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Intracranial electroencephalography features of young and old mice under midazolam administration

Yue Liu[a]*, Quanyong Yang[b,]* and Yiqing Yin[b]

Understanding the electroencephalography features of young and old patients treated with anesthetic drugs is important to allow accurate drug use in elderly patients. This study aimed to monitor the intracranial electroencephalography (in the cortex and hippocampus) in free-moving young and old mice under midazolam administration. Behavioral assessment revealed that compared with young mice, old mice had a longer immobility time with a similar midazolam dose. In both young and old mice, midazolam significantly suppressed the total, δ (0.5–4 Hz), θ (4–8 Hz), and α (8–12 Hz) power, and thus induced an increase in the relative β (12–30 Hz) and γ (30–140 Hz) power. Age had a main effect on the γ frequency; specifically, under normal conditions, old mice had a lower γ power than young mice. After midazolam administration, the relative power of high γ frequency (50–140 Hz) remained lower in old mice than in young mice. Our findings suggest that a lower γ power is indicative of an aging brain. ***NeuroReport*** **32: 1192–1197 Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

With the rising worldwide life expectancy, an increasing number of old patients will receive anesthetics for sedation and surgery. Since older patients are more sensitive to anesthetics than young patients, they require more attention and proper assessment of their sedation status [1,2].

Electroencephalograms can be used to monitor the anesthetic depth; however, electroencephalography features are significantly affected by age and the choice of anesthetic drugs [2]. Although numerous commercial electroencephalography-derived indices are currently available [3], they are not adjusted for age or drugs; moreover, it remains unclear whether electroencephalography-guided anesthesia can reduce postoperative delirium (POD) [4,5]. Therefore, there is a need to further examine the electroencephalography features of the old patients.

Benzodiazepines are widely used for sedation and anesthesia. However, benzodiazepine use in the old population is more likely to cause POD and other neurological adverse effects [6,7]. Midazolam is a fast-acting and short-lasting benzodiazepine that is widely used for ICU sedation and intraoperative anesthesia. This study aimed to monitor intracranial electroencephalography features in free-moving young and old mice under midazolam administration. Specifically, we aimed to analyze the electroencephalography spectrum in the cortex and hippocampus of young and old mice. Moreover, we explored the correlations between their electroencephalography features and behavioral activity. This study could facilitate our understanding of the brain state of elderly patients, as well as the midazolam effect on electroencephalography features.

Methods

Animals

We purchased 3- to 5-month-old (young) and 24- to 28-month-old (old) C57BL/6 N mice from Vital River Laboratories Animal Technology Co. Ltd. [Permit Number: SCXK (JING) 2012–0001; Beijing, China]. The animals were housed in a vivarium maintained at 22–23°C with a 12-h light on/off cycle. Food and water were available *ad libitum*. All experimental procedures and protocols were approved by the Experimental Animal Ethics Committee of the Academy of Military Medical Science of China (approval No. NBCDSER-IACUC-2015128) according to the United States National Institutes of Health Guideline for the Care and Use of Laboratory Animals.

Implantation of intracranial electroencephalography electrodes

Electrode implantation and recording were performed as previously described [8]. The electrodes were made

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of polyimide-insulated stainless-steel wires (outside diameter: 0.2 mm, Plastics One, Roanoke, Virginia, USA). The electrodes were implanted in the right hippocampal CA1 area (bregma, −2.3 mm; lateral, 2.0 mm; and depth, 2.0 mm) and left parietal cortex (bregma, −0.6 mm; lateral, 1.5 mm; and depth, 1 mm). A reference electrode was placed into the right rostral brain (bregma, +1.2 mm; lateral, 2.0 mm; and depth, 0.5 mm). The animals were allowed to recover for ≥7 days before further experiments. Subsequently, the implanted electrode locations were verified through histological brain assessments, as previously described [8].

Midazolam administration

Midazolam was obtained in an injectable form (Sandoz Canada Inc., Boucherville, Quebec, Canada). Mice received intraperitoneal injections of 0.2 mL saline and midazolam at doses of 0.5, 1, 2, and 4 mg/kg (diluted in 0.2 mL saline) successively. Different midazolam doses were tested at a 3-day interval to allow sufficient between-test recovery and to minimize potential acute tolerance [9].

