High Resolution Electroencephalography in Freely Moving Mice

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INTRODUCTION

Electroencephalography (EEG) is used to measure electrical potential differences on the brain scalp directly and has been used successfully as a widespread neuropsychological assessment tool. Changes in the regional power spectral density or connectivity strength in different brain regions have been studied with EEG, and tremendous efforts have been made to link these changes with neurological, physiological, or psychological correlates. Recent developments in the fabrication of microelectrodes and noninvasive imaging modalities have been promoted to identify the cellular and physiological sources of EEG rhythms (Goncalves et al. 2006; Lubenov and Siapas 2009). Additional ways to dissect the key elements of neural synchrony is genetic perturbation followed by electrophysiological and behavioral analysis in mice.

In recent years, the creation of genetically modified mice has allowed more comprehensive perspectives on the regulation of oscillatory dynamics usually through investigation of neurological, behavioral, electrophysiological, or anatomopathological comparisons to normal mice. These approaches have not only delivered better scientific understanding of brain oscillations but have also yielded potential drug targets for related diseases (Shin et al. 2008). Furthermore, some genes have been reported to affect spontaneous oscillations, e.g., gamma and delta (Joho et al. 1999) or cholinergic theta oscillations (Shin et al. 2005), or gamma oscillations (Llinas et al. 2007). However, most EEG recordings in mice lack spatial resolution, producing difficulties in determining the cortical origins of rhythms. Considering that field oscillations at different frequency bands have been observed in numerous cortical structures (Steriade 2000), the cortical relation to brain oscillations in conjunction with behavior remains to be described in transgenic mice. Despite its significance, lacking spatial information has always hindered the investigators to extend their data interpretation to network level. Recently Megevand et al. proposed a spatial mapping technique for modeling large-scale neuronal networks in mouse model using needle-type electrode cluster and mapped the event related potentials successfully in mouse model but restricted to anesthetized mice (Megevand et al. 2008).

A major technical problem in obtaining an EEG map in freely moving mice is maintaining a significant number of EEG electrodes on the mouse brain with consistent contact during a recording period. More specifically, all the electrodes must be maintained in the same range of impedance, and mechanical stability is required to allow repeated plug-in and -out actions when the application includes longitudinal monitoring. In this article, we present a novel technique to obtain spatially resolved information of mouse cortical EEG in absence of invasive surgery to the brain. We applied soft material as the carrier substrate and fabricated polyimide-based microelectrode array (PBM array) and produced platinum based microcircuit with lithography technique of which the biocompatibility has been proven (Lago et al. 2007). Within the limited area of exposed skull, 32 electrodes were imbedded. Topographical and dynamical analysis tools also have been developed to visualize the dynamical changes of spatially resolved mouse EEG.

First we demonstrated the validity and utility of mouse EEG by localizing the cortical foci of absence seizure. Although the absence seizure is well established by spike-wave discharges (SWDs), a paroxysmal oscillation in thalamocortical network (Steriade et al. 1993), the initiation mechanism of SWD is still under debate between cortical focus theory and centerncephalic theory (Meeren et al. 2005; van Luijteleraar and Stinnikova 2006). In this article, the initiation timings of SWD were obtained throughout the cortex, and the cortical foci in parietal and frontal regions were explored. This result is expected to be
used in identifying the specific contribution of neocortex and thalamus in the initiation of SWD in conjuncture with simultaneous recording of thalamus. Second, we confirmed the specific contribution of mouse EEG to genetic studies by characterizing irregular oscillation dynamics in cholinergic theta in phospholipase C (PLC)-β1 deficit mice. PLC-β1 knock-out mice lacks in cholinergic theta rhythms with a reduced spectral power in urethan-induced theta bands (Shin et al. 2005); however, whether the residual theta rhythm has the same origin with cholinergic theta is still unknown. Here we analyzed the phase dynamics of cholinergic theta in wild and PLC-β1 knock-out mice and confirmed that spatial coherency of cholinergic theta is absent in the mutant, suggesting different origin of the residual theta rhythm. Our methods can be readily tested in other knock-out mouse models and expected to contribute to investigation of the generating mechanism of EEG signals in molecular and cellular levels.

METHODS

Nanofabrication of bifurcated and soft microelectrode

The structures of PBM array were fabricated using a well-established nanofabrication process. The electrical contacts, the connection lines, and the interconnection pads were made of 300 nm thick platinum, deposited by sputtering on a spin-coated polyimide substrate (Pyralin 2611, HD Microsystems, Bad Homburg, Germany) of 5 μm thickness. After patterning of the metal layers using a photolithography process, a second layer of polyimide of the same thickness was spin-coated on top of the structure. The electrical contacts and the interconnection pads were then opened through selective reactive ion etching of the polyimide layer. Two connectors with 16 pins each (Omnetics Connector) were attached to the interconnection pads using a conductive glue to provide an interface to the recording equipment. To improve the electrical properties of the electrode, the contacts were platinumized by electroplating.

