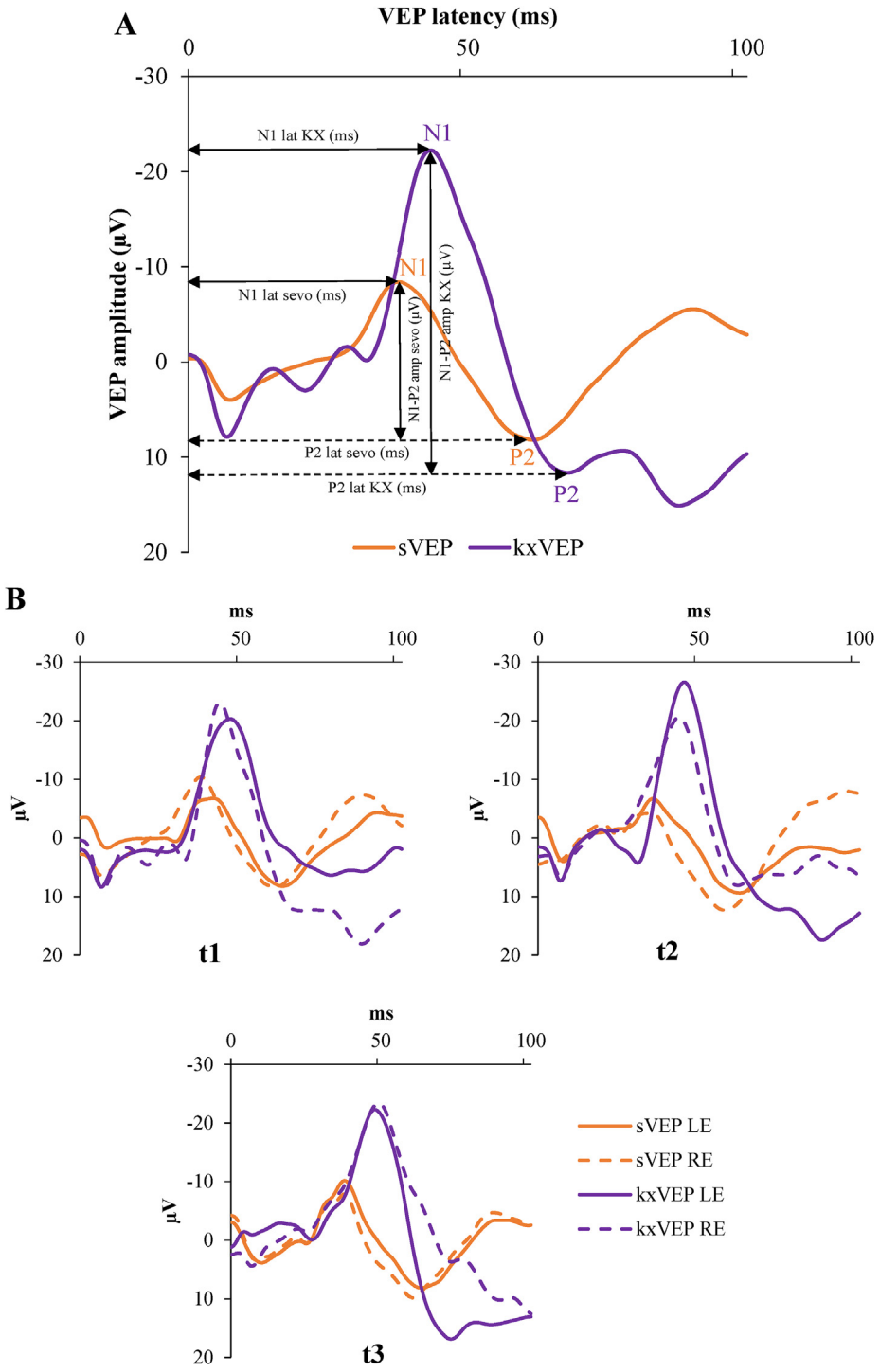

Figure 1. A: Representative VEP traces recorded from two Dark Agouti rats anaesthetized with sevoflurane (sVEP, orange lines) or ketamine-xylazine (kxVEP, purple lines), in which the N1-P2 complex is highlighted. B: Representative VEP traces recorded from left (LE, solid lines) and right eyes (RE, dotted lines) at different timepoints (t1, t2 and t3) under sevoflurane (sVEP) or ketamine-xylazine (kxVEP).

= 0.852) and kxVEP ($p = 0.119$). No significant effect of the factor "time" was detected in sVEP ($p = 0.294$) and kxVEP ($p = 0.390$). Moreover, there was no significant "time*eye" interaction in sVEP ($p = 0.427$) and kxVEP ($p = 0.570$). Therefore, the binocular average of N1-P2 amplitude was adopted for all subsequent analyses (Table S4).

