Neuroscience of Consciousness, 2018, 4(1): niy002

doi: 10.1093/nc/niy002
Research article

Moderate sedation induced by general anaesthetics disrupts audio-spatial feature binding with sustained P3 components in healthy humans

Takehiro Minamoto[1,*], Takashi Ikeda[2], Hongling Kang[3], Hiroshi Ito[4], Piyasak Vitayaburanananont[5], Aya Nakae[6], Satoshi Hagihira[3], Yuji Fujino[3], Takashi Mashimo[7] and Mariko Osaka[1]

[1]Center for Information and Neural Networks, National Institute of Information and Communications Technology, Osaka, Japan; [2]Research Center for Child Mental Development, 13-1 Takaramachi, Kanazawa-shi, Ishikawa, 920-8640, Japan; [3]Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan; [4]Technology Standardization Department, 1-31-4 Nishiochiai, Shinjuku-ku, Tokyo 161-8560, Japan; [5]Faculty of Medicine, Bangkok Metropolitan Administration Medical College and Vajira Hospital, Mahidol University 681 Samsen Rd, Vajiraphayaban, Dusit, Bangkok 10300, Thailand; [6]WPI Immunology Frontier Research Center, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan; [7]Toyonaka Municipal Hospital, 4-14-1 Shibahara, Toyonaka, Osaka 560-8565, Japan

*Correspondence address. Center for Information and Neural Networks, National Institute of Information and Communications Technology, 1-4 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel: +81-80-9098-3299; Fax: +81-6-6879-4687; E-mail: txminamoto@nict.go.jp

Abstract

Feature binding is considered to be the basis for conscious stimulus perception, while anaesthetics exert a gradient effect on the loss of consciousness (LOC). By integrating these two streams of research, the present study assessed the effect of two anaesthetic agents (i.e. propofol and midazolam) on audio-spatial feature binding. We also recorded the electrophysiological activity of the frontal channels. Using pharmacokinetic simulation, we determined the effect-site concentration (Ce) of the anaesthetics at loss of response to verbal command and eyelash reflex. We subsequently adjusted Ce to 75%, 50% and 25% of Ce-LOC to achieve deep, moderate and light sedation, respectively. Behavioural results showed that moderate sedation selectively disrupted feature binding. The frontal channels showed a P3 component (350–600 ms peristimulus period) following the presentation of audio-spatial stimuli at baseline and under moderate and light sedations. Critically, the late event-related potential component (600–1000 ms) returned to the pre-activated level (0–350 ms) at baseline and under light sedation but was sustained under moderate sedation. We propose that audio-spatial feature binding may require the presence of a P3 component and its subsequent and sufficient decline, as under anaesthetic-induced moderate sedation the P3 component was sustained and featured binding was impaired.

Key words: feature binding; P3; oscillation; propofol; midazolam

Received: 22 June 2017; **Revised:** 25 January 2018; **Accepted:** 29 January 2018

Highlights

- Effect of two anaesthetic agents (propofol and midazolam) on audio-spatial feature binding was assessed.
- Moderate sedation induced by general anaesthetics selectively disrupted feature binding.
- Irrespective of sedative levels, frontal channels showed P3 component in response to audio-spatial stimuli.
- The late event-related potential component did not decline to the pre-activated level under moderate sedation.
- Audio-spatial feature binding requires a P3 component with its subsequent and sufficient decline.

Introduction

Consciousness is a multifaceted construct and ranges from awareness of one's perceptions and sensation to self-awareness. Among the several properties of consciousness, feature binding refers to how our brain integrates the different properties of an object into one coherent construct, which is subsequently used for interpretation of the environment (Singer 2001). Therefore, feature binding is an essential precondition and a critical component for the emergence of a conscious stimulus precept. Feature binding has been well studied in the visual domain (e.g. Kahneman *et al.* 1992), but the same mechanism has been reported in the auditory (e.g. Hall *et al.* 2000) and multimodal domains (e.g. Mayr *et al.* 2011).

According to Zimmer *et al.* (2006), there are several types of binding, including binding of features within object tokens, between-object binding, relational binding and larger-unit binding, such as of scenes and events. In the present study, the first two types (i.e. binding features within object tokens and between-object binding) were our primary targets because we wished to focus on feature binding in working memory. Specifically, audio-spatial binding in working memory requires binding features within object tokens, because encoding of target stimuli requires object recognition, which demands short-term episodic representation of objects (Kanwisher 1987). Additionally, as working memory is typically required to maintain multiple items, between-item binding is needed (Zimmer *et al.* 2006).