Characterization of microelectrode

Before and after platinization, the electrodes were electrochemically characterized by means of impedance spectroscopy (measurement amplitude: 50 mV, frequency range: 10^-5 Hz). The measurements were performed using a three-electrode setup with a platinum counter electrode (PT 1800, Schott Instruments, Mainz, Germany) and a silver/silver chloride reference electrode (B 2920, Schott Instruments, Mainz, Germany) with 5 μm thickness. After patterning of the metal layers using a photolithography process, a second layer of polyimide of the same thickness was spin-coated on top of the structure. The electrical contacts and the interconnection pads were then opened through selective reactive ion etching of the polyimide layer. Two connectors with 16 pins each (Omnetics Connector) were attached to the interconnection pads using a conductive glue to provide an interface to the recording equipment. To improve the electrical properties of the electrode, the contacts were platinumized by electroplating.

Animal preparation and surgery

All surgical, handling, and experimental procedures were conducted in accordance with the guidelines for the Institutional Animal Care and Use Committee, following Act 1992 of the Korea Lab Animal Care Regulations and associated guidelines. The experiments on signal performance and pharmacologically driven seizure were performed with 7–8 wk female C57BL/6J-129S4/SvJae hybrid mice (19–23 g). The experiments on urethan-induced theta were performed on 10 wk old F1 homozygous male mice and wild-type littermates obtained by cross C57BL/6J(N8)PLC-β1± and 129S4/SvJae(N8)PLC-β1± mice, the genotypes of which were determined by polymerase chain reaction (PCR) analysis. For PBM-array implantation, the animal was anesthetized with an intraperitoneal dose of Avertin (2%, 20 μl/g), and then head-fixed in a stereotaxic apparatus (David Kopf Instruments, Model 902, Tujunga, CA). A 1 in incision was made at the midpoint of the scalp and exposed by micro clamps. Prior to placing the PBM array, the skull was wiped with a saline-soaked cotton ball to remove any tissue debris as well as to give moisture to the skull for better adherence to the PBM array. A wet condition helps the branches of PBM array be pulled to the skull by Van der Waals forces. The PBM array was carefully aligned so that the vertical midline met the midline of the skull and the bregma met the vertical midline and the upper edge of the third branch. An important aspect of the securing process is to ensure that the branches of PBM array are adhered to the skull prior to the administration of glass isomer. For electromyographic (EMG) recording, a Teflon-coated tungsten electrode was inserted into the dorsal neck and grounded with the PBM-array ground electrode. After positioning electrodes, self-curing glass isomer (Vivaglass CEM, Ivoclar Vivadent, Germany) was carefully coated over the PBM array to cement the PBM array and the bone. After curing, the incised skin was sutured. The animal was returned to a cage with no littermates under a thermal lamp for recovery.

EEG recording

For initial experiment signal performance, a Grass 8-16C amplifier (Grass Technologies, West Warwick, RI) was applied with band-pass filtering from 0.3 to 70 Hz with a 60 Hz notch. The analog signals were digitized by a 16-bit Digidata 1440A (Molecular Device) at a sampling frequency rate of 1 kHz. For experiments on seizure and theta mappings, EEGs were recorded with a SynAmps amplifier (Neuroscan,. El Paso, TX). The low impedance electrode on the left was used as a reference electrode, and the other low impedance electrode on the right was used as a ground electrode. Signals were recorded by Scan 4.3 (Neuroscan) at a sampling rate of 1,000 Hz with band-pass of 0.1–100 Hz. The dynamic range of mouse EEG recordings was within 100 μV and the impedance of skull-electrode measured by Scan 4.3 was <100 kΩ at test frequency of 30 Hz.

Data analysis

All the off-line analysis was carried out on a PC by means of in-house programs developed in the Matlab (Mathworks, Natick, MA) programming platform.

Statistical analysis

Descriptive statistical analysis of behavioral parameters before and after PBM-array implantation was performed by the paired r-test. As for the conventional parameters of EEG (e.g., frequency, power values, phase delay, coherence, and phase synchronization index), the Kruskal-Wallis one-way ANOVA test was applied. Each frequency band was filtered by Butterworth zero-phase symmetric using filter design toolbox in Matlab. All differences were regarded as significant if P < 0.05.

Detection of SWD

The characteristic patterns of SWD in rodent EEG are 3–5 Hz repetitive high-voltage negative spikes (20–50 ms of spike width, positive in case of depth-EEG) followed by larger high-voltage positive wave (~200 ms of wave width, negative in case of depth-EEG) lasting >1 s (van Luijtelaar and Coenen 1986). SWD extraction procedures are the following. 1) The Daubechies 5 wavelet analysis was performed and the filtered signals were reconstructed from decomposed signals at the 5th–8th levels, corresponding to 15.6–31.3, 7.8–15.6, 3.9–7.8, and 2.0–3.9 Hz, respectively in case of 1 kHz sampling rate. 2) The positive peaks larger than predetermined threshold values were detected. In this study, the threshold values were set...
to be $5 \times$ SD of baseline. 3) The interpeak distance in milliseconds was calculated. If the difference in neighboring interpeak distance is $<20$ ms and the values of interpeak distance are in the range of 180–250 ms, we defined the moment as equidistance intervals. 4) The SD of EMG was calculated at each equidistance interval and only quiescent moments were selected. 5) The rising and falling slopes to each peak within the equidistance interval were calculated. When rising is faster than falling repetitively, we considered the interval as SWD moment. The onset of SWD was defined at the moment of the first peak in the equidistance interval.