Two-way ANOVA for repeated measures, with "anaesthesia" as "between subjects" factor and "time" as "within-subjects" factor, revealed a significant effect of "anaesthesia" ($p = 0.022$). Post-hoc analysis showed a significantly higher N1-P2 amplitude for kxVEP compared with sVEP at all time points (t1: $p = 0.032$; t2: $p = 0.038$; t3: $p = 0.046$; Figure 4). On the other hand, no significant effect of "time" ($p = 0.683$) or "time-*anaesthesia" interaction was found ($p = 0.197$).

Regarding the repeatability of N1-P2 amplitude over time (Table S5), sVEP and kxVEP showed no significant differences in CoVw and RSEw (for both, Welch's t-test: $p = 0.574$; Figure 5). Inter-session ICC was statistically significant for both sVEP ($p < 0.0001$) and kxVEP ($p = 0.0009$; Table 1). Nonetheless, a higher ICC was found for VEPs acquired under sevoflurane (sVEP ICC = 0.753; kxVEP ICC = 0.642).

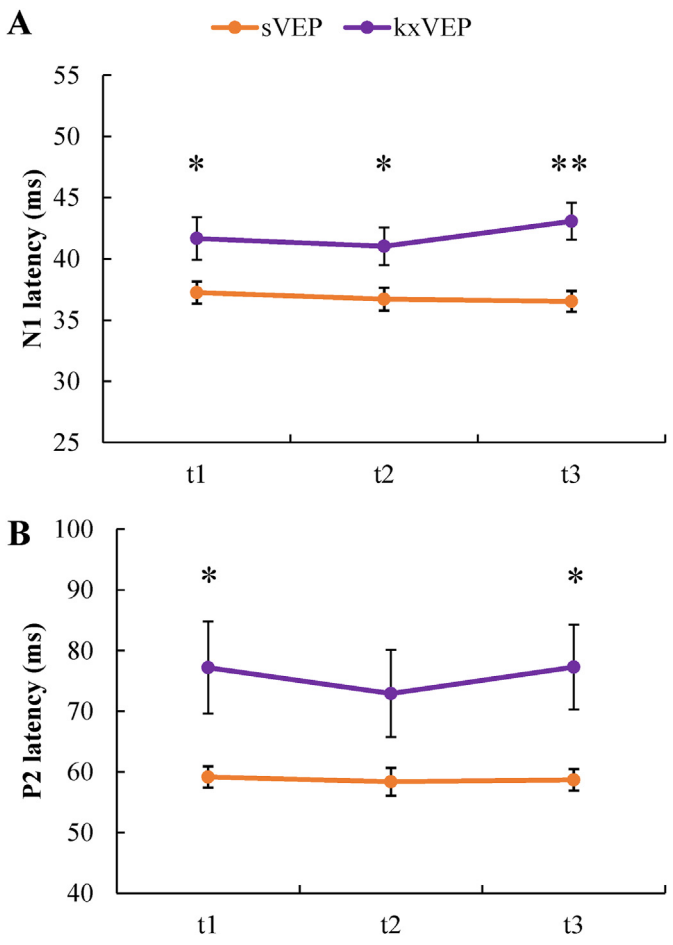

Figure 2. N1 (A) and P2 (B) latencies measured in Dark Agouti rats (left and right eyes averaged) anesthetized with sevoflurane (sVEP, n = 9, orange lines) or ketamine-xylazine (kxVEP, n = 9, purple lines) at different time points (t1, t2 and t3). Asterisks indicate significant differences at each time point between the two groups. Data are expressed as mean ± SEM (*: $p < 0.05$; **: $p < 0.01$).

