Epidural EEG Recordings Using Microchips in Behavioural Context

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ABSTRACT

Traditionally, behavioural and electrophysiological measurements in animals are performed separately; however their combined use has considerably enriched our understanding of, for example, memory-related processes. Especially single unit recordings paved the way and highlighted performance-specific firing characteristics of individual neurones (place cells, phase cell, head direction cell – see other contributions to this symposium). Less well explored is the correlation between behavioural activity and global brain activity recorded through the electroencephalogram (EEG), particularly in mice. This may be due to technical limitations both in terms of hardware and software, such as time-stamping of events and complex analytical issues.

We here present the first EEG recordings from freely moving mice equipped with wireless microchips (Neurologger – NewBehavior). Unique features of the devices include 4 recording channels, an accelerometer to determine 3-D activity, and an infrared sensor for time-stamping of external events. Devices were tested in two behavioural conditions in which previous use of video-observation systems has provided behavioural indexes widely used to define and explain task performance.

Author Keywords

Video observation, subcranial EEG recording, sleep, disease models, cognition.

INTRODUCTION

Recording of brain activity is pivotal to our understanding of brain function and malfunction, and has recently experienced a revival for translational medicine and the development of powerful computational tools for complex data analyses. The (EEG) determines global electrical activity of the brain and is generally recorded from the scalp at multiple recording sites in humans. Corresponding animal studies utilize both surface and depth EEG individually or simultaneously, and these may be recorded at rest or as event-related potentials.

EEG traces are oscillations with characteristic frequency ranges (1-50Hz) that depend on the vigilance state (e.g. wakefulness or sleep), the location of the recoding sites, and the general health status of the subjects. It comprises the summation of synchronous dendritic events of large neuronal populations, favored by the radial orientation of cortical dendrites [1].

Compared with other modern imaging tools such as PET and MRI, EEG also is non-invasive in humans, comes at considerably lower costs and the relative ease of recording, brain activity is directly measured, and the temporal resolution is excellent. EEG permits determination of essential physiological processes, and EEG spectra can be utilized to determine e.g. sleep stages, ageing, cognition, CNS disorders and drug actions, to name but a few. At the same time, the spatial resolution is poor as activity recorded is by and large from the most superficial layers of the brain.

In rodents (especially mice), typical EEG recordings comprise the surgical implantation of surface/depth electrodes under stereotaxic control, which are then anchored to the skull in a small head-stage. To discriminate between sleep and activity patterns, additional EMG Teflon-coated silver wires are implanted into either neck- or eyelid muscles [2,3]. Animals are then returned for recovery and housed individually to prevent interference with the head-stage during social contacts and aggressive episodes. For recordings, the head-stage is either connected to a wire-less transmitter/transponder positioned subcutaneously in a skin pocket, or flexible lightweight cables that are counterbalanced through a weight on a string running over roller-bearing pulleys [4].

This is a delicate set-up and restricts movement of mice spatially and can impinge on vigilance outcome by interference with sleep pattern (dangling cables will keep animals awake [5]. Therefore, the absence of tethering in a telemetric recording set-up would be preferred. At the same time, EMG silver wires undergo necrosis so that reliable recording of muscle activity will eventually cease after several weeks. This precludes repeated measurements of animals in a within-subject design during ageing studies. As a result, the vast majority of EEG studies are conducted as short-term experiments in between-subject, cross-sectional study designs. We here set out to refine and optimize longitudinal recording techniques by using a novel untethered recording device and provide proof of principal that such studies can be used for a range of scientific research questions including ageing, preclinical disease-model comparison and in behavioral situation with cognitive relevance.

METHODS

Subjects

Mice (female and male) were derived from different sources and in some experiments carried various transgenes to generate Alzheimer or schizophrenia-like phenotypes. They were maintained either on a C57Bl/6 x C3H background, or as C57BL/6 x CBA hybrids. All mice were group housed until surgery (3-5 per standard Macrolon cages), and kept in a controlled holding environment with a 12-hour light-dark cycle (lights on at 7am). The procedures concerning animal care and treatment were in accordance with international standards on animal welfare and Home Office (UK) regulations.