Intracranial electroencephalography recording

Electroencephalography signals obtained during baseline immobility were used as control data since the animals were mostly immobile after midazolam injections. Further, we analyzed electroencephalography signal changes collected 10–40 min after midazolam injections and compared them between young and old mice to minimize the effect of age-dependent differences in midazolam metabolism.

A dual-channel amplifier with extended head stages (Model-300; AM Systems Inc., Carlsborg, Washington, USA) was used to obtain electroencephalography recordings. Signals were collected within a 0.1–1000 Hz frequency band, amplified 1000 times, and digitized at ≥5 kHz (Digidata, 1300; Molecular Devices, Sunnyvale, California, USA). Spectral analysis was performed using pCLAMP software (version 10; Molecular Devices). The frequency spectrum was divided into δ (0.5–4 Hz), θ (4–8 Hz), α (8–12 Hz), β (12–30 Hz), low γ (30–50 Hz), and high γ (50–140 Hz) frequency bands. Moreover, the absolute power (mV^2) was defined as the total of the median power values for each frequency band.

Behavioral assessment

After saline or midazolam injection, the animal's behavior was monitored using a webcam (Logitech C525). A blinded researcher assessed the video data. The cumulative length of immobility time (with each immobile episode ≥10 s) within 10–40 postinjection minutes was recorded.

Statistical analysis

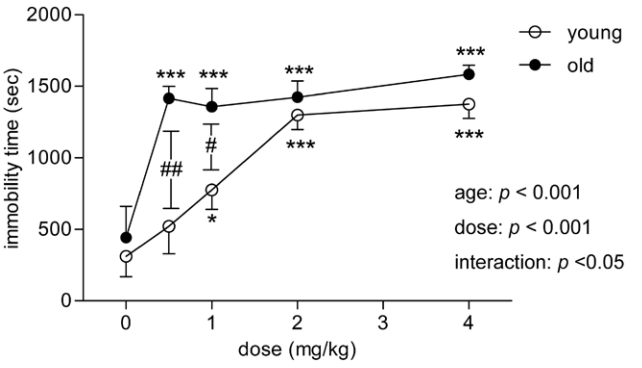
All statistical analyses were performed using the GraphPad Prism 6 software. Quantitative variables are expressed as the means ± SEM. An unpaired Student's *t*-test with Welch's correction was used for between-group comparisons of the average power of each frequency band. To examine the effect of midazolam dose and age on the absolute power of each band, we used two-way repeated-measures analysis of variance (TW-RM ANOVA) with the midazolam doses as repeated measures. Within-group comparisons were performed using the Bonferroni post-test. A P value <0.05 was considered statistically significant.

Results

There was no between-group difference in the immobility time under the baseline condition. After being given midazolam, all the mice were inactive (Fig. 1). TW-RM ANOVA revealed that the immobility time was affected by the midazolam dose ($P<0.001$) and age ($P<0.001$). There was a positive relationship between the midazolam dose and the stationary duration of the young mice. Specifically, in young mice, only a dose >1 mg/kg could significantly prolong the immobility time compared with the baseline. In the old mice, 0.5 mg/kg of midazolam could extend their immobility time. However, further increases in the doses did not extend the immobility time further. The effect of 0.5 mg/kg and 1 mg/kg midazolam on immobility time was significantly stronger in old mice than in young mice. This suggested that the old mice were more sensitive to midazolam than the young mice.