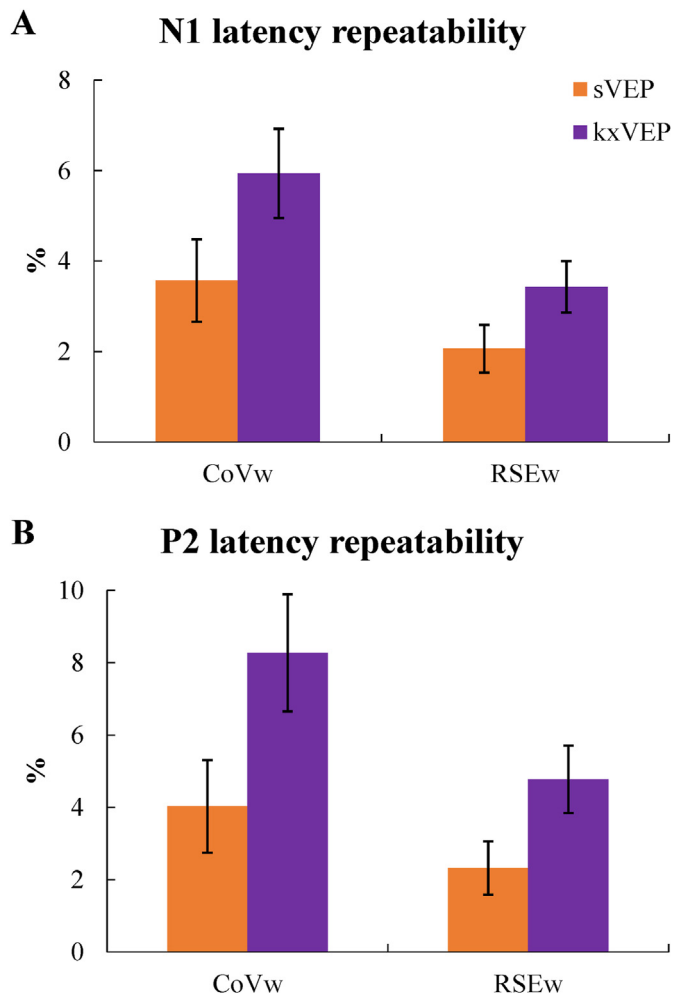

Figure 3. Inter-session Coefficient of Variation (CoVw) and inter-session Relative Standard Error (RSEw) calculated from N1 (A) and P2 (B) latencies obtained in Dark Agouti rats anesthetized with sevoflurane (sVEP, n = 9, orange columns) or ketamine-xylazine (kxVEP, n = 9, purple columns). Data are expressed as mean ± SEM.

3.4. SNR between sVEP and kxVEP

In order to assess the signal quality of VEP recording, the SNR was calculated at t1 for sVEP and kxVEP (Table S6). In particular, SNR derived from kxVEP was significantly higher than sVEP (Welch's t-test: $p = 0.048$; Figure 6).

3.5. Duration of VEP recording procedure

For each rat, every single VEP recording session was timed to find possible differences in the procedure duration of sVEP and kxVEP (Table S7). Specifically, the recording time was significantly longer for sVEP compared with kxVEP (Welch's t-test: $p = 0.004$; Figure 7).

Table 1. Inter-session ICCs of different VEP components from rats anaesthetised with sevoflurane or ketamine-xylazine (mean and 95% confidence intervals).

VEP component	anaesthesia	ICC	lower bound	upper bound	p-value
N1 latency	sevo	0.606	0.209	0.880	0.002
	kx	0.702	0.363	0.913	0.0002
P2 latency	sevo	0.711	0.353	0.918	0.0003
	kx	0.859	0.643	0.962	<0.0001
N1-P2 amplitude	sevo	0.753	0.443	0.930	<0.0001
	kx	0.642	0.271	0.892	0.0009

4. Discussion

This work was focused on VEPs recorded at three consecutive time-points in Dark Agouti rats anaesthetised with a volatile (sevoflurane mixed with 30% oxygen and 70% nitrogen) or injectable agent (mixture of ketamine *plus* xylazine) to explore the differences of N1 and P2 components in terms of latency, amplitude, repeatability and SNR.

VEP under ketamine-xylazine presented longer latency and higher amplitude of the N1-P2 complex, together with an increased SNR compared with sevoflurane anaesthesia. Therefore, ketamine-xylazine might have produced a deeper level of anaesthesia than sevoflurane, in particular during the initial phase of VEP recording, causing a significant increase of N1 latency. Accordingly, it has been previously reported that VEP latency increased together with the depth of anaesthesia (Ghita et al., 2013). During VEP recording sessions performed in this experiment, a relatively low concentration of sevoflurane (2%) was administered, which is approximately half the dose used for optimal surgical procedures in rats (4.1%; Tsukamoto et al., 2018). Regarding N1-P2 amplitude, its decrease under sevoflurane compared with ketamine-xylazine might occur because halogenated inhalational agents like sevoflurane and isoflurane produce a dose-dependent reduction in amplitude of cortically evoked responses (Sloan, 1998) and therefore VEPs (Kameyama, 1994; Jehle et al., 2009; Tanaka et al., 2020).

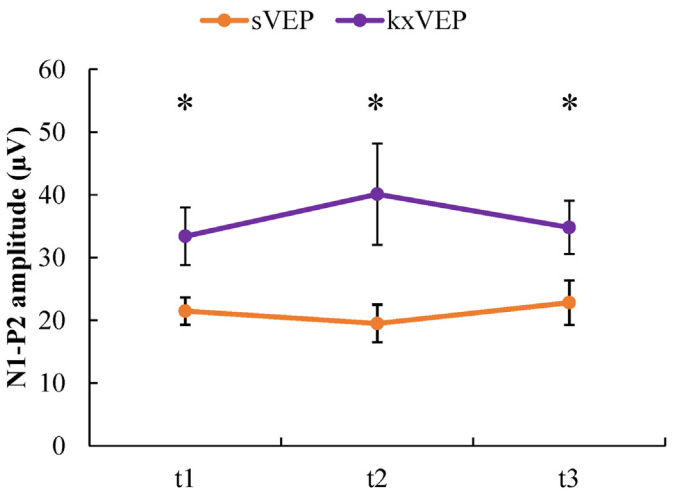

Figure 4. N1-P2 amplitude measured in Dark Agouti rats (left and right eyes averaged) anesthetized with sevoflurane (sVEP, n = 9, orange lines) or ketamine-xylazine (kxVEP, n = 9, purple lines) at different time points (t1, t2 and t3). Asterisks indicate significant differences at each time point between the two groups. Data are expressed as mean ± SEM (*: $p < 0.05$).