After surgery and at least 10 days recovery, mice were singly housed and assigned to the different experiments (see below), their recording quality tested and animals with poor signals were excluded from the study.

Surgery

Anesthesia was induced with 3% isoflurane in medical grade oxygen and maintained at 1.5% after the mouse was placed in a stereotaxic frame (Stoelting, US). The skull was exposed and epidural gold screw electrodes placed into bur holes at the following coordinates relative to Bregma [6] above i) medial prefrontal cortex (PFx: AP +2 mm/close to midline); and ii) parietal cortex and dorsal hippocampi (bilateral, AP -2 mm /1.5 mm lateral to midline). Surface recordings at this position are dominated by coherent hippocampal discharges [7] and will thus be referred to as the hippocampal recoding site. Reference and ground electrodes were placed at neutral locations superficial to parietal and occipital cortex respectively. Electrodes were assembled into a 7 pin adaptor and fixed to the skull by a mixture of Durelon dental cement and tissue glue. Once the cement had dried, animals were removed from the stereotaxic instrument, injected with 0.5 ml saline (intraperitoneal) and 0.01 µl analgesic (Temgesic; subcutaneous), returned to their home cages and placed in a heating cabinet for 60 minutes. Mice were weighed daily to monitor their recovery and at least 10 days recovery allowed before experiments commenced.

Home Cage Activity Monitor

Circadian activity was recorded in PhenoTyper home cages (Noldus, The Netherlands) through video observation techniques and XY coordinates recorded over 7 consecutive days as described previously [8]. PhenoTyper cages (30cm x 30cm x 35cm) have clear Perspex walls with a plastic floor covered with sawdust, fixed feeding station and water bottle attached to the front wall and an overhead infra-red sensitive camera connected a computer-assisted video tracking software (EthoVision 3.0, Noldus Information Technology, Wageningen, The Netherlands) for automatically recording of activity. Video tracking was performed at a rate of 12.5 samples /second. Ambulatory activity (distance moved) was extracted using the in-house 'Mnimi' software package with automatic exclusion of artifacts. Path length was averaged from a 5-day recording (excluding initial 2 days of habituation) into 1 hour bins and group means (+/- SEM) calculated for a 24hr period separated into light (sleep) and dark (activity) phases.

Object Recognition

Object recognition followed the protocol implemented by Good and Hale [9] with minor modifications. The apparatus was a white Perspex cylinder (50cm diameter; 50cm wall height), in which the animal's movement was recorded by an overhead camera and digitized to a PC-observation system (EthoVision Pro 3.1). Objects were tall to prevent mice from climbing onto them (15-20cm). Behavioural testing consisted of: 1) Habituation (2 days, 2 trials, 5 min, ITI: 2 min). During trial 1, the arena was empty; during trial 2, a single object was placed in its centre. 2) Object novelty: Presentation of two identical objects A (sample phase); objects were replaced by one identical (A) and one novel (B) object (test phase). 3) Spatial novelty: two novel objects C and D (sample phase); displacement of one object (test phase). Locomotor activity (path length) was determined and object exploration recorded (% time within in-object-zone (4cm)). Exclusion criteria: object bias during the sample phase or no exploration of objects.

Wireless EEG Recording and Analysis

EEG recordings commenced for 24 hours on day 3 of PhenoTyper recording. Wireless Neurologger (NewBehavior, Zurich, Switzerland) microchips weighing < 3g (approximately 10% of the body weight) were used for EEG registrations from freely behaving mice. Movement was detected via a built-in accelerometer. Neurologgers were set to record at 200 samples per second with a high pass filter of 0.25 and low pass filter of 70Hz, recordings were downloaded offline to a PC using USB plug-in docking stations.