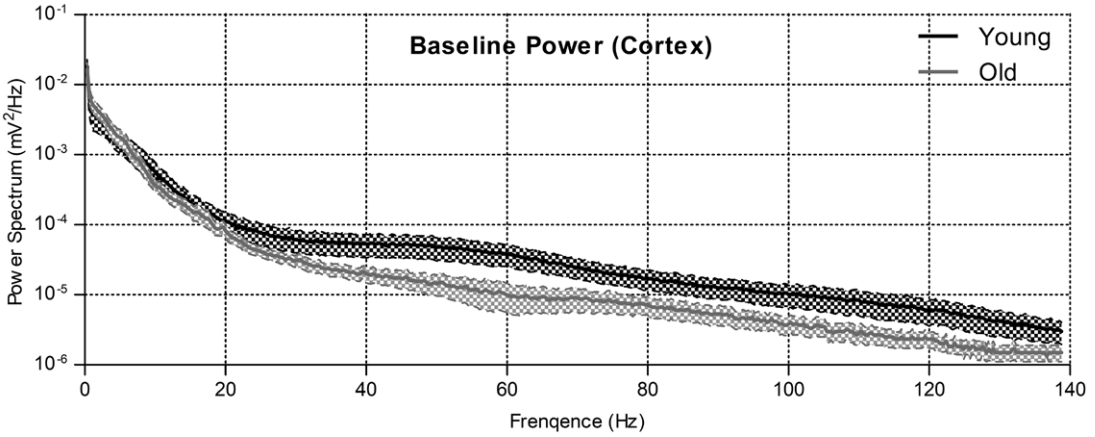
To determine the electroencephalography signal features in young and old mice after midazolam injection, we first analyzed the baseline power spectra of electroencephalography signals in the cortex and hippocampus in young and old mice (Fig. 2, Supplemental Figure

Fig. 1



The immobility time of young and old mice after receiving different midazolam doses. Young mice ($n=8$); old mice ($n=6$). Data are shown as mean ± standard error of the mean. Two-way repeated-measures analysis of variance for age and dose interaction: the effect of age, $P<0.001$; the effect of midazolam dose, $P<0.001$; interaction, $P<0.05$. Bonferroni post hoc analysis: *$P<0.05$, ***$P<0.001$, compared with baseline; #$P<0.05$, ##$P<0.01$, comparison between young and old mice receiving the same drug dose.

Fig. 2



Power spectra in the cortex of young and old mice under baseline condition. The shaded area between the dotted lines indicates the mean ± standard error of the mean.

1, Supplemental digital content 1, *http://links.lww.com/WNR/A648*). We calculated the average power of each frequency band, and no significant between-group difference in the δ, θ, α, and β frequencies were observed in both the cortex and the hippocampus region (Table 1). However, in the cortex, there was a moderate, nonsignificant decline in the low γ (30–50 Hz) power ($P = 0.0818$), and a significant decrease of high γ (50–140 Hz) power of the old mice compared with the young mice ($P = 0.0393$, Table 1). Moreover, there was a trend that the γ power of the hippocampus, especially the high γ power ($P = 0.0551$), was lower in old mice than in young mice (Table 1). These results indicated that, under baseline conditions, the old mice had a decreased high γ power than the young mice.

We further analyzed the intracranial electroencephalographs of young and old mice under midazolam administration. Figure 3 shows the absolute power of each frequency band in the cortex of young and old mice under different midazolam doses. There was a negative relationship between the midazolam dose and the total power of electroencephalography signals (Fig. 3a), with the power of the δ, θ, and α frequency bands decreasing more significantly (Fig. 3b–d). TW-RM ANOVA revealed a marked effect of midazolam dose on the δ, θ, α, and total power. The high γ power was significantly suppressed by 2 mg/kg midazolam in young, but not old, mice (Fig. 3g).

Further, we examined the relative power of each band, which is defined as the proportion of each frequency power in the total electroencephalography power. In all the mice, midazolam administration significantly increased the relative β and γ power in the cortex (Fig. 4). Additionally, age had a significant effect on the

Table 1. The average power spectra of each frequency band of young and old mice under baseline condition