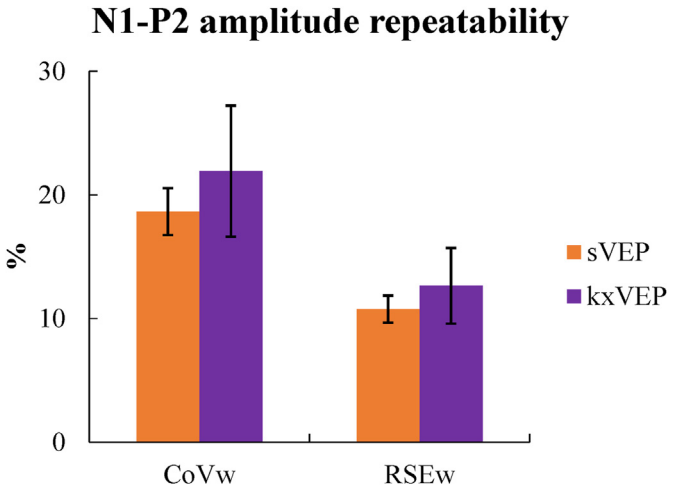

Figure 5. Inter-session Coefficient of Variation (CoVw) and inter-session Relative Standard Error (RSEw) calculated from N1-P2 amplitudes obtained in Dark Agouti rats anesthetized with sevoflurane (sVEP, n = 9, orange columns) or ketamine-xylazine (kxVEP, n = 9, purple columns). Data are expressed as mean ± SEM.

Moreover, the use of ketamine for somatosensory evoked potential recording has been associated with an increase in amplitude compared with the awake state (Schubert et al., 1990). Analogously, the employment of ketamine-xylazine for VEP recording led to a higher N1-P2 amplitude with respect to both awake condition and isoflurane anaesthesia (Charng et al., 2013). Overall, the anaesthetic states observed in this study were not similar in terms of EEG activity, since burst suppression and slow waves were more common with sevoflurane than ketamine-xylazine, thus causing an increase in the acquisition time due to the difficulty in obtaining clear VEP peaks over the recorded EEG. It should also be taken into account that rats subjected to volatile anaesthesia could have undergone poor oxygen saturation, resulting in VEP amplitude decrease, as reported during intraoperative monitoring in humans (Hayashi and Kawaguchi, 2017). On the other hand, the effect of injectable agents usually changes after administration, being lower towards the end than at the start of the experimental procedures. Therefore, the anaesthetic depth of ketamine-xylazine could be less stable

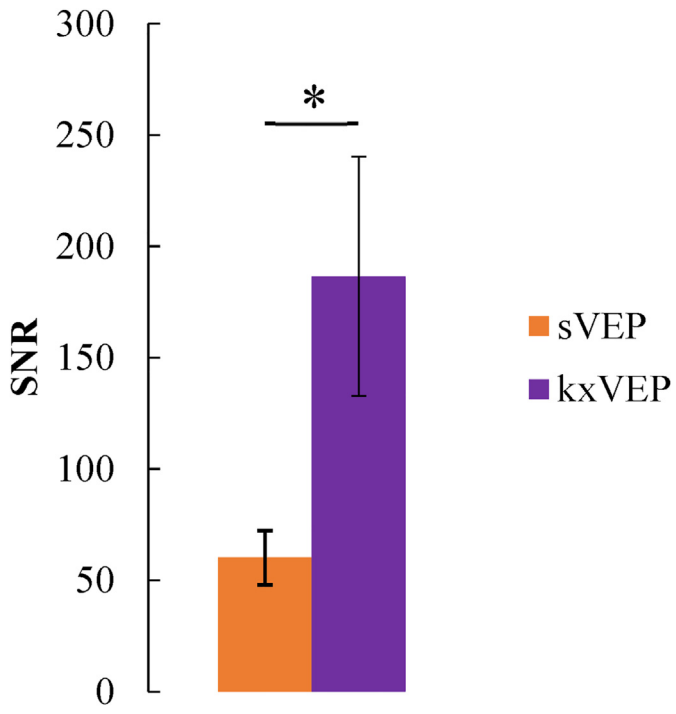

Figure 6. SNR calculated from Dark Agouti rats anesthetized with sevoflurane (sVEP, n = 9, orange column) or ketamine-xylazine (kxVEP, n = 9, purple column) recorded at t1. Data are expressed as mean ± SEM (*: $p < 0.05$).