### Topographic mapping

Prior to interpolation, each EEG channels was divided by normalization factor, which was defined by the average power of the range, 130 to 170 Hz within quiescent moment of the animal. Ellipsoidal boundary was arbitrarily drawn based on the electrode coordinates, and imaginary points were generated in a linearly spaced way between electrode positions. The potential value of the imaginary points were calculated from cubic spline interpolation method. For the boundary values, we adopted the 10 percentiles of the dataset. The topographic map was displayed by drawing contour of the matrix.

### Function connectivity

In case of epileptic signals, phase synchronization index was used to scale the synchrony level. The instantaneous phase, $\phi(t)$ of the measured EEG signal was obtained by Hilbert transform, $\pi^{-1} \text{P.V.} \int_{-\infty}^{t} s(t-\tau) d\tau$, where P.V. in front of the integral denotes the Caucy principle value (Piersol 1986). And we used the phase locking value, $\langle \exp[i \Delta \phi_{n,m}(t) \rangle \rangle$, as a synchronization index between nth and mth channels, where $\Delta \phi_{n,m}(t) = \phi_{n}(t) - \phi_{m}(t)$, and bracket notes an ensemble average, and $\langle \rangle$ means absolute values of the complex number (Rosenblum et al. 1996). Please note that the index ranges from 0 (independent fluctuations, i.e., $\Delta \phi_{n,m}$ is randomly or uniformly distributed) to 1 (perfect phase synchronization, i.e., $\Delta \phi_{n,m}$ through the observation time).

In case of oscillation signals, coherence is used as a measure of synchrony between EEG signals, calculated between pairs of signals as a function of frequency. The coherence $C_{xy}(f)$ for two continuous time series $x(t)$ and $y(t)$, having values in the interval from 0 to 1 (Bendat and Piersol 1986). A significance threshold for the coherence is then determined by $1.05^{1/\text{T}^{-1}}$, where $T$ is the number of EEG segments, as an estimate of the upper 95% confidence limit under the hypothesis of independence (Halliday et al. 1995).

### Phase dynamics of oscillation

The phase of the Hilbert transform of EEG signals is often used in determining the phase of the signals in the case of a narrow-band oscillation. A narrow-band oscillation can be expressed as $x(t) = A(t)e^{i\phi(t)}$, which resembles a sine wave with slowly varying frequency and amplitude or a superposition of sine waves with similar frequency. The amplitude $A(t)$ and the instantaneous phase $\phi(t)$ of the signal are uniquely determined by Hilbert transform. The phase relationship between two EEG signals has been widely applied to EEG studies, including the characterization of synchrony of human epileptic or noisy signals (Freeman et al. 2003; Lai et al. 2007), investigation of underlying control mechanisms of certain oscillations such as spindle or alpha/beta (Nikulin and Brismar 2006; Varela et al. 2001), and definition of large-scale integration among cortical areas (Engel et al. 1991; Roelfsema et al. 1997).

### RESULTS

**Development of bifurcated and soft electrode for mouse skull**

**STANDARD COORDINATES FOR MOUSE EEG.** The electrode arrangement for mouse EEG tomography needed to be covered the exposed skull and electrodes needed to be evenly distributed so that an accurate scalp potential map could be obtained for estimating the current sources inside the brain. The area of electrode coverage was collected from C57BL/J6 mice (weight range: 25–30 g), which were chosen due to the availability of neuro-anatomical atlases (Paxinos and Franklin 2008). The shape and size of the active electrodes were determined for a circle with a diameter of 500 $\mu$m, at which the maximal SNR of EEG was measured (Fig. 1A). Reference and ground electrodes were designed to be on both sides of the cerebellum. The null space between electrodes was cut off to enhance flexibility. The electrode array has a bifurcated structure with six branches on each side (Christmas tree shape), and each branch has two or three electrical contact points, covering the exposed skull in an evenly distributed manner (Fig. 1B). The interdistance between two neighboring channels was $1.42 \pm 0.31$ (SD) mm. The coordinates of the active electrode were decided to be in the middle of one cortical area based on the mouse brain atlas (Paxinos and Franklin 2008): $\pm 0.5/2.3$ (in mm, anteroposterior/lateral, secondary motor cortex, M2), $\pm 1.5/2.3$ (M2), $\pm 0.55/1.04$ (M2), $\pm 1.93/1.04$ (primary motor cortex, M1)), $\pm 2.12/0.48$ (primary somatosensory cortex, fore/hind limb regions, S1FL/S1HL), $\pm 3.5/0.48$ (primary somatosensory cortex, barrel field, S1BF), $\pm 1.12/0.96$ (medial parietal association cortex, MPTA), $\pm 2.88/0.96$ (S1BF), $\pm 4.05/0.16$ (secondary auditory cortex, AuV), $\pm 1.13/0.30$ (secondary visual cortex, V2), $\pm 2.24/0.32$ (primary visual cortex, binocular area, V1B), $\pm 4.13/0.30$ (primary auditory cortex, Au1), $\pm 0.5/0.14$ (V2), $\pm 0.24/0.14$ (primary visual cortex, monocular area, V1M), $\pm 4.05/0.14$ (temporal association cortex, TeA), under the condition that the vertical midline and the upper edge of the third branch lays exactly on the bregma point (origin of coordinate axes).