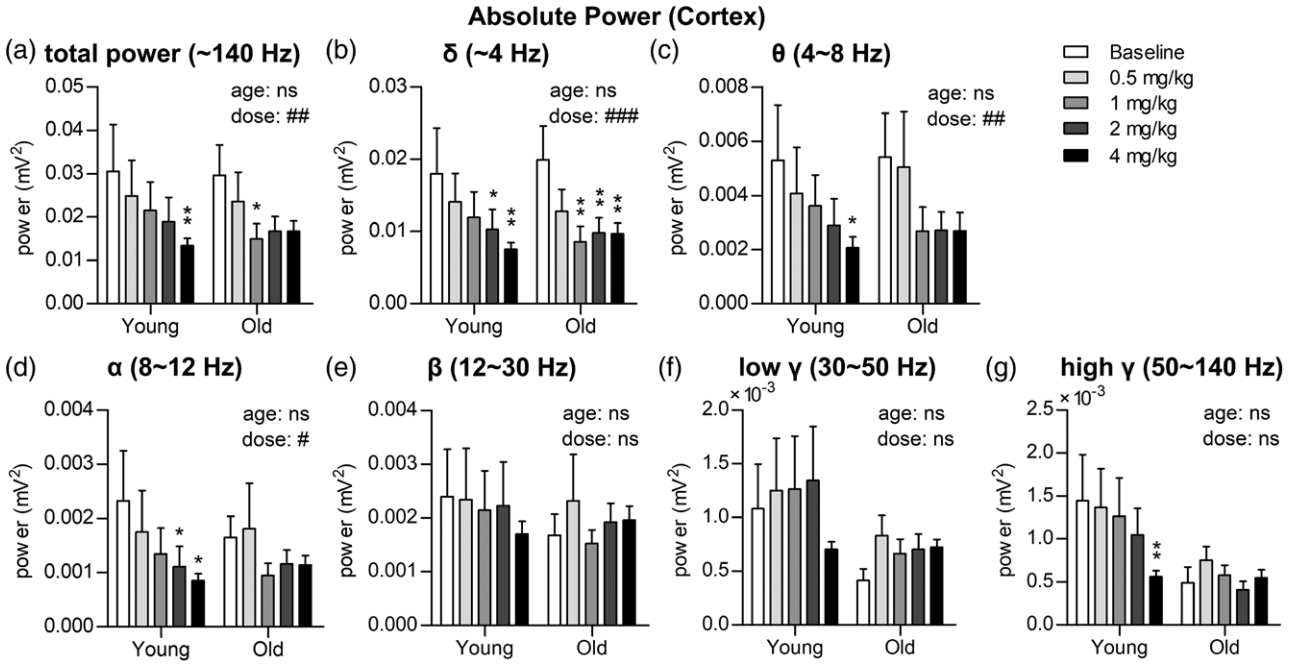
	Cortex		Hippocampus	
Frequency band	Young ($n=8$)	Old ($n=6$)	Young ($n=8$)	Old ($n=6$)
δ ($\times10^{-3}$)	4.50 ± 1.58	4.99 ± 1.16	4.56 ± 1.57	4.22 ± 1.31
θ ($\times10^{-3}$)	1.32 ± 0.51	1.36 ± 0.40	1.51 ± 0.50	1.78 ± 0.59
α ($\times10^{-4}$)	5.82 ± 2.31	4.11 ± 0.99	6.69 ± 2.31	5.30 ± 1.75
β ($\times10^{-4}$)	1.33 ± 0.49	0.93 ± 0.22	1.45 ± 0.49	1.19 ± 0.42
Low γ ($\times10^{-5}$)	5.40 ± 2.08	2.06 ± 0.54 ($P=0.0818$)	5.52 ± 2.09	2.35 ± 0.80 ($P=0.0967$)
High γ ($\times10^{-5}$)	1.61 ± 0.50	0.54 ± 0.21 ($P=0.0393$)	1.65 ± 0.59	0.54 ± 0.19 ($P=0.0551$)

The data are shown as the means ± SEM. To compare between the young and old group, the *P* value was calculated using an unpaired Student's *t*-test with Welch's correction.
δ, 0.5–4 Hz; θ, 4–8 Hz; α, 8–12 Hz; β, 12–30 Hz; low γ, 30–50 Hz; high γ, 50–140 Hz.

relative power of high γ frequency ($P < 0.001$, by TW-RM-ANOVA). Compared with young mice, old mice had a lower relative γ power. Notably, midazolam and age had a similar effect on the relative power of hippocampal and cortical electroencephalography signals (Supplemental Figures 2 and 3, Supplemental digital content 1, *http://links.lww.com/WNR/A648*).

