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Spatial-temporal patterns of electrocorticographic spectral changes during midazolam sedation

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Abstract

Objective—To better understand ‘when’ and ‘where’ wideband electrophysiological signals are altered by sedation.

Methods—We generated animation movies showing electrocorticography (ECoG) amplitudes at eight spectral frequency bands across 1.0 to 116 Hz, every 0.1 second, on three-dimensional surface images of 10 children who underwent epilepsy surgery. We measured the onset, intensity, and variance of each band amplitude change at given nonepileptic regions separately from those at affected regions. We also determined the presence of differential ECoG changes depending on the brain anatomy.

Results—Within 20 seconds following injection of midazolam, beta (16–31.5 Hz) and sigma (12–15.5 Hz) activities began to be multifocally augmented with increased variance in amplitude at each site. Beta-sigma augmentation was most prominent within the association neocortex. Augmentation of low-delta activity (1.0–1.5 Hz) was relatively modest and confined to the somatosensory-motor region. Conversely, injection of midazolam induced attenuation of theta (4.0–7.5 Hz) and high-gamma (64–116 Hz) activities.

Conclusions—Our observations support the notion that augmentation beta-sigma and delta activities reflects cortical deactivation or inactivation, whereas theta and high-gamma activities

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CONFLICT OF INTEREST

None of the authors have potential conflicts of interest to be disclosed.

contribute to maintenance of consciousness. The effects of midazolam on the dynamics of cortical oscillations differed across regions.

Significance—Sedation, at least partially, reflects a multi-local phenomenon at the cortical level rather than global brain alteration homogeneously driven by the common central control structure.

Keywords

Electrocorticography (ECoG); high-frequency oscillations (HFOs); pediatric epilepsy surgery; video-EEG; animation movie

INTRODUCTION

Midazolam is a commonly-used benzodiazepine, whose effects include alleviation of anxiety and restlessness while effectively reducing the risk of recurrent seizures. Electrophysiological correlates of benzodiazepine sedation have been studied in human brain primarily using scalp EEG recording, and the common observations include amplitude augmentation of beta (16–31.5 Hz), sigma (12–15.5 Hz) and delta (<3.5 Hz) activities (Schulte am Esch et al., 1990; Veselis et al., 1991; Hering et al., 1994; Liu et al., 1996; Schnider et al., 1996). Beta-sigma oscillations have been considered to comprise idling rhythms signaling the status quo to the underlying cortex (Neuper and Pfurtscheller, 2001; Engel and Fries, 2010), whereas delta oscillations reflect inhibited neuronal states (Steriade and Timofeev, 2003; Nishida et al., 2008; Vyazovskiy et al., 2011). Unanswered questions still exist regarding sedation-induced electrographic changes, partly because scalp EEG recording has a poor spatial resolution. It remains unknown whether sedation-induced alteration of neuronal activities would be uniform across regions or differ depending on the brain anatomy. The answer to this question is expected to increase the understanding of clinical symptoms elicited by sedation. It also remains unknown how high-frequency oscillations at >60 Hz would be altered by sedation with benzodiazepine. Such high-frequency activity is considered to be an excellent summary measure of *in situ* cortical activation, as its amplitude augmentation induced by sensory and cognitive tasks is tightly correlated to increased firing rate on single neuron recording (Ray et al., 2008) and accurately localizes the relevant functionally-important cortex in brain surgery (Lachaux et al., 2012; Kojima et al., 2013). Furthermore, such high-frequency activity coupled with theta rhythm (4–7.5 Hz) has been proposed to maintain consciousness, awareness, as well as cognitive function during the awake state (Canolty and Knight, 2010; Long et al., 2014; Szczepanski et al., 2014; Yaffe et al., 2014). Accurate measurement of such high-frequency oscillations in human brains, particularly from deep sites such as medial-temporal regions, is possible on intracranial electrocorticography (ECoG) recording.

