Reliability of VEP Recordings Using Chronically Implanted Screw Electrodes in Mice

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Purpose: Visual evoked potentials (VEPs) are widely used to objectively assess visual system function in animal models of ophthalmological diseases. Although use of chronically implanted electrodes is common in longitudinal VEP studies using rodent models, reliability of recordings over time has not been assessed. We compared VEPs 1 and 7 days after electrode implantation in the adult mouse. We also examined stimulus-independent changes over time, by assessing electroencephalogram (EEG) power and approximate entropy of the EEG signal.

Methods: Stainless steel screws (600- μ m diameter) were implanted into the skull overlying the right visual cortex and the orbitofrontal cortex of adult mice (C57Bl/6J, $n = 7$). Animals were reanesthetized 1 and 7 days after implantation to record VEP responses (flashed gratings) and EEG activity. Brain sections were stained for glial activation (GFAP) and cell death (TUNEL).

Results: Reliability analysis, using intraclass correlation coefficients, showed VEP recordings had high reliability within the same session, regardless of time after electrode implantation and peak latencies and approximate entropy of the EEG did not change significantly with time. However, there was poorer reliability between recordings obtained on different days, and a significant decrease in VEP amplitudes and EEG power. This amplitude decrease could be normalized by scaling to EEG power (within-subjects). Furthermore, glial activation was present at both time points but there was no evidence of cell death.

Conclusions: These results indicate that VEP responses can be reliably recorded even after a relatively short recovery period but decrease response peak amplitude over time. Although scaling the VEP trace to EEG power normalized this decrease, our results highlight that time-dependent cortical excitability changes are an important consideration in longitudinal VEP studies.

Translational Relevance: This study shows changes in VEP characteristics over time in chronically implanted mice. Thus, future preclinical longitudinal studies should consider time in addition to amplitude and latency when designing and interpreting research.

Introduction

The mouse visual system is a widely used model to assess changes in sensory processing in studies of developmental plasticity, as well as in preclinical models of ophthalmological diseases.^{1,2} A common method to objectively assess visual system function and visual performance is by recording visual evoked potentials (VEPs).³ VEPs are generated by combined

post synaptic visual cortical activity in response to a visual stimulus and are typically recorded from electrodes positioned in or above the primary visual cortex (V1).⁴ Longitudinal studies of VEP responses are common (e.g., in the context of developmental cortical plasticity, or repeated drug trials),⁵ and as such, it is important to establish the reproducibility of the VEP response not only within a session but also across multiple sessions over time.

VEP response reliability is affected by methodo-

logical factors, such as variability in electrode placement and stability,^{6,7} as well as by intrinsic changes within the animal, such as baseline visual cortical excitability and activity of other brain regions.⁸ Chronically implanted electrodes reduce variability due to methodological factors, with superior reliability across recording sessions and greater VEP amplitudes compared with subdermal needle electrodes.⁹ This improved reliability is likely due to the fixed electrode position across recording sessions, and greater stability in electrode placement.

Chronic electrode implantation improves reliability, but it also damages the underlying brain tissue. Injury occurs not only in cases with mechanical trauma from electrode penetration of the cortex,^{10,11} but also in relatively noninvasive implantation procedures (e.g., epidural implantation),¹² which can cause cortical compression injury. The initial injury and the secondary response results in time-dependent changes in neuronal excitability,¹³ potentially confounding longitudinal analysis of VEPs. With needle-stick injury, there is an initial spike in activity followed by cortical depression, attributed to the mechanical stimulation.¹⁴ Similarly, models of focal compression injury show initial decreases in cortical excitability, followed by hyperexcitability within hours of injury,^{13,15} which was sustained for at least 24 hours.¹⁶ Although a definitive time-course following injury has not been established, some studies report hyperexcitability months after injury,¹⁷ while others report a return to normal levels.¹⁸

Given that chronically implanting electrodes causes injury and resultant excitability changes, VEP studies using implanted electrodes generally employ a 'recovery period' after implantation, allowing time for excitability to stabilize. However, duration of this recovery period varies greatly between studies, ranging from 3 days to several weeks, and often varies between subjects.^{9,12,19} Although longitudinal studies in mice are common, no studies have systematically assessed whether a recovery period is necessary for reliable VEP responses in the mouse, nor how the VEP response waveform changes over time.