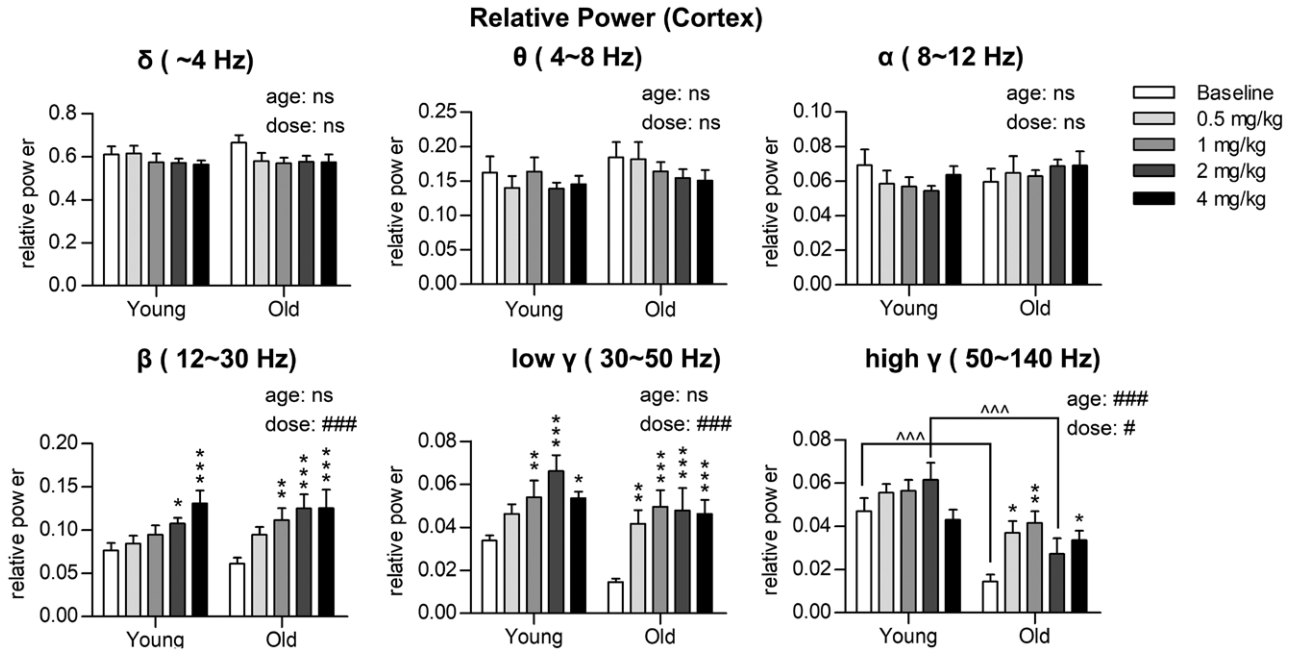
Taken together, the main effect of midazolam on electroencephalography signals was suppressing the power of δ, θ, and α frequencies, which lowered the total power. Moreover, midazolam administration resulted in a dose-dependent increase in the relative β and γ power. These midazolam effects were similar among the old and young mice. However, behavior assessment revealed that the old mice were more sensitive to midazolam than the young mice; further, they tended to exhibit a longer immobility time with a low midazolam dose. Regarding electroencephalography analysis, the old mice had a

Fig. 3



The power of each frequency band in the cortex of young and old mice. Young mice (n=8); old mice (n=6). Data are shown as mean±standard error of the mean. Two-way repeated-measures analysis of variance was used to compare the effect of dose and age. The results are shown on the upper right of each graph: ns, no significance, #P<0.05, ##P<0.01, ###P<0.001. Bonferroni post hoc analysis: *P<0.05, **P<0.01, compared with baseline.

Fig. 4



The relative power of each frequency band in the cortex of young and old mice. Young mice (n=8); old mice (n=6). Data are shown as mean±standard error of the mean. Two-way repeated-measures analysis of variance was used to compare the effect of dose and age. The results are shown on the upper right of each graph: ns, no significance, #P<0.05, ##P<0.01, ###P<0.001. Bonferroni post hoc analysis: *P<0.05, **P<0.01, compared with baseline (saline infusion); ^^^P<0.001, compared with young mice under a similar drug dose.

lower high γ frequency power under baseline conditions; moreover, even with midazolam administration, the relative power of high γ frequency was significantly lower in the old mice than in the young mice.