In the present study of patients with focal seizures who underwent extraoperative ECoG recording (Asano et al., 2009), we first generated animation movies showing amplitudes at eight spectral frequency bands across 1.0 to 116 Hz, every 0.1 second, on three-dimensional surface images of individual patients. We determined the onset, intensity, and variance of each band amplitude change at given nonepileptic brain sites. We specifically expected that midazolam would increase the amplitudes of beta, sigma, and delta activities but would decrease those of theta and high-gamma (64–116 Hz) activities. We also expected that the

spectral patterns of midazolam-induced ECoG changes would differ across the ‘somatosensory-motor’, ‘visual’, ‘auditory’, ‘medial-temporal (also known as memory system)’ regions and ‘association neocortex’. The latter expectation is based on the general notion that early evoked responses in primary sensory regions are often preserved during anesthesia, deep sleep, and vegetative state (Alkire et al., 2008); the association neocortex is hemodynamically deactivated during ‘vegetative state’ as well as ‘seizures characterized by loss of consciousness’ such as absence and complex partial seizures (Laureys, 2005).

METHODS

Patients

The inclusion criteria included: (i) patients with drug-resistant focal seizures receiving intravenous administration of midazolam for the purpose of sedation, as a part of clinical management, during extraoperative ECoG recording at Children's Hospital of Michigan in Detroit. The exclusion criteria included: (i) previous history of epilepsy surgery and (ii) structural lesions diffusely involving the sampled hemisphere (Wu et al., 2011). The study was approved by the Institutional Review Board at Wayne State University, and written informed consent was obtained from the guardians of all patients.

Subdural electrode placement

Platinum grid and strip electrodes (10 mm intercontact distance, 4 mm diameter) were implanted, to subsequently localize the focus responsible for habitual seizures and the cortical regions essential for important functions (Asano et al., 2009; Kumar et al., 2012; Supplementary Figure S1). A three-dimensional MRI was created for each patient with the location of electrodes directly defined on the brain surface (Alkonyi et al., 2009; Matsuzaki et al., 2015). Using automatic parcellation of cortical gyri on FreeSurfer software (Desikan et al., 2006), subdural electrodes were assigned anatomical labels (Pieters et al., 2013; Matsuzaki et al., 2015; Supplementary Figure S2).

Extraoperative video-ECoG recording

Extraoperative video-ECoG recordings were obtained for three to six days using a 192-channel Nihon Kohden Neurofax 1100A Digital System (Nihon Kohden America Inc, Foothill Ranch, CA, USA; Brown et al., 2008; Nariai et al., 2011). The sampling frequency was set at 1000 Hz with the amplifier band pass at 0.016–300 Hz. Sites affected by artifacts were excluded from further analysis. ECoG signals were then re-montaged to a common average reference as previously discussed in detail (Wu et al., 2011). ‘Seizure onset zones’ as well as ‘spiking zones’ (defined as non-seizure onset zones but still affected by interictal spikes) were visually determined (Asano et al., 2009; Zijlmans et al., 2011), and the remaining sites were defined as ‘nonepileptic regions’ in the present study. In general, interictal spike discharges (i.e.: epileptiform discharges between seizure events) are most frequently generated by the seizure onset zone, and propagate to the surrounding regions (Asano et al., 2003). All patients underwent resective surgery based on the video-ECoG, functional brain mapping, and neuroimaging data.

Time-frequency analysis of ECoG signals

The analysis period of interest in the present study was five minutes immediately following a bolus injection of midazolam, which was given for the purpose of sedation during interictal state. The periods during or immediately after seizures were not included in the analysis. The dose of midazolam given to each patient is presented in Table 1. Following the administration of midazolam, patients were not assigned particular tasks, but their heart rates and breathing patterns were closely monitored.