**NANOFABRICATION OF BIFURCATED STRUCTURE.** Figure 1C shows a photograph of the complete electrode structure, including the connector. The PBM array was made thin to sit on the mouse skull naturally with no introduction of additional force. For the electrode substrate, we used polyimide. Polyimide films with various thicknesses from 5 to 40 $\mu$m were tested for adherence to the mouse skull. We found that 10 $\mu$m is a critical thickness for quick placement of the electrodes. After wiping the skull with a saline soaked cotton ball, a 10 $\mu$m thickness film easily adhered to the skull with no additional force or glue and remained for hours, whereas a 15 $\mu$m thickness film was easily detached from the skull due to the tension of the film.

The nanofabricated microelectrode had an impedance range of 2–3.5 k$\Omega$ in the frequency range of $10^{-10}^4$ Hz. As an example, Fig. 1D shows the absolute values of impedance and $E$ shows phase shifts of the platinum contacts showing the electric performance of the sample. All the electrodes exhibited an exponential decrease in impedance with respect to frequency; this is a characteristic behavior for a general electrode-electrolyte interface.
Each electrical contact was aligned to the interconnection pad, which was arranged to fit two double-row microconnectors (NSD-16-VV, Omnestics Connector, Minneapolis, MN). The microconnectors were attached to the electrode pads with the aid of conductive glue. An instant adhesive was applied to encapsulate the edges of the connector to enhance the mechanical stability. The integrity of the electrode and connector connection eased implantation dramatically and also made the study subjects accessible to the recording machine on demand.

**SCREW-TYPE ELECTRODE VERSUS PBM ARRAY.** We compared the signal quality between the PBM array and the conventional screw electrode by simultaneous measurement of both types during epilepsy simulation. In this application, one mouse (25 g, 10 wk) was anesthetized with Avertin (2%, at a dose of 20 ml/kg bodyweight) and mounted in a stereotaxic apparatus (Lab Standard, Stoelting). The film electrode was carefully cut to cover only left hemisphere. One screw electrode was placed on the right lobe (AP: +2.5 mm, L: +2.5 mm), and the contact of the film electrode was placed in a symmetrical position to the screw electrode with respect to the midline lobe (AP: +2.5 mm, L: +2.5 mm). The reference electrode was implanted on the center of frontal lobe and bifurcated into two connectors to be used as a common reference. The ground electrode was placed over the occipital lobe 2 mm dorsal to the lambda point. We applied γ-butyrolactone (GBL, Sigma) to the animal with a dosage of 75 mg/kg to induce bilaterally synchronous spike-wave discharge (SWD) epileptic patterns. The horizontal bars indicate the SWD epileptic events. The SNR curves show that the range 400–500 mm has a relatively large SNR. B: arrangement of high-density mouse EEG electrodes. C: photo of fabricated PBM. Approximate dimensions of PBM array are noted. The layered structure of the PBM array is shown in the inside of the box. D: the absolute impedance (|Z|) in ohm of the PBM array plotted with respect to the measurement frequencies. Standard deviations are marked as error bars. E: phase shifts in degrees plotted with respect to the measurement frequencies. Standard deviations are marked as error bars. F: EEG recordings from a mouse after administration of γ-butyrolactone (GBL). Both electrodes were located symmetrically around the midline. The EEG signal of the film electrode was multiplied by 10. In both EEG figures, spike-wave discharge (SWD) epileptic patterns were observed. The horizontal bars indicate the SWD epileptic events. G: The SNRs of film and screw type electrodes for delta (1–4 Hz), theta (5–8 Hz), alpha (9–12 Hz), beta (12–30 Hz), gamma (30–60 Hz). The bars represent the average of SNR from 5 mice and the error bars are the SE. The SNR of film and screw electrodes did not show any statistical significance (1 way ANOVA test for significance level, $P = 0.05$). The SNR values were calculated as defined in A. H: mouse with exposed skull after placement of PBM. Any membrane layers on the skull were removed and a small amount of saline was applied over the skull for adhesion of the electrode to the skull. I: a freely moving mouse in 1 of our EEG recording sessions.