Discussion

In this study, we measured the electroencephalography signals in the mouse cortex and hippocampus. Midazolam dose-dependently suppressed the total power, as well as the δ, θ, and α power, and thus induced an increase in the relative β and γ powers, in both the cortex and hippocampus. The old mice were more sensitive to midazolam than young mice; moreover, under normal conditions, the old mice had a lower γ power than the young mice. Furthermore, after given midazolam, the relative power of high γ frequency in the old mice remained lower than that in the young mice.

There have been human studies on the midazolam effects on the electroencephalography features mainly using scalp electroencephalography recording technique. Commonly reported findings to include a lower background electroencephalography activity, with an accompanying increase and decrease in β and α activity, respectively. These changes have been reported in young healthy volunteers [10–13], patients with epilepsy [14,15], and elderly patients [16,17]. Consistent with these findings, we found that in both young and old mice, midazolam dose-dependently suppressed the total power and α frequency of the electroencephalography signals. Moreover, it increased the relative power of β frequency.

Under baseline conditions, we observed a lower trend of γ power in the old mice than in the young mice. The γ frequency is indicative of neural communication [18]; however, it is difficult to measure in humans using scalp electrodes given its low power compared with other frequencies and its proneness to interference by the electromyographic signals [19,20]. Therefore, the in-vivo intracranial electroencephalography monitor technique is more reliable for γ frequency analysis. Studies in experimental animals and human patients with intracranial electroencephalography monitoring for neurosurgery have reported that the γ frequency, especially the high γ frequency (>44 Hz), increases with memory formation and learning [21–23]. Compared with young rats, aging rats have reduced 40–70 Hz γ rhythm in the anterior cingulate cortex [24], which is consistent with our results. Furthermore, rats with lower γ power have been found to move more slowly [24]. Although we did not observe a significant reduction of behavioral activity under normal conditions in old mice, we suspect that the lower γ power may be indicative of the aging brain.

Aging individuals are more sensitive to anesthetics than younger individuals. To achieve the same anesthetic state, elderly patients have been reported to require much lower doses of anesthetic drugs than younger patients [1]. Similarly, we found that 0.5 mg/kg midazolam could significantly inhibit activity in the old, but not young, mice. Compared with young patients, elderly patients have been reported to show a greater decrease in the relative α power during propofol and sevoflurane anesthesia [25]. However, there have been few studies comparing electroencephalography signatures between young and old participants under midazolam sedation. In our study, there was no difference in the midazolam effect on the α and β frequencies between the young and old mice, which may not explain the susceptibility of old mice to midazolam. Nevertheless, we found a significant age effect on the relative power of high γ frequency, which was lower in old mice than in young mice. Given the lower baseline γ power in the old mice, we suspect that the lower γ frequency may be indicative of an aging brain and more sensitivity to midazolam.

This study has several limitations. The behavior assessment was relatively simple, and cognitive function studies may more accurately evaluate the midazolam effects. Smaller doses of midazolam, for example, 0.25 mg/kg or 0.125 mg/kg, should be studied in the old mice since only 0.5 mg/kg midazolam could cause a peak effect on the immobility time in old mice. Further, there is a need for studies with larger sample sizes to achieve statistical significance and stronger results.

In conclusion, midazolam can inhibit the power of δ, θ, and α; moreover, it can increase the relative β and γ power in both young and old mice. Under baseline conditions, old mice had a lower γ power than young mice. Further, under midazolam administration, the relative power of high γ frequency remained lower in old mice. A lower γ power may indicate a more sensitive state of a mouse to midazolam. Given the unreliability of γ frequency recording during human scalp electroencephalography measurements, the importance of the γ band may have been ignored. Further studies are required to explore the role of γ frequency in brain aging and the effects of anesthetic drugs.

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Conflicts of interest

There are no conflicts of interest.

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