We generated animation movies showing ECoG amplitudes at eight bands, every 0.1 second, on three-dimensional surface images of given patients, and determined when, where, and how much ECoG amplitudes were altered following the administration of midazolam. The spectral frequency bands of interest were defined as below: low-delta (1–1.5 Hz), high-delta (2–3.5 Hz), theta (4–7.5 Hz), alpha (8–11.5 Hz), sigma (12–15.5 Hz), beta (16–31.5 Hz), low-gamma (32–56 Hz), and high-gamma (64–116 Hz) activities.

ECoG voltage signals were transformed into the time-frequency domain using a complex demodulation technique (Papp and Ktonas, 1977) incorporated in BESA® EEG V.5.1.8 software (BESA GmbH, Gräfelfing, German; Hoechstetter et al., 2004). At each channel, we measured the percent change in amplitude at a given frequency band, in steps of 0.5 Hz and 100 milliseconds, relative to the mean amplitude in a control period for 20 seconds prior to the administration of midazolam (Nagasawa et al., 2012; Matsuzaki et al., 2015). We subsequently superimposed ECoG amplitude measures to each individual three-dimensional MRI (Brown et al., 2008; Nariai et al., 2011; Toyoda et al., 2014), yielding animation movies of amplitude changes at eight frequency bands (Figures 1 and 2; Video S1 and S2). We provided the detailed methodology in the Supplementary Document.

Assessment of the effect of interictal spike discharges on ECoG signals

The following assessment was performed, in part, to determine if sedation-induced ECoG changes in the seizure onset and spiking zones would be affected by the unwanted effect of interictal spike discharges. ECoG amplitudes at eight frequency bands (averaged across patients) were plotted as a function of time, separately for (i) nonepileptic, (ii) spiking, and (iii) seizure onset zones. We determined ‘when’, ‘where’, and ‘at what frequency band’ the ECoG amplitude was increased or decreased compared to that during the control period, using studentized bootstrap statistics followed by Bonferroni correction (Davison and Hinkley, 1997; Terwee et al., 2010). By comparing the plots each other (Figure 3), we determined if short-lasting wideband amplitude-augmentation due to interictal spike discharges (Nagasawa et al., 2012) would involve the seizure onset and spiking zones but not the nonepileptic regions.

We also plotted ‘variance in ECoG amplitudes of each frequency band within each 20-second time window’ over the five-minute period following the administration of midazolam (Figure 4). A large variance would indicate a larger degree of waxing and waning of ECoG amplitudes at a given frequency band at a given site. We predicted that variance in ECoG amplitudes would increase diffusely within the nonepileptic regions, based on the previous observations of periodic waxing and waning of beta and sigma

activities on scalp EEG during sedation and deep anesthesia (Wolter et al., 2006; San-juan et al., 2010). We also predicted that variance would be vastly increased during the time windows affected by interictal spike discharges within the seizure onset and spiking zones but not within the nonepileptic regions.

Assessment of the effect of midazolam on ECoG signals at the nonepileptic regions

The following analyses were applied to the nonepileptic regions alone, in order to rule out the potential effect of interictal spike discharges on ECoG signals. We plotted ECoG amplitudes at each of the eight frequency bands, averaged across patients (Figure 5), separately for (a) 'somatosensory-motor', (b) 'visual', (c) 'auditory', (d) 'medial-temporal', and (e) 'association neocortex' regions (Supplementary Figure S2).

Mixed-model analyses using SPSS Statistics 22 (IBM Corporation, Armonk, New York) subsequently determined if the degree of ECoG amplitude change of a given frequency band (averaged across time and electrode sites) in a brain region significantly differed from those in the other regions. Thereby, 'brain region' was treated as a fixed effect with 'age' and 'midazolam dose (mg/kg)' treated as covariates, whereas 'individual electrode' was treated as a random effect. This analytic approach is expected to reduce the confounding effects of age and midazolam dose, which varied across patients, on ECoG amplitude measures.