**FIG. 1.** Mouse skull electroencephalographic (EEG) electrodes: polyimide based microelectrodes (PBM). A: the signal-to-noise ratio (SNR) for various sizes of electrical contacts in PBM. EEG bi- and monopolar recordings were obtained on the mouse skull. SNR was calculated for delta, δ (1–4 Hz) and theta, θ (5–8 Hz) rhythms. The SNR (dB) was obtained by calculating $10 \log(\frac{P_{\text{signal}}}{P_{\text{noise}}})$, where $P_{\text{signal}}$ is the mean power spectral density for corresponding frequency band and $P_{\text{noise}}$ is the mean power spectral density between 190 and 230 Hz. The SNR curves show that the range 400–500 mm has a relatively large SNR.
wave-density (Snead et al. 1999). We observed that the SWD occurred ∼30 min after the administration of the GBL. In our study, the frequency of SWD was ∼9–13 Hz. Figure 1F shows a sample time series showing SWD collected from both film and screw electrodes. The signal level of the film electrode was 10 times less than that of the screw electrode under the same amplification settings, but waveform and frequency information was successfully delivered with the film electrode.

The implantation of the film microelectrodes took less effort and time compared with the conventional screw electrodes. Figure 1H shows the dorsal view of the mouse with electrodes on its skull. After applying dental cement for fixation, we sutured the incised scalp. The microconnector enabled us to connect the recording wires in a convenient manner and the multichannel EEG acquisition can be performed in a freely moving condition (Fig. 1I). The detailed procedure is described in METHODS.

EEG recordings in freely moving mice

After implantation and fixation of the film microelectrode array on mice skulls using dental cement, no severe disorder, such as limpnosed on later, was observed throughout the study.

LONG-TERM STABILITY OF EEG RECORDINGS FOR LONGITUDINAL STUDY. The levels of SNR were evaluated in longitudinal recording over a month of six freely behaving animals to explore the long-term stability of the EEG signals. With EEG, the definitions of signal and noise are unclear due to the complex properties of EEG recordings. We selected theta rhythms as signals in the frequency range from 4 to 8 Hz. For the background noise, the frequency range of 190–230 Hz was chosen to avoid the harmonics of 60 Hz line noise and/or any other physiological rhythms. Figure 2 shows the average and SD of temporal development of SNR from 8 to 41 days after surgery divided by the SNR on the 8 days after surgery. We observed that the signal quality was maintained for ∼1 mo after surgery and dramatically deteriorated after this period.

MOVEMENT ARTIFACTS. The recording duration and the quality of the data are highly dependent on mechanical stability versus movement of the subject. A co-registration of video, motion sensor, and EEG recordings assured no significant changes occurred during mild movements such as sniffing, drinking water, or licking paws. On inspecting the raw EEG results, protruding artifacts sometimes appeared; e.g., when the animal bumped into a wall (Fig. 3A) or jumped with a 180° turn (Fig. 3B). On the other hand, scratching the ear, which is a very perturbing moment in terms of interface noise, did not evoke either a transient or lasting artifact (Fig. 3C). The example in Fig. 3D shows the power of EEG rhythms while an animal jogged on a treadmill with three different speeds (5, 10, 15 cm/s). The activity level is the vector magnitude of three dimensional accelerometer signals. Some rhythms showed increased power compared with the resting state, but no correlation to activity levels was found. Our successive recordings suggested that the condition of the connector jack or insulation of the wire lead were crucial in barricading any unwanted noise.

Functional brain mapping of mouse EEG

Spatiotemporal mapping of multichannel EEG signals to the mouse brain is similar to other EEG topographic mapping techniques in human EEG. However, in mouse EEG, the cortex region covered by the electrode is limited to the frontal, central, parietal and upper temporal regions, which restricts the precise identification of a zone of an EEG event in terms of depth. While the potential mapping delivers surface information, nevertheless noninvasive cortical localization of EEG in functioning mouse can image the generation or dynamics of certain rhythms or epileptic signals or event-related signals in gene modified animals.