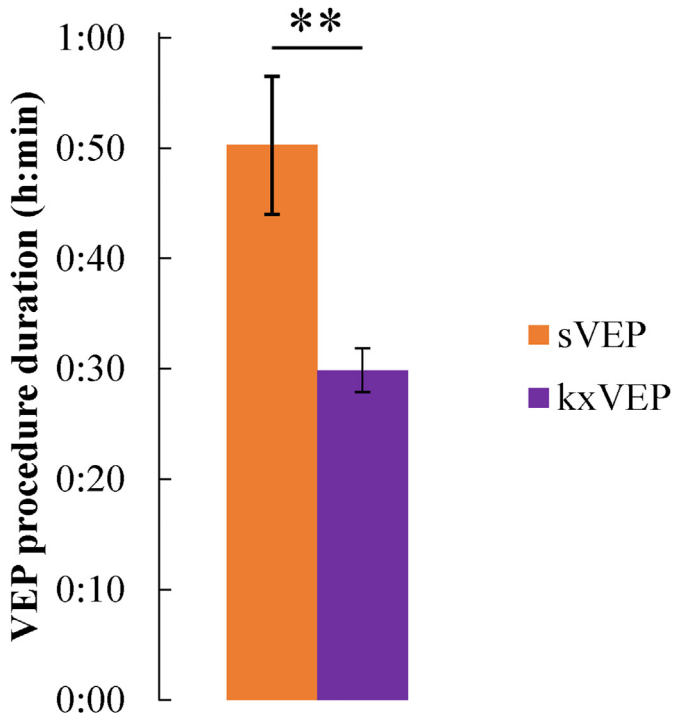

Figure 7. VEP procedure duration (expressed in h:min:s) timed in Dark Agouti rats anesthetized with sevoflurane (sVEP, n = 27 recordings, orange column) or ketamine-xylazine (kxVEP, n = 27 recordings, purple column). Data are expressed as mean ± SEM (**: $p < 0.01$).

compared with sevoflurane; however, this issue had been partially buffered by the fixed time frame of VEP recording procedures, in particular to the time elapsed between anaesthetic administration and flash stimulation.

Concerning VEP repeatability over time, CoVw and RSEw were comparable between the two anaesthetic regimens for amplitude and latency, with the latter that showed a trend in favour of sevoflurane, especially for the P2 component, probably due to the different pharmacokinetics of volatile *versus* injectable agents. Similar repeatability values for rats anaesthetised with sevoflurane were already observed with epidermal (Santangelo et al., 2018) and epidural electrodes (You et al., 2011a). Thanks to a controlled gas flow, the absorption of sevoflurane resulted in more stability to the detriment of ketamine-xylazine, which underwent the physiological fluctuations caused by body metabolism, resulting in increased SEM measured at all timepoints. However, for N1 latency, the CoVw of the two anaesthesia ranged between 2 and 6 %, which indicates acceptable repeatability (around 5%; Campbell et al., 2007). Size and location of the cortical region involved in generating the potential, together with skull thickness, contribute to increasing VEP amplitude variability (Barton et al., 2019); therefore repeatability indices were worse than those related to latency, as expected. Inter-session ICC was highly significant for both anaesthetic regimens, demonstrating a strong correlation between timepoints and hence a good repeatability. Nevertheless, some differences between the two anaesthetic agents were observed. For latency of N1 and P2, VEPs recorded under ketamine-xylazine displayed a higher ICC. On the other hand, for N1-P2 amplitude sevoflurane led to a higher ICC, suggesting that an optimal anaesthetic regimen for VEP recording should be chosen also considering if latency or amplitude is the primary variable of interest.

To understand which anaesthesia allowed to obtain clearer VEP signals, SNR of the N1 component was evaluated. Under ketamine-xylazine, the N1 peak was better discernible from the background noise when compared with VEPs under sevoflurane. Accordingly, VEPs under ketamine-xylazine displayed a significantly higher SNR. As already reported in previous studies (Santangelo et al., 2018; Castoldi et al., 2020), N1 was the only VEP component always clearly detectable under sevoflurane, while P1 and P2 were less recognisable, even if still present. On the other side, with ketamine-xylazine it was possible to easily identify the peaks of all main VEP components. The bioelectrical signal quality during neurophysiological recordings plays an important role in evaluating brain function both in clinical and preclinical settings. The SNR determines the quality and reliability of VEP recordings (Turetsky et al., 1988), being critical to detect the different VEP components that could be hidden throughout the EEG recorded over the visual cortex: the higher the signal over the noise, the sharper the recorded EP, which is a key point to obtain clear electrical waveforms. The SNR obtained in rats anaesthetised with sevoflurane was comparable to a previous work (Santangelo et al., 2018). However, injectable anaesthesia showed a three times higher SNR than volatile anaesthesia, facilitating N1 peak detection during VEP analysis. Indeed, inhalational anaesthesia as sevoflurane, isoflurane and desflurane cause burst suppression of EEG (Johnson and Taylor, 1998; Antunes et al., 2003; Murrell et al., 2008; McIlhone et al., 2014). On the other hand, ketamine is a non-competitive N-Methyl-D-Aspartate (NMDA) receptor antagonist and the block of NMDA receptors induced by ketamine leads to consequent disinhibition of glutamate release and activation of AMPA receptors (Moran et al., 2015). This mechanism could enhance visual cortex excitability, increasing VEP amplitude and SNR compared with sevoflurane, whose molecular effects are transient and less selective, such as $GABA_A$ and glycine receptor agonism, two-pore potassium channel activation and excitatory neurotransmitter receptor antagonism (Lee et al., 2013). Since VEPs are electrical signals that are extracted from the EEG, when an alternative option is possible, inhalant agents are less recommendable for VEP recordings (You et al., 2015).