RESULTS

Ten patients satisfying the inclusion and exclusion criteria were studied. The mean dose of midazolam was 0.050 mg/kg (range: 0.032–0.069 mg/kg) (Table 1). None of the patients had a sign of over sedation such as hypoventilation. The linear change of heart rate over the time was -0.24 per minute (standard deviation: 0.44), on average, across the study patients.

Animation movies of ECoG changes

As best presented on Videos S1 and S2, injection of midazolam elicited augmentation of beta and sigma activities, with an increase in the degree of waxing and waning over the time at given sites. Each of the waxing of beta-sigma amplitudes multi-focally involved spatially-restricted regions rather than an entire lobe simultaneously at a given 100 ms time window. A single waxing-and-waning cycle of beta-sigma amplitudes appeared to range from <1 to several seconds. Beta-sigma augmentation was noted in the widespread regions and most prominent within the association neocortex (Figures 1 and 2). Visual assessment of animation movies for all 10 patients failed to appreciate a consistent pattern of spatial propagation of beta or sigma augmentation (e.g.: rostral-caudal or lobe-to-lobe direction).

Augmentation of low-delta activity was relatively modest and often appeared to be confined to the somatosensory-motor region. Conversely, theta activity appeared diffusely attenuated.

Midazolam-induced ECoG changes at nonepileptic, spiking, and seizure-onset regions

A total of 770, 237, and 105 electrodes were classified as the nonepileptic, spiking and seizure-onset sites free from artifacts, and included in the analyses below. The initial amplitude change in the overall nonepileptic regions consisted of augmentation of beta

activity starting at +13 seconds (Figure 3A); augmentation of sigma and low-gamma activities was subsequently noted. Theta-attenuation reached the statistical threshold at +89 seconds. High-gamma activity was briefly attenuated at +28 seconds but returned to the baseline level afterwards. The maximum effect size of beta-augmentation averaged across all nonepileptic sites was +0.75 (i.e.: +75% increase instantaneously compared to the control period), whereas those of theta- and high-gamma attenuation were -0.37 and -0.08.

At given nonepileptic sites, variance in beta-amplitudes was increased (Figure 4A) during the time window between +20 and +40 seconds, when beta-augmentation diffusely took place. The mean variance in beta-amplitudes was +3.3 (+330% increase compared to the control period) afterwards; in other words, midazolam-induced beta-augmentation was accompanied by a 3.3 times larger degree of periodic waxing and waning between +40 and +300 seconds. Variance in low-gamma and sigma amplitudes showed the similar dynamic changes, though their effect sizes were smaller. Variances in amplitudes of the other bands were much smaller than those of beta-, sigma- and low-gamma-amplitudes (Figure 4A).

In the seizure onset region, steep augmentation of wideband activities intermittently masked the aforementioned ECoG amplitude changes (Figure 3C), whereas no steep amplitude augmentation was appreciated in the nonepileptic or spiking regions (Figures 3A and 3B). At the seizure onset sites, variance in wideband ECoG amplitudes vastly increased intermittently during the time windows affected by interictal spike discharges (Figure 4C), whereas no vast increase of variance was appreciated at nonepileptic or spiking sites (Figures 4A and 4B). These observations suggest that midazolam-induced ECoG-changes in the seizure onset zones cannot be generalized to the general population; conversely, we failed to detect the unwanted effects of interictal spike discharges on ECoG signals at nonepileptic sites in our patient cohort.