CHARACTERIZATION OF SEIZURE ONSET: A CASE OF SPIKE-WAVE-DISCHARGE. Absence seizure were recorded on mice skulls with PBM array by systemic administration of GBL (50 mg/ kg) (Snead and Bearden 1980). GBL is known to generate SWD in thalamocortical networks (Ishige et al. 1996). Sample traces of EEG and EMG with SWD episode are exhibited in Fig. 4A and the SWDs robustly occurred in GBL model. The EMG was simultaneously recorded in the dorsal neck, and only motionless SWD episodes were exclusively extracted. The absence seizure globally was observed in all channels, and it was noticeable that strong signals were detected favorably in the parietal and frontal cortex. The power was evaluated both for nonepileptic and SWD intervals and the spatial distribution of ensemble average of power ratio during SWD with respect to the baseline was mapped in Fig. 4B (n = 10, 1 mouse). The same length of time interval at 2 s after cessation of SWD was picked as baseline. Compared with the baseline, power of the signal increased significantly particularly in somatosensory area including barrel, limb area, whereas the power of SWD in posterior parietal or occipital lobe did not increase significantly during SWD. We found that the power pattern of SWD did not hold the hemispheric symmetry. In our 10 SWD samples, only 2 samples had SWD dominance in right hemisphere, and the other SWD had preference to left hemisphere. The cross-channel connectivity was mapped by evaluating phase synchronization index for every pair of channel, and only the pairs of increased connectivity with statistical significance were overlaid in Fig. 4B. It is noticeable that the cross-channel connectivity increased during absence seizure mostly in the ipsilateral way except the connection between prefrontal and
In our 30 channel EEG measure-
intraperitoneal injection of urethan (0.8 g/kg). PLC-
depicted in C
 hippocampal theta rhythms (Shin et al. 2005). As expected, the mice have been shown have a lack of cholinergic, type II, EEG mapping technique to map a deficiency of hippocampus availability of valid behavioral mouse models. We applied our cholinergergic theta rhythms in PLC
Characterization of rhythm phenotype: a case of irregular
cases, the probability of onset area shows that left parietal cortex dominantly leaded the absence seizure appeared SWD within the whole channels. It is noticeable that the first peak of the SWD and the ensemble averages of the occipital cortex. The SWD onset was identified by detecting the first peak of the SWD and the ensemble averages of the onset time, \( \tau \) in milliseconds and the peak strength in \( \mu \)V were depicted in C and D, respectively. \( \tau \) was defined by the time delay of the first peak at the corresponding channel to the firstly appeared SWD within the whole channels. It is noticeable that left parietal cortex dominantly leaded the absence seizure within the cortex. Although there are a heterogeneity in onset patterns, for example, frontal onset was observed in some cases, the probability of onset area shows that left parietal corresponding to primary somatosensory cortex leads the SWD in the cortex as shown in Fig. 4E.
Characterization of rhythm phenotype: a case of irregular cholinergergic theta rhythms in PLC \( \beta 1 \) knock-out mouse
The advantage of studies using transgenic mice is in the availability of valid behavioral mouse models. We applied our EEG mapping technique to map a deficiency of hippocampus theta rhythms in the phospholipase C (PLC)-\( \beta 1 \) \( \sim \)/\( \sim \) mouse by intraperitoneal injection of urethan (0.8 g/kg). PLC-\( \beta 1 \) \( \sim \)/\( \sim \) mice have been shown have a lack of cholinergic, type II, hippocampal theta rhythms (Shin et al. 2005). As expected, the EEG map of PLC-\( \beta 1 \) +/- mouse shows robustly synchronized theta oscillations (Fig. 5A). Over the entire cortex, the theta oscillations occur dominantly with almost zero time lags. On the other hand, as characterized by previous hippocampus local field recordings, PLC-\( \beta 1 \) \( \sim \)/\( \sim \) mice showed a deficit of cholinergic theta oscillation. In our 30 channel EEG measure-
ments, rhythms were not completely abolished in all the channels, however. Average power spectra for all the channels re-affirmed that PLC-\( \beta 1 \) \( \sim \)/\( \sim \) mouse had diminished theta rhythms with frequencies lower than wild-type littermates (Fig. 5E). Topographic maps of theta amplitude plot the regions of the brain where theta activity are the most prominent (Fig. 5, C and D). Additionally, the long-range connectivity of neuronal activity has also been derived from the coherence of theta at two different sites and is depicted as connecting lines on the topographic maps. The coherence map shows that the cholinergic theta in PLC-\( \beta 1 \) +/- mouse maintains coherence over the brain, and additionally shows that the frontal area has dynamics independent of theta. On the other hand, theta exists in PLC-\( \beta 1 \) \( \sim \)/\( \sim \) mice, but in a spatially local and incoherent way, implying that the network generating theta is partially broken down, nonexistent, or modified.
From a temporal point of view, these power changes in theta bands are associated with impairments in the ability of distributed networks to establish precise synchronization of neuronal assemblies oscillating at theta frequencies. The distributions of phase differences between theta rhythms at two different sites were evaluated for all combinations and plotted in Fig. 5F. The distribution of sharp peaks around zero was found in case of PLC-\( \beta 1 \) +/- mice theta oscillations, whereas a broad distribution of phase differences were seen in association with PLC-\( \beta 1 \) \( \sim \)/\( \sim \) mouse rhythm. The theta oscillations in PLC-\( \beta 1 \) +/- mice were formulated in a synchronous manner over the brain, and the theta oscillations in PLC-\( \beta 1 \) \( \sim \)/\( \sim \)
mice occurred in an asynchronous manner. Considering that urethane-induced theta rhythms are synchronous (Buzsáki 2002; Buzsáki et al. 1986; Fox et al. 1986), the irregular propagation of theta rhythms in PLC-β1/H9252/H11002 mice suggests that the theta power observed in PLC-β1/H9252/H11002 mice might have different origins from urethane-triggered resonances shown in the wild type.