Another stream of study concerning consciousness is loss of consciousness (LOC) induced by general anaesthesia (Hemmings *et al.* 2005; Alkire *et al.* 2008; Franks 2008; Brown *et al.* 2011; Purdon *et al.* 2015). Several theories have been proposed for the mechanism involved in LOC induction, including reduction of the thalamic metabolism, deactivation of the neocortex and disruption of cortical integration and cortical information capacity (Alkire *et al.* 2008; Changeux 2012). Recent human and rodent studies have shown that general anaesthesia induces LOC by disrupting cortical connectivity across regions, such as the feedback connectivity from the prefrontal to the posterior parietal cortex (Ferrarelli *et al.* 2010; Ku *et al.* 2011; Lewis *et al.* 2012; Lee *et al.* 2013).

Experimentally modified pharmacological disruptions of the bound representation would allow studying the neural mechanisms of consciousness by measuring the neural state during the disruption phase. In the present study, using a pharmacokinetic simulation procedure, we controlled the concentration of general anaesthetic agents (i.e. propofol and midazolam) in the brain to experimentally disrupt audio-spatial binding in working memory and performed electroencephalogram (EEG) recordings of the frontal channels, which appear to be involved in the thalamo-cortical loop or fronto-parietal connectivity (Ching *et al.* 2010; Ku *et al.* 2011; Supp *et al.* 2011).

According to the cognitive unbinding theory, consciousness is built on synthesis of cortical modules by top-down feedback processing, which depends on the prefrontal cortex and fronto-parietal network (Mashour 2004, 2013). As the prefrontal cortex is known to play a critical role in coordinating brain modules by biasing the activity of those modules in accordance with a given goal (Desimone and Duncan 1995), it is reasonable to hypothesize that integration of different features, which depends on distinctive cognitive modules, is impaired due to dysfunction of the prefrontal cortex by general anaesthetic agents. Accordingly, the present study investigated the effect of general anaesthetic agents on the electrophysiological activity of the frontal sites during anaesthesia-induced mild sedation. Especially, we were interested in the P3 component of the brain event-related potential (ERP). The ERP is known to occur 300–600 ms following the presentation of a target stimulus in the odd-ball task and has been proposed to play a significant role in updating the cognitive representation of the environment (Donchin and Coles 1988). We were particularly interested in the component because our task demanded continuous updating of feature binding. Hence, we predicted that general anaesthesia affects the P3, which impairs efficient updating of audio-spatial binding.

In addition to the ERP amplitude, we performed preliminary analysis for oscillatory power (see Supplementary Materials) because feature binding is known to depend on neural oscillations. Initially, gamma-band oscillations in the visual perpetual regions were shown to be the primary neural mechanism for perceptual feature binding (Singer 2001). However, recent studies have argued that gamma-band oscillations are not specific to feature binding but are the fundamentals for cortical computation that mediates the interplay between neuronal dynamics and structural neuronal connectivity (Fries 2009). Other studies have revealed significant roles of the beta- and alpha-band oscillations, which provide a basis for multiple feature binding by forming large-scale neural networks. Especially, object representation in working memory is proposed to depend on simultaneous oscillation across alpha-, beta- and gamma-band oscillations (Palva and Palva 2007, 2011). Thereafter, coordination across multiple frequency bands is likely to allow for bound object representations by integrating multiple brain regions, which results in highlighting goal-related neural representations, especially in working memory. As the anaesthetised LOC is reported to accompany increases in alpha-/beta-band oscillations in the frontal cortex (Purdon *et al.* 2013), we hypothesized that general anaesthesia would increase alpha-/beta-band oscillations, which are associated with disruption of feature binding.

Materials and Methods

Participants

Twenty healthy men participated in the study [mean age = 22.93 years, standard deviation (SD) = 3.13 years]. The participants were recruited through local and on-campus advertisements, and their participation was voluntary. All participants

reported no history of neurological disorders prior to the experiment. We received document-based informed consent from each participant after giving detailed explanation of the study. The study protocol was approved by the ethical committee board of Osaka University Medical Hospital. Participants received a monetary reward as compensation for the 3-day session.