Midazolam-induced ECoG amplitude changes at distinct nonepileptic brain regions

Out of 770 nonepileptic sites, 184, 58, 20, 22, and 486 were located within the 'somatosensory-motor', 'visual', 'auditory', 'medial-temporal', and 'association neocortex' regions. The initial amplitude change consisted of augmentation of beta activity, starting at +13 seconds, was detected in the association neocortex (Figure 5). Beta-augmentation was particularly prominent in the association neocortex (Figure 5E). A mixed-model analysis revealed significant effects of 'association neocortex', 'age', and 'midazolam dose' on the degree of beta-augmentation (averaged between +20 and +120 seconds). The fixed effects of 'association neocortex', 'age', and 'midazolam dose' was 0.06 ($F=4.4$; $p=0.036$; 95%CI: 0.004 to 0.12), -0.046 ($F=207.0$; $p<0.001$; 95%CI: -0.052 to -0.040), and 6.2 ($F=18.3$; $p<0.001$; 95%CI: 3.4 to 9.1), respectively. In other words, 'association neocortex' was associated with 6% larger beta-augmentation compared to the remaining regions; each increase in age by year was associated with 4.6% decrease of beta-augmentation; each increase of 0.01 mg/kg of midazolam increased beta-augmentation by 6.2%. Augmentation of sigma- and low-gamma activities likewise involved the widespread brain regions.

Augmentation of low-delta activity, for which the effect size was smaller than that of beta-augmentation, was most prominent in the somatosensory-motor region (Figure 5A); the onset was +31 seconds. A mixed-model analysis suggested that 'somatosensory-motor

region' was associated with 17% larger low-delta augmentation compared to the remaining regions ($F=59.0$; $p<0.001$; 95%CI: 12.6 to 21.2%); each increase in age by year was associated with 1.4% increase of low-delta augmentation ($F=46.3$; $p<0.001$; 95%CI: 1.0 to 1.9%). No significant effect of 'midazolam dose' on low-delta augmentation was found ($F=1.5$; $p=0.2$).

Theta attenuation, while involving the widespread brain regions, was particularly prominent in the visual region (Figure 5B). A mixed-model analysis suggested that 'visual region' was associated with 9.4% larger theta-attenuation compared to the remaining regions ($F=12.7$; $p<0.001$; 95%CI: 4.2 to 14.6%); each increase of 0.01 mg/kg of midazolam enhanced theta-attenuation by 1.9% ($F=7.0$; $p=0.009$; 95%CI: 0.5 to 3.3%). No significant effect of 'age' on theta-attenuation was found ($F=1.5$; $p=0.2$).

Visual assessment of the plots (Figure 5D) suggests that high-gamma attenuation was minimal or modest in all but the medial-temporal region. A mixed-model analysis failed to demonstrate a significant effect of 'medial-temporal region' on the degree of high-gamma attenuation ($F=0.9$; $p=0.3$).

DISCUSSION

Beta and sigma augmentation

The most prominent changes induced by midazolam, commonly noted across all 10 patients, include augmentation of beta (16–31.5 Hz) and sigma (12–15.5 Hz) activities involving broad cortical regions. The observed onset of beta-augmentation as early as +13 seconds, taken together with the correlation between medication dose and the degree of amplitude augmentation, suggests that beta-augmentation reflects the direct effect of midazolam rather than the subsequent behavioral changes induced by midazolam. Beta and sigma activities shared the similar dynamic changes in our patient cohort. These observations are highly consistent with the notion that midazolam enhances the status quo signal to the underlying cortex, by augmenting the idling rhythms reflected by beta-sigma oscillations (Neuper and Pfurtscheller, 2001; Engel and Fries, 2010). A number of previous ECoG studies reported that the amplitudes of beta and sigma activities in functionally-important cortices become smaller during sensorimotor and cognitive tasks and larger during resting state (Lachaux et al., 2012).

Multi-focality of beta and sigma augmentation is best appreciated on movies

A novel observation in the present study was the manner of beta-sigma augmentation characterized by an increase in the degree of waxing and waning over the time at each site without simultaneously involving the entire lobes at each 100-ms time window (Videos S1 and S2). Each of the waxing of beta-sigma amplitudes appeared to involve multiple spatially-restricted regions without a consistent sequence pattern. A single waxing-and-waning cycle of beta-sigma amplitudes ranged from <1 to several seconds. Such complex dynamic changes in amplitude could not be appreciated in our previous study, which measured ECoG amplitudes every 4.1 seconds using Fourier Transformation (Nishida et al., 2009). Multi-focality of beta-sigma augmentation across sites infers that many of local