The duration of testing procedures is a relevant aspect in neurophysiological examination, especially in preclinical settings, because a protracted examination time may be detrimental for the experimental outcomes and could impact the recovery time and animal welfare. From this point of view, ketamine-xylazine has proved to be more advantageous than sevoflurane, since a shorter duration of the entire procedure was evident from the analysis of VEP recording times.

Regarding the limitations of this study, the first was the employment of only female rats. Since it has been shown that sex effects may be present in rat VEP responses (Dyer and Swartzwelder, 1978; Frenk and Taylor, 1978), in a future study it could be useful to repeat the same experiment on male rats to check if the observed findings are valid for both sexes. The second limitation was the higher mean amplitude and SD obtained in the left eyes of rats anaesthetised with ketamine-xylazine. This issue might be due to the first eye tested that was often the right eye (17/27 of the first recorded eyes), when probably the level of anaesthesia was deeper and more stable. Despite these marked discrepancies, no significant difference was detected, therefore amplitude values from left and right eyes were averaged for subsequent analyses. For future studies, it would be better to alternate the first eye that undergoes VEP recording with ketamine-xylazine to minimize the inter-eye variability. A further weakness of this work was the lack of simultaneous electroretinogram and/or ophthalmic examination before VEP recording. These procedures would have allowed the detection of sporadic malformations (such as microphthalmia) and post-inflammatory lesions (corneal opacities, cataracts and retinal lesions) that could occur in Dark Agouti rats, therefore affecting VEP outcomes.

In conclusion, during VEP recording in Dark Agouti rats, sevoflurane and ketamine-xylazine revealed reliable latency and amplitude values, which remained stable across all three time points of this study. Nevertheless, sevoflurane assured a more stable level of sedation, resulting in higher amplitude repeatability in terms of inter-session ICC, whereas ketamine-xylazine anaesthesia determined a more detailed VEP profile, higher latency repeatability in terms of inter-session ICC, absence of EEG suppression, better SNR and quicker recording procedure.

Overall, VEP characterisation under different anaesthesia regimens could be useful for investigations requiring biomarkers of optic nerve function in animal models of neurological diseases. In preclinical research, a deeper knowledge of biomarker properties would allow a *bona fide* monitoring of the different stages of the disease, as well as reliable testing of new treatments, consequently bringing benefits to patients with visual pathway defects caused by neuropathological conditions.

Declarations

Author contribution statement

Valerio Castoldi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Raffaele d'Isa: Analyzed and interpreted the data; Wrote the paper.

Silvia Marenna: Performed the experiments.

Giancarlo Comi: Contributed reagents, materials, analysis tools or data.

Letizia Leocani: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by Regione Lombardia (POR FESR 2014–2020) within the framework of the NeOn project (ID 239047) and by the Italian Ministry of Education, University and Research through the IVASCOMAR project (CTN01_00177_165430, Cluster Tecnologico Nazionale Scienze della Vita "Alisei").

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2021.e08360.

References

Aggarwal, A., Brennan, C., Shortal, B., et al., 2019. Coherence of visual-evoked gamma oscillations is disrupted by propofol but preserved under equipotent doses of isoflurane. Front. Syst. Neurosci. 13, 19.

Antunes, L.M., Golledge, H.D., Roughan, J.V., et al., 2003. Comparison of electroencephalogram activity and auditory evoked responses during isoflurane and halothane anaesthesia in the rat. Vet. Anaesth. Analg. 30, 15–23.

Armstrong, R.A., 2013. Statistical guidelines for the analysis of data obtained from one or both eyes. Ophthalmic Physiol. Opt. 33, 7–14.

Barton, J.L., Garber, J.Y., Klistorner, A., Barnett, M.H., 2019. The electrophysiological assessment of visual function in Multiple Sclerosis. Clin. Neurophysiol. Pract. 4, 90–96.

Bolay, H., Gürsoy-Ozdemir, Y., Unal, I., et al., 2000. Altered mechanisms of motor-evoked potential generation after transient focal cerebral ischemia in the rat: implications for transcranial magnetic stimulation. Brain Res. 873, 26–33.