In this study, we use chronically skull-implanted screw electrodes in the mouse to compare reliability and waveform features in VEP responses obtained 1 day (within the typical recovery period) and 7 days (allowing a recovery period) after implantation. To examine whether this change was specific to visual stimuli and to gain insight into the nature of changes over time, we also examined the change in resting brain activity (electroencephalogram [EEG] power

spectra) and assessed amplitude-independent EEG structural complexity using approximate entropy (ApEn). In line with previous studies,^{20,21} we test whether scaling to resting EEG activity on the day of recording can be used to improve reliability of VEP recording data across days. Such scaling has been shown to decrease variability within animals over time and between animals, hence increasing reliability.²¹ Furthermore, we examined cortical tissue to identify whether implantation caused damage that could contribute to response changes over time.

Methods

Animals

A total of seven male C57BL/6J mice (10-weeks old, body weight 21–25 g) were used in the study. Mice were obtained from the Animal Resources Centre (Canningvale, Perth, Australia) and transferred to the PreClinical Facility at The University of Western Australia (Crawley, Perth, Australia). Mice were housed in standard cages, maximum of five per cage, with food and water ad libitum and a 12 hour light/dark cycle. All animals were housed individually after surgery to implant screw electrodes. Five mice underwent chronic screw implantation and repeated EEG and VEP recordings. Two additional mice were euthanized either 2 days (11-weeks old, body weight: 25 g) or 7 days (10-weeks old at implantation, body weight: 26 g) after screw implantation. These mice were used to assess extent of cortical damage caused by the screw implant, with no further experimental testing. These animals were housed under the same conditions as the cohort used for electrophysiological data. All procedures were conducted in accordance with National Institutes of Health guidelines, with approval from The University of Western Australia Animal Ethics Committee.

Surgical Procedure for Chronic Screw Implantation

All benches, surgical tools and equipment were sterilized with 70% ethanol, in accordance with aseptic techniques. All mice were weighed and anesthetized using a single intraperitoneal (i.p.) injection of ketamil (75 mg/kg, ketamine hydrochloride, Ilium; Troy Laboratories Pty. Ltd, Glendenning, NSW, Australia) and medetomidine (1 mg/kg, medetomidine hydrochloride, Ilium; Troy Laboratories Pty. Ltd).²² Following anesthesia, the scalp was

shaved from the neck to the snout and between the ears. Animals were secured into a mouse adapted (Model 68,030) stereotaxic frame (Model 68,001; RWD Life Sciences Co, Pty., Nanshan, China). The scalp was disinfected with 70% ethanol and a longitudinal incision made to expose the skull. The skin was retracted and the underlying connective tissue blunt dissected from the bone using a scalpel blade.

Electrode positions were determined using the mouse brain atlas in stereotaxic coordinates.²³ A fine-point marker pen was used to mark the surface of the skull at bregma and for the placement of the screws in the skull overlying the right monocular primary visual cortex for the recording electrode (V1, 3.6-mm caudal to bregma and 2.3-mm lateral) and an area of the right prefrontal cortex for the reference electrode (2.0-mm rostral to bregma and 0.5-mm diameter).

A small pilot-hole (0.5-mm diameter) was drilled at the screw placement marks, using a hand drill with stainless steel metal drill bit (Model No: A3162 #114,731, Titex Drills; K2 Engineering, Perth, Australia). Bone fragments were cleared away from the surface surrounding the hole using a tissue dampened with 70% ethanol, avoiding any contact with the exposed brain. Any fragments within the hole were carefully removed using fine forceps.

Two stainless steel pan-head screws (M0.6 × 2-mm length; Micro Fastenings Ltd., West Sussex, UK) were stabilized with fine forceps while being screwed into the pilot-holes. Once the thread of the screw obtained purchase into the bone it was tightened further, lowering the screw enough to make contact with the surface of the brain without penetrating into the cortex itself (Fig. 1).