cortical regions independently respond to the effect of midazolam rather than that cortical activities are globally, homogeneously, and simultaneously altered by the common central control structure. Furthermore, there was no single focus obviously responsible for generation and propagation of beta-sigma augmentation in our patient cohort. Midazolam has been suggested to alter the amplitude of beta-band oscillations by altering tonic GABAA receptor-mediated currents in cortical neurons (Yamada et al., 2007; Christian et al., 2015; Prokic et al., 2015). It is plausible to hypothesize that midazolam-induced sedation would, at least partially, reflect a multi-local phenomenon at the cortical level, as proposed in spontaneous sleep (Krueger et al., 2008) and propofol-induced deep anesthesia (Lewis et al., 2013).

Beta augmentation is most prominent in the association neocortex

Another novel observation was that association neocortex and younger age were independently associated with a larger degree of beta-augmentation. This observation might suggest that the association neocortex is more sensitive to the effect of midazolam, compared to the other studied regions. This is also consistent with the hypothesis that altered neural activities in the association neocortex are particularly responsible for induction of a state of sedation. Previous imaging studies using positron emission tomography (PET) demonstrated that sedation using benzodiazepine reduced global cerebral blood flow and metabolism particularly in the association neocortex (Foster et al., 1987; Matthew et al., 1995; Gillin et al., 1996; Schreckenberger et al., 2004). Functional imaging and ECoG studies of patients with temporal lobe epilepsy previously reported that loss of consciousness during a seizure event was associated with reduced cerebral blood flow and altered neural processes involving the frontal-parietal association neocortex (Blumenfeld et al., 2004; Englot et al., 2010). Other imaging studies of patients with absence epilepsy likewise reported that loss of consciousness during a seizure event was associated with hemodynamic deactivation of the association neocortex (Bai et al., 2010; Moeller et al., 2010). Furthermore, imaging studies reported that functional connectivity within the association neocortex was reduced during propofol-induced anesthesia (Huang et al., 2014) as well as brain injury-induced vegetative state (Boly et al., 2009). Significance of the observation that younger children showed a larger degree of beta-augmentation is currently unknown. Some studies reported that a larger dose of midazolam was required to sedate younger children (Kain et al., 2007), whereas others failed to find such an age effect (Fraone et al., 1999; Coté et al., 2002).

Low-delta augmentation in the somatosensory-motor region

Another novel observation in the present study was low-delta (1.0–1.5 Hz) augmentation confined to the somatosensory-motor region with the onset of augmentation at +31 seconds. Conversely, high-delta (2.0–3.5 Hz) augmentation failed to reach the statistical significance. Our observations indicate the presence of differential effects of midazolam on the somatosensory-motor region and association neocortex. This is in line with the general notion that early evoked responses in the primary somatosensory region are often preserved during anesthesia, deep sleep, and vegetative state (Sloan et al., 1989; Alkire et al., 2008), when the association neocortex is deactivated (Laureys, 2005). A study of adult patients undergoing spinal surgery using transcranial magnetic stimulator reported that

administration of midazolam had the least effect on motor-evoked potentials (Sihle-Wissel et al., 2000). Low-delta augmentation in the somatosensory-motor region might reflect the inhibition of the underlying cortical function as the result of sedation; low-delta activity during slow-wave sleep is generally associated with long-lasting neuronal hyperpolarization and cellular silence at the level of intracellular recording (Timofeev et al., 2001).