Brusa, A., Jones, S.J., Plant, G.T., 2001. Long-term remyelination after optic neuritis: a 2-year visual evoked potential and psychophysical serial study. Brain: J. Neurol. 124 (Pt 3), 468–479.

Cambiaghi, M., Teneud, L., Velikova, S., et al., 2011. Flash visual evoked potentials in mice can be modulated by transcranial direct current stimulation. Neuroscience 185, 161–165.

Campbell, M.J., Machin, D., Walters, S.J., 2007. Medical Statistics: a Textbook for the Health Sciences, fourth ed. John Wiley & Son, Chichester West Sussex; Hoboken, NJ.

Charng, J., Nguyen, C.T., He, Z., et al., 2013. Conscious wireless electroretinogram and visual evoked potentials in rats. PLoS One 8 (9), e74172.

Castoldi, V., Marenna, S., d'Isa, R., et al., 2020. Non-invasive visual evoked potentials to assess optic nerve involvement in the dark agouti rat model of experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein. Brain Pathol. 30, 137–150.

Castoldi, V., Marenna, S., Santangelo, R., et al., 2018. Optic nerve involvement in experimental autoimmune encephalomyelitis to homologous spinal cord homogenate immunization in the dark agouti rat. J. Neuroimmunol. 325, 1–9.

Dyer, R.S., Swartzwelder, H.S., 1978. Sex and strain differences in the visual evoked potentials of albino and hooded rats. Pharmacol. Biochem. Behav. 9, 301–306.

d'Isa, R., Castoldi, V., Marenna, S., Santangelo, R., Comi, G., Leocani, L., 2020. A new electrophysiological non-invasive method to assess retinocortical conduction time in the Dark Agouti rat through the simultaneous recording of electroretinogram and visual evoked potential. Doc. Ophthalmol. 140 (3), 245–255.

Faul, F., Erdfelder, E., Buchner, A., Lang, A.G., 2009. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav. Res. Methods 41 (4), 1149–1160.

Frenk, H., Taylor, A.N., 1978. Sex differences in averaged visual evoked potentials during food intake in rats. Brain Res. Bull. 3 (1), 1–5.

Ghita, A.M., Parvu, D., Sava, R., et al., 2013. Analysis of the visual evoked potential in anaesthesia with sevoflurane and chloral hydrate : (Variability of amplitudes, latencies and morphology of VEP with the depth of anesthesia). J. Med. Life 6, 214–225.

Gordon, J.A., Stryker, M.P., 1996. Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J. Neurosci. 16, 3274–3286.

Graham, S.L., Klistorner, A., 2017. Afferent visual pathways in multiple sclerosis: a review. Clin. Exp. Ophthalmol. 45 (1), 62–72.

Griffith, R.W., Humphrey, D.R., 2006. Long-term gliosis around chronically implanted platinum electrodes in the Rhesus macaque motor cortex. Neurosci. Lett. 406, 81–86.

Hayashi, H., Kawaguchi, M., 2017. Intraoperative monitoring of flash visual evoked potential under general anesthesia. Korean J. Anesthesiol. 70 (2), 127–135.

Halliday, A.M., 1993. Evoked Potentials in Clinical Testing. Churchill Livingstone, Edinburgh; New York.

Holder, G.E., Celesia, G.G., Miyake, Y., et al., 2010. International Federation of Clinical Neurophysiology: recommendations for visual system testing. Clin. Neurophysiol. 121, 1393–1409.

Imas, O.A., Ropella, K.M., Wood, J.D., et al., 2004. Halothane augments event-related gamma oscillations in rat visual cortex. Neuroscience 123, 269–278.

Jehle, T., Ehlken, D., Wingert, K., et al., 2009. Influence of narcotics on luminance and frequency modulated visual evoked potentials in rats. Doc. Ophthalmol. 118, 217–224.

Johnson, C.B., Taylor, P.M., 1998. Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. Br. J. Anaesth. 81, 748–753.

Kameyama, Y., 1994. Effect of isoflurane and sevoflurane on evoked potentials and EEG. Masui 43 (5), 657–664.

Karagkouni, A., Alevizos, M., Theoharides, T.C., 2013. Effect of stress on brain inflammation and multiple sclerosis. Autoimmun. Rev. 12, 947–953.

Koo, T.K., Li, M.Y., 2016. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J. Chiropr. Med. 15 (2), 155–163.

Kuroda, K., Fujiwara, A., Takeda, Y., et al., 2009. Effects of narcotics, including morphine, on visual evoked potential in rats. Eur. J. Pharmacol. 602, 294–297.

Land, R., Kapche, A., Ebbers, L., Kral, A., 2019. 32-channel mouse EEG: visual evoked potentials. J. Neurosci. Methods 325, 108316.

Lee, U., Ku, S., Noh, G., et al., 2013. Disruption of frontal-parietal communication by ketamine, propofol, and sevoflurane. Anesthesiology 118, 1264–1275.

Leocani, L., Comi, G., 2000. Neurophysiological investigations in multiple sclerosis. Curr. Opin. Neurol. 13, 255–261.