Once both screws were inserted, dental cement (Poly-F Plus; Densply GmbH, Mannheim, Germany) was prepared using one scoop of cement to four drops of distilled water and applied around the shaft of the screws and over the exposed skull to seal the wound (Fig. 1B). Importantly, the head of the screws remained above the surface of the dental cement. The cement was allowed to set for 10 minutes before removing the mouse from the stereotaxic frame and applying Xylocaine gel (2% lignocaine hydrochloride; AstraZeneca, North Ryde, NSW, Australia) to the scalp wound as a topical anesthetic.

Following surgery, we administered a reversal agent, atipamezole (1 mg/kg, subcutaneous injection, atipamezole hydrochloride; Ilium, Troy Laboratories) to aid awakening.²² Mice were kept warm on a heat

pad until ambulatory then returned to their cage with softened food and water available.

Electrophysiology

Electrodes

A 'quick-release' electrode using an artery clamp (Part No:18,052-03; Fine Science Tools, Foster City, CA) and silver (Ag) wire (Part No:781,500; A-M Systems, Australia) was developed to connect and disconnect the electrodes to the implanted screws quickly and easily, minimizing the chance of dislodging or damaging the screws. The stainless-steel clamps were electro-etched (12 V DC, 3 M NaCl) to assist in forming a stable solder join between the clamp and silver wire. A ground electrode was constructed from a shortened 23-G hypodermic needle by removing the plastic syringe-attachment, placing silver wire into the needles' electroetched blunt end and soldering the wire into place.

EEG and VEP Recordings

EEG and VEP traces were recorded 1 and 7 days after screw implantation. In each recording session, resting activity (EEG) was recorded, followed by four VEP trials. VEP trials were repeated after a 10-minute break, followed by a second EEG recording, thus obtaining both EEG and VEP trials within the same anaesthetic session (intrasession) and across days (intersession).

Mice were anesthetized for recordings using the same anaesthetic protocol as for surgery. We used a homoeothermic heat pad and rectal thermometer system (TC-1000; CWE Inc., Ardmore, PA) to maintain body temperature at $37.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. While mice were under anesthesia their eyes were treated with Luxyal eyedrops (sodium hyaluronate 1.5mg/mL; AFT Pharmaceuticals, Sydney, NSW, Australia) to prevent dryness of the corneas.²⁴ The pupil remained clear and was not artificially dilated.

Anesthetized mice were placed within an electrically grounded Faraday cage to attenuate erroneous electrical artifacts, and dark adapted for 10 minutes prior to recordings.⁴ The recording electrode was connected to the V1 screw while the reference electrode was connected to the prefrontal cortex screw. The ground electrode needle was inserted subcutaneously into the neck. Sinusoid gratings (0.4 cycles per degree, 0° orientation) were generated using MATLAB (MathWorks Inc., Natick, MA) and sent to a ViSAGE (Cambridge Research Systems, Rochester, UK) for display on a calibrated CRT monitor (ViewSonic PF817; Sony, Tokyo, Japan). The mon-

Figure 1. Chronic implantation of screws in the mouse skull for recording visual evoked potentials. (A) Image of the screw implants into the skull overlying the visual cortex (recording screw, indicated by the *yellow arrow*) and the frontal cortex (reference screw). Bregma is indicated by the *asterisk*. (B) Dental cement applied to the scalp of anesthetized mice to secure screws in place. (C) Experimental set up for recording visual evoked potentials and EEG from the anesthetized mouse, showing attachment of electrodes to the implanted screws via modified artery clamps. (D) Representative photomicrograph of Nissl stained V1 section. *Black arrow* indicates slight compression of the V1 cortex, used in confirming screw location.

itor was positioned at 200 ± 10 mm from the mouse's head, perpendicular to the left eye. Each trial consisted of 32 averaged samples of 1178 ms: 100 ms before the stimulus trigger, 500 ms displaying the stimulus, and the remaining time after stimulus offset. Four consecutive trials were recorded from each animal at each time-point and averaged for analysis. VEP responses were amplified 10,000 times (AC coupled, Neurolog NL104; Warner Instruments, Hamden, CT), bandpass filtered between 5 and 500 Hz with a 50-Hz notch filter (Neurolog NL115; Warner Instruments). The signal was sampled at 2 kHz using PowerLab running Scope software (v4.1.1;

ADInstruments, Bella Vista, NSW, Australia). We confirmed that VEP responses were not artefacts by placing a cardboard 'blank' over the screen, blocking light exposure to the mouse, which abolished all VEP responses. Three more trials were recorded with the 'blank' removed, verifying return of the VEP response (data not shown). We also confirmed that resistance of the screws did not change between day 1 and 7.