Theta and high-gamma attenuation induced by midazolam

Our observation of broad attenuation of theta activity (4.0–7.5 Hz) during the sedated state is consistent with the notion that likeliness of cortical activation and effective communication in distributed cortical areas are modulated by theta activity. Intracranial studies of animals and humans suggest that cortical activation reflected by high-gamma augmentation frequently coincides with the trough or peak of underlying theta oscillations during sensory, motor, and cognitive tasks; at the same time, theta oscillations become coherent across sites (Jacobs and Kahana, 2009; Axmacher et al., 2010; Canolty and Knight, 2010; Yanagisawa et al., 2012; Ito et al., 2013). The greater degree of midazolam-induced theta attenuation in the visual regions cannot be explained merely by the effects of potential eye closure during the sedated state, because alpha activity, which eye closure generally accentuates, was also attenuated together with theta activity in the visual region (Figure 5B).

Attenuation of high-gamma activity (64–116 Hz), though relatively brief and modest, was noted in the present study (Figure 4 and Figure 5D). Failure to observe a large effect size in high-gamma activity can be partly explained by the absence of assigned tasks before and after the administration of midazolam. Task-related high-gamma augmentation in functionally-important cortex has been reported to be a short-lasting phenomenon strictly locked to the timing of a task (Crone et al., 2006). We cannot rule out the possibility that under-sampling relative to the association neocortex may have resulted in failure to find more robust and sustaining alteration of ECoG amplitudes in some regions of interest including the medial-temporal region, which had only 22 non-epileptic sites eligible for analysis.

Low-gamma activity (32–56 Hz), sharing the dynamic changes similar to beta activity, was augmented following the administration of midazolam. Our observation is consistent with the results of a previous scalp study, reporting that loss of consciousness induced by propofol was associated with augmentation of EEG amplitude at 25–40 Hz (Murphy et al., 2011). Our observation also supports the notion that local cortical activation is reflected by high-gamma augmentation better than low-gamma augmentation (Lachaux et al., 2012; Kojima et al., 2013).

Methodological considerations

The presence of focal epilepsy does not infer that the rest of brain is all abnormal; the findings of motor and sensory-related ECoG changes are remarkably similar between healthy monkeys and humans with focal epilepsy (Fukuda et al., 2008; Ray et al., 2008; Lachaux et al., 2012). The consensus in the neuroscience community is that intracranial ECoG studies serve as essential brain mapping techniques and complement non-invasive neurophysiology studies (Crone et al., 2006; Lewis et al., 2013). To increase the

generalizability of our ECoG findings, we analyzed amplitude measures in the nonepileptic regions separately from those in the seizure onset and spiking zones. Indeed, the dynamic changes of ECoG amplitudes in the seizure onset zone were severely affected by the effects of interictal spike discharges (Figure 3C). The seizure onset sites were reported to generate task-related high-gamma augmentation less frequently than the remaining sites (Kojima et al., 2013). Thus, ECoG measures in the seizure onset zones cannot be used to make a generalizable conclusion. In the present study, conversely, we failed to find the objective evidence of such an unwanted effect of epileptiform discharges on ECoG signals sampled from nonepileptic regions (Figures 3A and 4A). Likewise, spiking zones (defined as non-seizure onset zones but still affected by interictal spikes) were apparently free from intermittent and steep augmentation of wideband activities (Figures 3B and 4B). Failure to visualize a noticeable effect of interictal epileptiform discharges on midazolam-induced ECoG changes within the spiking zone may be attributed to the smaller amplitude and rate of interictal epileptiform discharges compared to those in seizure onset zones (Hufnagel et al., 2000; Asano et al., 2003). We previously reported that the effect of interictal spike discharges on task-related high-gamma augmentation was confined to the seizure onset zone (Brown et al., 2012). Taken together, we believe that ECoG measures derived from the nonepileptic regions reasonably reflect those expected in the general population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

Midazolam multifocally augmented beta, sigma and low-delta activities.

Midazolam attenuated theta and high-gamma activities.

The effects of midazolam on ECoG oscillations differed across brain regions.

Figure 1. Snapshots of animation movie of ECoG amplitude changes in Patient #1

Sequential changes in ECoG amplitudes at eight spectral frequency bands are presented. The amplitudes of beta, sigma, and low-gamma activities were increased, particularly in the association neocortices (red arrows). Conversely, theta activity was attenuated. L.: Low. H.: High. Video S1 presents the dynamic changes of ECoG amplitudes on the medial, lateral, and inferior surfaces at -20 to +300 seconds.