Maertz, N.A., Kim, C.B., Nork, T.M., et al., 2006. Multifocal visual evoked potentials in the anesthetized non-human primate. Curr. Eye Res. 31, 885–893.

Marenna, S., Castoldi, V., d'Isa, R., et al., 2019. Semi-invasive and non-invasive recording of visual evoked potentials in mice. Doc. Ophthalmol. 138, 169–179.

McIlhone, A.E., Beausoleil, N.J., Johnson, C.B., et al., 2014. Effects of isoflurane, sevoflurane and methoxyflurane on the electroencephalogram of the chicken. Vet. Anaesth. Analg. 41, 613–620.

Moran, R.J., Jones, M.W., Blockeel, A.J., et al., 2015. Losing control under ketamine: suppressed cortico-hippocampal drive following acute ketamine in rats. Neuropsychopharmacology 40, 268–277.

Murrell, J.C., Waters, D., Johnson, C.B., 2008. Comparative effects of halothane, isoflurane, sevoflurane and desflurane on the electroencephalogram of the rat. Lab. Anim. 42, 161–170.

Nisipeanu, P., Korczyn, A.D., 1993. Psychological stress as risk factor for exacerbations in multiple sclerosis. Neurology 43, 1311–1312.

Onofrj, M., Harnois, C., Bodis-Wollner, I., 1985. The hemispheric distribution of the transient rat VEP: a comparison of flash and pattern stimulation. Exp. Brain Res. 59, 427–433.

Paxinos, G., Watson, C., 2014. The Rat Brain in Stereotaxic Coordinates, seventh ed. Elsevier, Academic Press, Amsterdam.

Porciatti, V., Pizzorusso, T., Maffei, L., 1999. The visual physiology of the wild type mouse determined with pattern VEPs. Vis. Res. 39, 3071–3081.

Roth, S., Dreixler, J., Newman, N.J., 2018. Haemodilution and head-down tilting induce functional injury in the rat optic nerve: a model for peri-operative ischemic optic neuropathy. Eur. J. Anaesthesiol. 35, 840–847.

Santangelo, R., Castoldi, V., D'Isa, R., et al., 2018. Visual evoked potentials can be reliably recorded using noninvasive epidermal electrodes in the anesthetized rat. Doc. Ophthalmol. 136, 165–175.

Schubert, A., Licina, M.G., Lineberry, P.J., 1990. The effect of ketamine on human somatosensory evoked potentials and its modification by nitrous oxide. Anesthesiology 72, 33–39.

Schuelert, N., Dorner-Ciossek, C., Brendel, M., et al., 2018. A comprehensive analysis of auditory event-related potentials and network oscillations in an NMDA receptor antagonist mouse model using a novel wireless recording technology. Phys. Rep. 6, e13782.

Siegel, J., Sisson, D.F., Driscoll, P., 1993. Augmenting and reducing of visual evoked potentials in Roman high- and low-avoidance rats. Physiol. Behav. 54, 707–711.

Sloan, T.B., 1998. Anesthetic effects on electrophysiologic recordings. J. Clin. Neurophysiol. 15, 217–226.

Ström, L., Ekesten, B., 2016. Visual evoked potentials in the horse. BMC Vet. Res. 12, 120.

Szarowski, D.H., Andersen, M.D., Retterer, S., et al., 2003. Brain responses to micro-machined silicon devices. Brain Res. 983, 23–35.

Tanaka, R., Tanaka, S., Ichino, T., Ishida, T., Fuseya, S., Kawamata, M., 2020. Differential effects of sevoflurane and propofol on an electroretinogram and visual evoked potentials. J. Anesth. 34 (2), 298–302.

Tomiyama, Y., Fujita, K., Nishiguchi, K.M., et al., 2016. Measurement of electroretinograms and visually evoked potentials in awake moving mice. PLoS One 11, e0156927.

Tsukamoto, A., Niino, N., Sakamoto, M., et al., 2018. The validity of anesthetic protocols for the surgical procedure of castration in rats. Exp. Anim. 67, 329–336.

Turetsky, B.I., Raz, J., Fein, G., 1988. Noise and signal power and their effects on evoked potential estimation. Electroencephalogr. Clin. Neurophysiol. 71, 310–318.

You, Y., Gupta, V.K., Chitranshi, N., et al., 2015. Visual evoked potential recording in a rat model of experimental optic nerve demyelination. J. Vis. Exp., e52934

You, Y., Klistorner, A., Thie, J., et al., 2011a. Improving reproducibility of VEP recording in rats: electrodes, stimulus source and peak analysis. Doc. Ophthalmol. 123, 109–119.

You, Y., Klistorner, A., Thie, J., et al., 2011b. Latency delay of visual evoked potential is a real measurement of demyelination in a rat model of optic neuritis. Invest. Ophthalmol. Vis. Sci. 52, 6911–6918.