Resting EEG was recorded at the start and end of each recording session for 90 seconds at a 20-kHz sample rate using PowerLab running LabChart (v5.1; ADInstruments). To obtain a measure of baseline noise and for ApEn analysis, in one mouse we also

recorded EEG from the time of Lethobarb injection and for 51 seconds after death, confirmed with concurrent ECG recording. Only the recording after death was included in ApEn analysis.

Data Analysis

EEG traces were Fast Fourier transformed using AxoGraph (v1.5.4; Axon Instruments Inc., Berkeley, CA) to obtain EEG power spectra up to 30 Hz.²⁵ VEP traces were normalized to 0 as baseline for each trial. Characteristic peaks were automatically defined for the pooled VEP trace for day 1 and 7, respectively, using area under the curve (AUC) analysis in Graphpad Prism (v6, GraphPad Software Inc., San Diego, CA). Where consecutive peaks did not cross the 0 line (and thus could not be automatically identified in the AUC analysis), they were manually defined at response reversal using cursors in AxoGraph. We analyzed raw VEP response peak-to-peak amplitudes and latencies for each time point (first and second recordings for both day 1 and 7). We also analyzed peak-to-peak amplitudes and latencies scaled to EEG power (≤ 30 Hz).

We scaled responses within-subjects, by multiplying VEP trace at each time point (recording session), by the contribution of EEG power in that session to the mean EEG power of the four recording sessions²¹:

$$\text{ScaledVEP} = \text{VEP}_{\text{raw}} \cdot \left(\frac{\text{EEG}_{\text{mean}}}{\text{EEG}_{\text{session}}} \right) \quad (1)$$

Approximate Entropy

For approximate entropy (ApEn) analysis, EEG traces sampled at 20 kHz, were downsampled to 2 kHz, to allow analysis of a broader time window in each data segment. Custom Matlab functions were used to randomly select 10 segments of 1024 consecutive data points (corresponding to 511.5 ms of EEG trace) from downsampled EEG traces. ApEn was calculated for each of the 10 data segments, with number of dimensions (m , length of data points being compared) fixed at 2, tolerance threshold (r , window value for matching pairs) at 0.2 multiplied by standard deviation (SD) of the data segment, as described previously.²⁶ ApEn values were obtained for 10 data segments per time-point for each animal, from which the mean was calculated and used in subsequent statistical analyses to compare time-points. To aid interpretation of ApEn data, we also measured ApEn, using the same procedure, for two control waveforms: a 40 Hz sine wave and Gaussian

white noise (SD: 0.6), which were generated with Labview (v 11.0; National Instruments, Austin, TX) with the same number of samples and sample rate (2 kHz) as used in EEG data collection.

Statistical Analyses

We used repeated measures multivariate analysis of variance (MANOVA) to compare peak-to-peak amplitudes and peak latencies between day 1 and 7, with raw and scaled VEP traces analyzed separately. Significant MANOVA results were followed up with Sidak-corrected post hoc tests, restricted to comparisons between days, within each peak-to-peak measure. To analyze EEG power (< 30 Hz) and ApEn we used two-way repeated measures ANOVAs, comparing the factors: day (day 1 and 7) and session (first and second recording session, within the same day). Reliability was analyzed using intraclass correlation coefficients (ICC).²⁷ Mean ICC values were further analyzed for difference between intrasession and intersession reliability using one-way repeated measures ANOVA. Mean intrasession ICCs were also analyzed for equivalence, using the confidence interval (CI) method.²⁸ The level for significance was set at P less than 0.05. Statistical analyses were performed using GraphPad Prism and SPSS (v20; IBM Corporation, Armonk, NY).