Data retrieved were transformed with Matlab 7 (The MathWorks Inc., Natick, USA) and imported into SleepSign (Kissei Comtec Co. Ltd, Nagano, Japan) for

vigilance staging and extrapolation of spectral power (Fig. 1B). Vigilance stages (wakefulness, NREM and REM sleep) of 4 sec epochs/bins were identified based on combined Fast Fourier Transform (FFT; delta / theta ratio from hippocampal EEGs) and accelerometer activity (body movement). Automated staging was followed by visual inspection and corrections excluding any movement-related artifacts from spectral analyses. FFTs were finally calculated for each epoch with a resolution of 0.77 Hz, Hamming window smoothed and averaged. The EEG power spectra (1-20 Hz, PFx and hippocampus) for each vigilance state were normalized relative to the absolute maximum power over all frequency bands and averaged for each genotype/age-group for hippocampus and PFx (spectral bands: delta: 0.5–5 Hz, theta: 5–9 Hz, alpha: 9 –14 Hz and beta: 14 –20 Hz).

For object recognition tests, vigilance staging was not required as animals were exploring the environment continuously. Recording with EthoVision was set such that both objects were surrounded by virtual in-object-zones. Entry of the animal into such zones triggered a TTL pulse for time-stamping of the event through infra-red lights onto the Neurologger. Averaging of power spectra was conducted dependent on spatial distribution and FFT analysis followed outline above. Spatial distribution pattern were averaged and compared in all stages of the test.

Histology

After recordings, mice were terminally anaesthetized and intra-cardially perfused with saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (PFA). Brains were removed, post-fixed in PFA overnight, photos taken of brain surfaces to determine electrode/screw locations based on indentation marks, wax embedded and sectioned (5µM).

Statistical Analysis

PhenoTyper and object recognition data are expressed as group means (+/- SEM) and statistically assessed using analysis of variance (ANOVA, 2-way or 1-way, with repeated measure where appropriate) with group (age and genotype) and time of the 12 h light/dark cycles as factors, followed by planned paired comparisons (t-test with Bonferroni correction) to determine the source of reliability.

For EEG power spectral analysis, a 2-way factorial ANOVA was conducted using group (genotype/age) and frequencies as discriminators. Post-hoc planned paired comparisons and frequency specific analyses were carried out on preselected frequency bands to determine effects between genotypes. The significance level in all calculations was set to $P<0.05$.

RESULTS

Experiment 1: Both activity and EEG were recorded by Neurologger microchips for at least 24 hours in PhenoTyper (Noldus) home cages. Circadian activity was measured as ambulation by EthoVision and accelerometer-based determination of movement. This enabled assessment of sleep patterns, vigilance staging and quantification of stage-specific EEG power. Recordings are sensitive to age- and genotype specific variations and can thus be used for a range of studies.

Experiment 2: Mice equipped with EEG recording devices were tested in an object recognition paradigm. Video observation (EthoVision) generated spatial distribution maps in predefined zones (with familiar of novel objects), that form behaviourally relevant endpoints. These maps were space-stamped onto the continuous EEG to enable quantitative analysis of global EEG according to spatial distribution patterns. Fast Fourier Transformation of spectra confirmed alterations in spectral power specific to the spatial location and/or familiarity of the object.

CONCLUSION

The combination of video-observation with quantitative cable-free EEG recording provides a major step towards a combined psycho-physiological approach needed to improve translational tools in neurosciences. Together, they deliver powerful multidisciplinary attempt to provide both systems and cellular information, and they are applicable to a wide range of research projects. Wireless devices such as the Neurologger enable long-term home cage and training-induced EEG observations in a within-subject design. Such a longitudinal study design is favourable for preclinical studies of experimental models that monitor different stages of disease progression. Such studies are also relevant in terms of reduction of animal numbers and cost savings for extensive drug studies.

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