Figure 2. Snapshots of animation movie of ECoG amplitude changes in Patient #2

Sequential changes in ECoG amplitudes at eight spectral frequency bands are presented. The amplitudes of sigma, beta, and alpha activities were increased, particularly in the association neocortices (red arrows). Low-delta activity was intermittently augmented in the somatosensory-motor cortex (yellow arrow). Theta activity was rather attenuated. Video S2 presents the dynamic changes of ECoG amplitudes on the medial, lateral, and inferior surfaces at -20 to +300 seconds.

Figure 3. ECoG amplitude changes at nonepileptic, spiking, and seizure-onset regions
 Using an eight-second smoothing, the sequential changes of ECoG amplitudes at eight spectral frequency bands are presented from -10 to $+280$ seconds relative to the onset of midazolam injection. It should be noted that application of an eight-second smoothing made the peak and trough of amplitude alteration less sharp, whereas all statistical analyses were performed on raw ECoG amplitude measures without smoothing. Light-green line: low-delta amplitude. Gray: high-delta. Blue: theta. Orange: alpha. Pink: sigma. Red: beta. Brown: low-gamma. Black: high-gamma. (A) Y-axis: Grand average amplitude measures within the

entire nonepileptic regions. The amplitude of beta activity was augmented by >0.4 (i.e.: $>40\%$ compared to the control period between -20 and 0 seconds). (B) Within the entire spiking regions (spiking sites defined as non-seizure onset sites but still affected by interictal spikes). (C) Within the entire seizure onset regions. Large interictal spikes within the seizure onset regions intermittently contaminated the dynamic changes of midazolam-induced ECoG changes, as reflected by steep augmentation of wideband activities at $+25$, $+65$, $+90$, $+110$, and $+140$ seconds. Such unwanted effects of interictal spike discharges were not visible within the nonepileptic or spiking regions.

Figure 4. Variance of ECoG amplitudes at nonpileptic, spiking, and seizure-onset regions
 Variance of ECoG amplitudes (Y-axis) within a given 20-second time window (X-axis) is plotted for each spectral frequency band. (A) Grand average variance measures within the entire nonpileptic regions. (B) Within the entire spiking regions. (C) Within the entire seizure onset regions.

Figure 5. ECoG changes at five distinct nonepileptic regions

The sequential changes of ECoG amplitudes derived from the nonepileptic regions are presented, using an eight-second smoothing. (A) Y-axis: Grand average amplitude measures within the somatosensory-motor regions. (B) Within the visual regions. (C) Within the auditory regions. (D) Within the medial-temporal regions. (E) Within the association neocortex.

Table 1

Patient profiles

Patient	Age at surgery (year and months)	Gender	Midazolam dose (mg)	Midazolam dose (mg/kg)	Seizure onset zone	Pathology
1	1y 10m	Female	1	0.069	Rt T	Dysplasia
2	4y 0 m	Female	2	0.062	Rt T	Gliosis alone
3	8y 5m	Male	2	0.05	Rt T	Dysplasia associated with Tumor
4	11y 11m	Female	2	0.039	Lt TPO	Dysplasia
5	12y 0m	Female	2	0.049	Lt FP	Dysplasia
6	12y 11m	Male	2	0.032	Lt P	Dysplasia
7	13y 8m	Female	4	0.05	Rt P	Dysplasia
8	15y 10m	Male	4	0.06	Rt F*	Dysplasia
9	17y 5m	Female	2	0.036	Lt T	Dysplasia
10	17y 10m	Male	4	0.056	Lt F	Dysplasia

* : Only non-habitual seizures were captured.

Lt: left. Rt: right. F: frontal. T: temporal. P: parietal. O: occipital.