Confirmation of Screw Location

All mice were euthanized 7 to 14 days after implantation by i.p. injection of Lethobarb (pentobarbitone) and transcardially perfused with sodium chloride (0.9%) and 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brain was removed and stored in 4% paraformaldehyde then transferred to 30% sucrose in phosphate buffered saline (PBS) until cryosectioning. Brains were sectioned in the coronal plane at 40 μm using a cryostat (Leica, Wetzlar, Germany) and Nissl stained using a protocol modified from Makowiecki et al.²⁹ Slides were immersed in PBS for 2 minutes, then transferred to ethanol with 5% glacial acetic acid solution and heated to 50°C to 60°C using a microwave (1 minute). Sections were then rehydrated in PBS (1 minute), stained in prewarmed (55°C) cresyl violet acetate (0.5%; Proscitech, Townsville, Queensland, Australia) for 8 minutes before differentiation in ethanol with 5% glacial acetic acid for up to 13 minutes. Sections were then dehydrated in ethanol (3 minutes, repeated twice) and xylene (2 minutes, repeated) before mounting with Entellan (Merck, Darmstadt, Ger-

many). Nissl-stained sections were then examined using bright field microscopy to confirm screw location immediately dorsal to V1, based on visible cortical compression from screw implantation (Fig. 1D) and cross-referenced to regions defined by the mouse brain atlas.²³

Assessment of Cortical Damage

To assess the extent and progression of any cortical damage from screw implantation, two mice were implanted with screws that had the base of the threaded shaft doped in DiI dye (1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine; Life Technologies, Carlsbad, CA; dissolved in dimethylformamide, Merck, Kenilworth, NJ). Mice were euthanized and transcardially perfused either 2 days (to allow time for DiI transport) or 7 days after implantation, and brains sectioned at 20 μ m in parallel series, as described in the preceding paragraph. One series was processed for an assay for apoptotic cell death using terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL). For TUNEL, sections were incubated with fluorescein-12 dUTP, processed according to the manufacturer's instructions (Dead End Fluorometric TUNEL System; Promega, Alexandria, NSW, Australia). A second series was stained for the expression of glial fibrillary acidic protein (GFAP) to monitor the astrogliotic response.³⁰ For GFAP, sections were incubated with anti-GFAP (Dako, Carpinteria, CA) overnight at 4°C and signal detected using anti-rabbit-Alexa 488. Both series were counterstained with Hoechst 33,342 (Sigma, St. Louis, MO) and imaged with a Nikon fluorescent microscope (Nikon Instruments Inc., Tokyo, Japan) and cooled digital camera (QuantiFIRE; Optronics, SIC Inc., Goleta, CA).

Results

VEP Response Changes Seven Days After Screw Implantation

Consistent with previous reports in rodents,³¹ all animals showed characteristic VEP responses with alternating positive and negative peaks (Fig. 2A, B). We use demarcations of peaks described previously.³¹ On day 1, all animals displayed a positive peak after stimulus presentation, followed by a major negative peak (N1), and an additional positive (P2) and slowly rising negative peak (N3). In line with previous studies, some mice also had a smaller P2-N2-P3

component. As this component was not present for all mice, it was not analyzed further. On day 7 only 2 of 5 (40%) of animals retained a positive P1 that was greater than 10% of the maximum amplitude. Fisher's exact test on proportions of animals with versus without a distinct, positive P1 failed to reach significance ($P = 0.06$). For subsequent analyses, P1 was defined as the point of maximum amplitude preceding reversal of polarity at the onset of the N1 major peak. On both day 1 and day 7, Off-responses were characterized by a positive and major negative peak (Off-P and Off-N, respectively), followed by smaller oscillations which, due to greater variability between trials and between mice, were not analyzed further.