**DISCUSSION**

Here we report on a new method for high-resolution EEG mapping in freely behaving mice using a nanofabricated microelectrode array and neuroimaging techniques. This method solves the problem of limitations in acquiring spatial information using conventional mouse EEG by allowing EEG using multiple electrodes distributed over the skull. To our knowledge, there is no comparable method for EEG mapping of freely moving mice that can be used to obtain functional brain imaging phenotypes in mutant mice.

Use of nanofabricated PBM array was the key element in achieving a high-density electrode configuration in our method. PBM array have been used for human electrocorticograms (ECoG) (Mercanzini et al. 2007; Rubehn et al. 2009) and rat ECoG (Yeager et al. 2008) but have not been applied to mouse EEG to the best of our knowledge. We chose this material because of the light-weight and flexible characteristics, the biocompatibility that has been proven in cell viability tests (Sun et al. 2008), and results from morphological studies after chronic implantation (Lago et al. 2007). The impedance spectrum of PBM array is relatively wide and tunable mostly by adjusting the size of electrical contact. In chronic recordings, PBM array has been proved to have great signal fidelity in recording cell-level activities (Rousche et al. 2001) and cortical potentials in addition to demonstrating long endurance in nerve (Stieglitz et al. 2000) or cortex (Rubehn et al. 2009; Yeager et al. 2008) stimulation. In our study, determining the correct impedance range for the electrodes was an important issue. The SNR for different sizes of electrical contacts (Fig. 1A) narrowed down the impedance range and pilot recordings with different pitch sizes lead to the setting of the current design parameters. In the future, more rigorous studies must be carried out in regard to the impedance between brain and electrode interface, which we expect will be a function of the impedance of the electrode, frequency of the rhythm, and conductivity of the tissue.
Advantages of spatially resolved mouse EEG

During the absence seizure in both hemispheres using one electrode anchored by a microscrew and PBM array on the other hemisphere, the bilaterally synchronized SWDs were recorded producing almost identical waveforms with neither frequency nor phase distortion (Fig. 1F). This suggests that PBM array will successfully replace microscrew electrodes for skull EEG recording, especially when spatial information is desired. From the perspective of measurement capabilities, a flexible, ultra-thin, film-type, high-density EEG electrode offers a number of advantages compared with existing screw type electrodes. First, film-type electrodes can reduce surgery times dramatically with no need for invasive surgery to the skull or brain. This not only produces less stress in the animal but also shortens the recovery time after surgery. Our experience with PBM array informed that recording immediately after cement cured is possible. Second, PBM arrays do not require an anchoring process on the skull and, hence, do not induce hemorrhaging in the brain during surgery. A blood clot under the skull has been the main source of artifacts unrelated to brain activation. Third, the flexibility of fabrication with polyimide enables us to make null spaces between electrodes, and a secondary electrode or needle can be applied for multi-modal recording or simultaneous microinjection. In sum, application of PBM array reduces the stress not only in the animal but also in the experimenter by delivering efficient and more successful way of implantation.

FIG. 5. EEG recordings of theta rhythm in the mouse brain. Simultaneous cortical recordings from 30 channel EEG under urethane anesthesia in PLC-β1+/+ (A) and PLC-β1−/− (B) mice. Left: ordered raw traces; right: the amplitude of theta rhythm filtered by zero-phase Butterworth filter with passband from the 4–8 Hz bands are color-coded. Up and down phases of theta rhythms have red and blue colors, respectively. Black dots mark the positive peak of theta waves. Lack of theta coherence between channels appeared in a PLC-β1−/− mouse. Topography and the coherence map of theta rhythms in (C) PLC-β1+/+ and (D) PLC-β1−/−. The colorbar indicates the values of power spectral density (mV²/Hz) in the theta band (4–8 Hz). Black dots indicate the channel positions. Lines were drawn between 2 channels the coherence of which exceeded the significant threshold, which was 0.79 to visualize the connectivity. The power and coherence maps of PLC-β1+/+ show that the cholinergic theta are globally synchronized. In PLC-β1−/−, theta oscillation locally present and coupling between oscillations are spatially segregated. E: average power spectrum of 30 EEG channels. Here the x and y axes represent frequency and power spectral density, respectively. Error bars represent the SD across the channels. The plots show peaks at 5.3 and 3.9 Hz in PLC-β1+/+ and PLC-β1−/− mice, respectively. Note that theta rhythm in PLC-β1−/− mouse was not totally abolished. H: the distribution of phase differences between 2 different sites for theta rhythms in PLC-β1+/+ (black) and PLC-β1−/− (red). Sharp peak around 0 phase difference in PLC-β1+/+ indicates the temporal delays of theta oscillation in different cortical regions are small and oscillations are locked to each other. On the other hand, the presence of broad distribution in PLC-β1−/− indicates that the oscillations are not dynamically coupled to each other.
Physically, the spatial distribution of the electric source is estimated by a potential map in space. The electromagnetic model of the synchronized synaptic action in neuronal groups is a current dipole (Nunez and Srinivasan 2006), and the location and size of a neuronal circuit generating the synchronized behavior can be gauged from the potential map. Therefore multichannel recording is required for the characterization of synchronization. Figure 5, C and D, shows the potential maps of theta rhythms in wild and transgenic mice, respectively. This topographic information discerns the cortical regions involved in certain rhythms or patterns and cine modes can be generated to visualize the generation, propagation, and termination of the waves. Furthermore, higher-level cognitive mechanisms are associated with functional relationships between different brain regions, and one channel EEG is not adaptable to the study of multiple-range connectivity of the brain during cognitive processes. The spectral coherence between different regions of brain visualized the cortical connectivity of the mouse brain. In summary, recruiting more electrodes presents not only spatiotemporal dynamics of neuronal synchrony but also the functional connectivity of different cortical regions.