Raw VEP peak-to-peak amplitudes significantly decreased by day 7 (ANOVA, $F_{1,4} = 150.3$, $P < 0.001$). Post hoc tests (Sidak correction) showed this decrease was significant for all peaks (all $P < 0.05$; Fig. 2C). Peak latencies were not significantly different between days (ANOVA, $F_{1,4} = 4.66$, $P = 0.10$, Fig. 2E). Additionally, latencies of specific peaks were very consistent between day 1 and 7 (mean ICC = 0.996), indicating high reliability of latencies across days.

VEP Responses are Reliable Within Sessions, but not Between Sessions

Reliability was assessed within animals using ICC for the whole trace. Within the same recording session (intrasession) reliability was high, indicated by high mean intrasession ICC values on both day 1 and 7 (Fig. 2F). Furthermore, intrasession reliability did not decrease significantly between days (ANOVA, $P = 0.52$). Also supporting that intrasession ICC values were equivalent at day 1 to 7, equivalence analysis using one-tailed CI method²⁸ showed that the upper and lower limits of the 90% CI boundaries (lower bound = -0.26 , upper bound = 0.05) were within the -0.3 bounds of indifference (i.e., nonmeaningful), as previously defined in ICC reliability analyses.³² In contrast, reliability between day 1 and 7 (intersession) was unacceptably low (Fig. 2F). CI analysis with Sidak correction for multiple analyses showed a significant difference between mean intersession ICC and mean ICC values for either day (day 1, $P = 0.004$, day 7, $P = 0.01$), indicating significantly decreased reliability across the two recording sessions, compared with the high reliabilities within blocks of the same recording session.

Figure 2. Reliability of visual evoked potential (VEP) responses 1 and 7 days after implanting screws. (A) Individual trials from a representative animal, showing all eight recording trials for both day 1 and 7. Note similarity between trials of the same day, compared with across days. (B) Mean of VEP trials for the same animal for day 1 and 7, showing raw trace and trace after scaling to EEG power.

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Shaded area (A, B) represents period of stimulus presentation. Trace includes 100 ms prior to presenting the stimulus. (C) Mean peak-to-peak VEP response amplitudes (raw) of each waveform component, 1 and 7 days after screw implantation. (D) Mean peak-to-peak amplitudes scaled to EEG power. (E) Mean latency for specific peaks; *dashed line* represents point at which stimulus was switched off. There was no significant difference in latencies between day 1 and 7 (ANOVA, $P > 0.05$). (A–E) *error bars*, SEM. (F) Mean within-subject ICC values with 95% CIs for intrasession VEP traces on day 1 and 7 and intersession ICC values. * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$.

EEG Power Decreases After 7 Days Screw Implantation

Previously, EEG power has been used to as a normalizing factor to scale VEP responses, reducing variability due to baseline EEG power or noise.²¹ To

assess whether scaling to EEG could be used to reduce variability, we first analyzed EEG changes between day 1 and 7 of screw implantation. At 7 days after screw implantation, raw EEG trace amplitudes decreased compared with 1 day after implantation in all mice (Fig. 3A). There was significantly

Figure 3. Comparison of EEG measures recorded 1 day after screw implantation to recordings after 7 days. (A) Representative raw EEG traces recorded 1 (grey) and 7 days (black) after screw implantation. The first and second recording sessions within the anaesthetic session are shown, offset. (B) Fast Fourier transformed EEG data from the same representative animal as shown in (A), showing power spectra (≤ 30 Hz). (C) Mean EEG power up to 30 Hz for the first and second recording blocks within each a single anaesthetic session. (D) Mean approximate entropy values for 10 data segment samples from each day (day 1 and 7) and each recording block within the same anaesthetic session (first and second). There were no significant differences between time-points, indicating that EEG structural complexity does not change significantly over time. ApEn values obtained from EEG trace from a mouse post euthanasia (death trace) and artificially generated control waveforms are shown for comparison with waveforms of known complexity: 40 Hz sine wave (high predictability and low complexity), and Gaussian white-noise (low predictability, high complexity). There were no significant differences between sine wave ApEn and any other time point, whereas, ApEn values for all time points differed significantly to the death trace and Gaussian white noise. ** $P < 0.01$. **** $P < 0.0001$.

decreased EEG power (≤ 30 Hz) between day 1 and 7 (ANOVA, $F_{1,4} = 38.67$, $P = 0.003$), but no significant difference between first and second recording blocks within the same anaesthetic session (ANOVA, $P > 0.05$; Fig. 3B, C). After scaling to EEG power (see Methods), VEP response amplitudes were no longer significantly different between day 1 and 7, (ANOVA, $F_{1,4} = 0.37$, $P = 0.58$, Fig. 2D).

Approximate Entropy

We used approximate entropy to assess changes in EEG structural complexity over time, independent of amplitude changes. There were no significant differences in mean ApEn either within days ($F_{1,4} = 0.28$, $P = 0.62$) or between day 1 and 7 ($F_{1,4} = 0.06$, $P = 0.81$, Fig. 3D), suggesting that EEG traces had similar structural complexity and predictability across different time points. In a separate analysis, we showed that mean ApEn values for all time points were not significantly different to the artificially generated sine-wave (ANOVA, Sidak corrected post hoc, all P values > 0.05), and confirmed that ApEn values were significantly lower than the EEG trace after death and the generated Gaussian white noise waveform (all P values < 0.001 , Fig. 3D). The death trace EEG contains only the baseline electrical noise, without any neural activity, and therefore, likely estimates the maximum possible ApEn with our recording equipment and electrode configuration. Similarly, the Gaussian white noise is a random signal, with high complexity and low predictability, thus ApEn value for white noise data segments would be close to the maximum ApEn value possible with the sampling parameters used.

Assessment of Cortical Damage From Chronically Implanted Screws

In all mice, there was visible compression of the cortex underlying the screw, however, cortical layers and cells remained intact. The extent of compression was similar in the two animals euthanized 2 or 7 days after screw implantation (Fig. 4A, B). TUNEL staining was negative (Fig. 4C, D), indicating that there were no dying cells in this region at either time point. At both 2 and 7 days, this was accompanied by localized glial activation, as shown by GFAP expression around the site of compression (Fig. 4E, F). We confirmed glial activation was the result of the screw implant, as GFAP was not expressed in V1 of the left hemisphere, where there was no implanted screw (Fig. 4G, H).

Figure 4. Photomicrographs of V1 sections for assessment of cortical damage at 2 and 7 days after screw implantation. (A, B) Dil (red) from Dil-doped screw and Hoechst counter-stain showing compression of V1 cortex underlying the screw, with cortical layers remaining intact. (C, D) TUNEL-stained adjacent V1 sections, negative for label, indicating cortical cells were not dying at either time point. (E–H) V1 cortical sections stained with GFAP to identify activated astrocytes associated with injury. GFAP is expressed in the cortex underlying the screw and appear similar between the two time points (E, F). GFAP was not expressed in the contralateral hemisphere, without an implanted screw electrode (G, H).

Discussion

Our study shows that even after a very short recovery period (1 day), chronically implanted screw electrodes can reliably record VEP responses in mice within the same recording session. However, we show that VEP response amplitudes and EEG power decrease 7 days after implantation, and that there is poorer reliability between recordings sessions a week apart. Despite changes in amplitude across days, waveform patterns and latencies remained consistent, as did approximate entropy. Taken together, these results suggest that when using chronically implanted screw electrodes in mice, a prolonged recovery period after implantation is unnecessary when examining acute changes in VEP peak-to-peak amplitudes (e.g., between-subject designs). Additionally, waveforms and latencies are stable and reliable between 1 and 7 days after implantation, suggesting longitudinal changes in VEP peak latencies and waveform characteristics can be examined without a lengthy post implantation recovery period. However, as VEP amplitudes significantly decreased between 1 and 7 days, assessment of within-subject changes in VEP peak amplitude or EEG power over long periods of time is problematic. Consistent with previous studies,^{20,21} we show that scaling VEP responses to EEG improves reliability across days. However, we also show localized GFAP expression, suggesting a biological origin for EEG and VEP variability (as opposed to change in recording). As such, whether this scaling method is appropriate should be carefully considered in context of the research question.