Localization of absence seizure onset in mice

The investigation of generating mechanisms of SWDs during absence seizure has been one of the most popular topics in neuroscience and specific contribution of brain regions or cells or neurons or molecules are still under investigation. A systemic diagram incorporating molecular to systemic level needs to be built. Our demonstration showing the SWD onset from intact brain of behaviorally unrestricted mice is one trial to understand the molecular backgrounds of SWDs in system level. We observed that the initiation of cortical SWD happened in a focal manner. Mostly the parietal and frontal cortex initiated SWD, and by scoring the probability of seizure onset, the left primary somatosensory cortex was the dominant leader of SWD. This result is in line with previous finding with a rat model with spontaneous absence seizure showing that SWDs are initiated in the facial region of the somatosensory cortex (Meeren et al. 2002). Recently genetic studies have contributed to the understanding of generation of absence seizure in the molecular level, for instance, the α1G subunit of low-voltage-activated T-type Ca²⁺ channels in thalamocortical relay neurons are involved in the genesis of absence seizure (Kim et al. 2001); on the other hand, phospholipase C β4 in the thalamic reticular neuron conduces absence seizure (Cheong et al. 2009). However, it is true that these genes have a role as modulator rather than exterminator or source for SWDs. A dynamic mapping of SWD in these genetic models is expected to visualize in which moment or which circuit these genes are involved in generation of SWD.

Phase synchrony and dispersion with EEG in mice

Some opinions hold that the process of integration is based on the interplay between phase locking and phase dispersion across different bands and at different moments in time (Varela et al. 2001), and EEG phase synchrony reflects that exact timing of communication between distant but functionally related neural populations (Sauseng and Klimesch 2008). One of the primary issues in high-density mouse EEG is how precisely the locking and dispersion of phase can be tractable in terms of temporal and spatial resolution. Recently published work on hippocampal theta oscillations beautifully demonstrated the propagation of theta along the septotemporal (vertical) axis of the hippocampus using multichannel silicon probes (Lubenov and Siapas 2009). An equiphase contour was observed within layers in their study, and therefore the traveling wave nature was not observable with our horizontally arranged PBM. However, the topography of theta phase offsets across the brain was demonstrated (Fig. 5A and B). Theta oscillations occurring in PLC β1 +/+ mice were synchronized with zero delay (Fig. 5A), whereas theta oscillations in the PLC β1 −/− mice were locked to each other both in and out of phase (B). Over the entire period of time, the phase differences between two sites in PLC β1 +/+ mice remained small, whereas those values in PLC β1 −/− mice had a broad distribution (Fig. 5H). This evidence clearly shows that PLC β1 −/− mice do not have a global clock. This information is useful especially when some residual rhythms remain in the power spectrum (Fig. 5C), but decisions need to be made whether their origin is the same as for wild-type mice.

Expected contribution of transgenic mice to the understanding of mechanisms underlying EEG rhythms

Recordings from EEG are now taken very seriously as important signals from the brain. As noted in the introduction, EEG in transgenic mice has been used to dissect brain rhythms related to genetic deficiency, which are directly linked to signaling via related receptors or ion channels, eventually disturbing signal transmission in the network. The most intriguing questions arise when the altered rhythm observed in mutant mice is correlated with cognitive or behavioral dysfunctions. It may not be the most significant example, but PLCβ1 knock-out mice, lacking cholinergic theta waves (Shin et al. 2005), showed locomotor hyperactivity and sensorimotor gating deficits as well as cognitive impairments (Koh et al. 2008; McOmish et al. 2008). The historical data of Lennox (Lennox and Davis 1950) and related works on human EEG in twins (Pelliccioli and Garioni 1955) suggested that there exists syndrome-specific genotypes. Apart from linkage to a specific pathological gene, integration of electrophysiological, pharmacopathological, or neuroanatomical studies with recordings from mouse brains will allow identification of molecular, neuronal or circuitry targets as potential causes of pathology. The in vivo study of brain wave mechanics over the entire brain will add complementary knowledge to the current neuroscience database in an effective way.

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DISCLOSURES

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