Changes Over Time

Characteristic VEP waveform components were reliably present at both day 1 and 7. The different sensitivity of specific peaks to changes over time may depend on source activity: the N1 major-component of the waveform is an indicator of afferent activity from thalamocortical excitation, whereas later components reflect action potentials within the cortex itself.¹² Thus, the N1 component may be less vulnerable to cortical circuit changes which could occur with screw implantation compared with later components in the waveform. Consistent with this, N1 was less variable between days and between animals. Previous work in mice reported similar waveforms for VEPs recorded using implanted or single use tungsten microelectrodes.³³ However, the responses recorded here using implanted screw

electrodes have greater amplitude (our study: >70 μV ; Huang et al.³⁴: 30 μV) and are in line with previous experiments using screw electrodes in mice¹⁹, validating our technique.

Possible Reasons for Change Over Time

Although VEP responses showed the characteristic waveform pattern, and most components remained consistent between days, we show a significant decrease in peak-to-peak amplitudes over 7 days. In contrast to studies in awake mice, where repeated exposure to the same stimulus increased peak-to-peak amplitudes,^{33,35} the significant decrease in EEG power we observed suggests that the VEP peak-to-peak amplitude decrease was not stimulus-specific. Furthermore, the high intrasession, but low intersession, reliability suggests that the change in VEP response amplitudes between day 1 and 7 is unlikely to be due to differences in measurement capabilities (e.g., screw conductivity). This is also consistent with there being no change in electrode resistance or a significant difference in ApEn value over time. If amplitude changes resulted from methodological issues, such as introducing additional random noise, ApEn values would increase between days, as confirmed by our examination of ApEn of the EEG trace after death and the white-noise control signal.

This suggests that changes over time may be more likely due to factors within the mouse, for example, damage and inflammation response from the screw implant.^{36,37} However, our examination of cortical damage does not clearly support this: although we observed glial activation (GFAP staining), associated with inflammation,³⁰ this appeared similar for both day 1 and day 7, as did the extent of V1 compression. Similarly, the reduced VEP peak amplitudes do not appear to be due to V1 cell death, as TUNEL staining was negative at both day 1 and 7. As TUNEL labels dying, rather than dead, neurons we cannot rule out possible cell death occurring between day 1 and 7. However, pyknotic nuclei were not evident at either time point, suggesting cell death is not responsible for the decline in VEP amplitude and EEG power after 7 days of screw implantation. Other electrode configurations may face similar issues. This is especially pertinent because microelectrode implantation is arguably more invasive, with evidence of glial scarring and inflammation following needlestick injuries,^{11,30} and cell death arising from the foreign body response to chronically implanted microelectrodes,¹⁰ although no systematic comparisons of cortical excitability over time have been carried out.

Although we found that amplitude decreased, the characteristic waveform shape and latencies of the VEP response were consistent between day 1 and day 7. This is consistent with findings in mild compression injury models. Studies in rats show cortical compression results in immediate cortical depression, followed by a period of hyperexcitability,¹³ present 24 hours after injury,¹⁶ which may explain the increased amplitude of VEP responses shown here. However, gross cell morphology and sensory response waveform are not abnormal following mild compression injury (no cell death).^{38,39} As we did not find any evidence of cell death, this is consistent with our finding of reliable and reproducible VEP response peaks and latencies between day 1 and 7, albeit with decreased amplitude.

Another possibility is that, rather than directly affecting excitability, the mild cortical compression disrupted equally oriented dipoles within the cortical tissue, which determine the polarity of the waveform.⁴⁰ With compression, there may be decreased distance between sinks and sources within the cortex, such that there is greater synchrony, thereby increasing amplitude.¹² The extent of cortical compression may reduce with time, and therefore, affect the balance of orientation and distance from the electrode.

Conclusions

These results indicate that VEP responses can be reliably recorded even after a relatively short recovery period but show changes in amplitude over time. It will be important for future studies to determine whether VEP response amplitudes stabilize or continue to decrease. Nevertheless, our results suggest a prolonged recovery period after implantation is unnecessary for acute studies. Although we demonstrate that scaling to EEG can normalize the amplitude decrease over time, we also highlight that VEP study design should take into account that method of recording may introduce time-dependent changes in cortical excitability, potentially confounding longitudinal experiments.

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