Drug administration

Participants were randomly assigned to either the propofol or the midazolam group, and they received the respective drug through intravenous administration. We used two agents to avoid a drug-specific effect. Both drugs are known to act on the $GABA_A$ receptor (Changeux 2012) and to reduce the metabolic rate throughout the brain (Veselis *et al.* 1997; Alkire *et al.* 1998). Target-controlled infusion was employed to maintain the drug concentration. Before the start of the study, we placed an intravenous catheter on the left forearm. In the propofol group, the drug was infused by a target-controlled infusion pump (Terufusion Syringe Pump TE-371, Terumo Co., Tokyo, Japan), which can maintain target blood concentration (Cb) based on population pharmacokinetics (Gray and Kenny 1998). This pump can calculate the putative effect-site (brain) concentration (Ce) as well as the Cb of propofol. In the midazolam group, the drug was infused by a standard infusion pump (Terufusion Syringe Pump TE-332 S, Terumo Co). We manipulated the midazolam infusion speed by referring to the results of a pharmacokinetic simulation with the TIVAtrainer software (Ver.8, EuroSIVA), which could also calculate both Ce and Cb.

Subsequently, we gradually increased Ce by manipulating the infusion speed and determined the Ce when a participant failed to respond to verbal commands and to show eyelid-closure reflexes [Observer's Assessment of Alertness/Sedation (OAA/S) score = 1]. We defined this Ce as Ce at loss of response (Ce_LOR). The Ce was adjusted to 3/4, 2/4 and 1/4 of the Ce_LOR to achieve deep, moderate and light sedation, respectively. Specific values (mean and SD) of Ce and bispectral index are available in Kang *et al.* (2017), where the present experiment was conducted as a part of the study. We use the term 'LOR' strictly here because participants may not have been able to respond but could have been conscious. However, 'LOR' is believed to be mostly equivalent to 'LOC'. We manipulated the infusion pump to keep the aimed Ce constant during the experiment at each level of sedation.

Experimental task

An audio-spatial working memory task was administered both during the drug-free baseline period and while the participants were anaesthetized. Figure 1 illustrates a trial sequence of the task. Two auditory stimuli were presented consecutively from speakers located on the left and right sides of a surgical bed on which the participants laid. We used auditory stimuli of three frequencies (600 Hz, 1200 Hz and 2400 Hz), which were easily discriminable, and their sound level was adjusted for each participant (i.e. 72 dB for most participants, and slightly above or below for others; specific values were missing during the process of data analysis). Each trial began with a human-voice start cue that made it easier for participants to identify the ending of the previous trial. Following the cue, two memoranda were presented. Each memorandum was presented for 1000 ms with a 1000-ms inter-stimulus interval. Participants were instructed to remember both the pitch and spatial location of each memorandum. A delay of 2000 ms was inserted before the presentation of

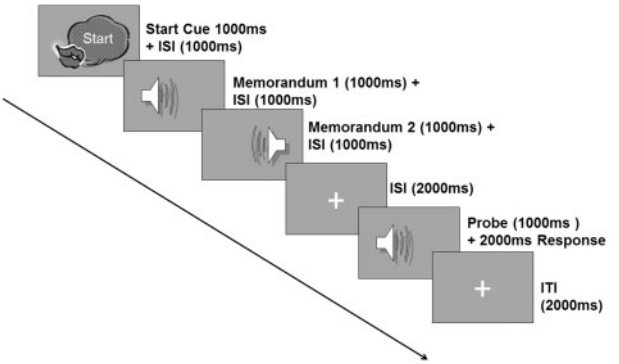

Figure 1. A schematic diagram of the audio-spatial working memory task. Two auditory stimuli were consecutively presented following a cue stimulus. The stimuli were presented for 1000 ms from either a left or a right speaker. Participants were required to remember two features of the stimuli (frequency and spatial location). A probe stimulus was presented 2000 ms after completion of the initial stimulus, during which the participant was asked if it was the same or different from one of the previous stimuli. The probe stimulus was either an identical item to the memorandum, an item whose features were switched across two memoranda, or a novel item in a given trial.

a probe stimulus. After the probe stimulus was presented for 1000 ms, participants were instructed to judge whether the probe stimulus was identical to the one they had just remembered. A match response was requested only when the probe stimulus had identical auditory and spatial properties to one of the two memoranda ('same' condition). Non-match responses were requested when the auditory and spatial properties of the probe stimulus were switched across two memoranda (e.g. auditory property of the first memorandum and spatial property of the second one; 'switch' condition), or when the sound frequency of the probe stimulus was different from those in the memoranda ('new' condition). Responses were retrieved by a subject response pad (Cambridge Research Systems Ltd., Kent, UK). A participant pressed a right button with the middle finger of the right hand for the same judgment and the point finger for a different judgment. The task consisted of 32 trials; half of the trials involved the 'same' condition, one quarter the 'switch' condition and the final quarter the 'new' condition. Therefore, the response ratios for match and non-match trials were equal. The order of the experimental conditions was randomized with a restriction that specific conditions not be repeated more than five consecutive times. If anaesthetic agents disrupt audio-spatial binding, accuracy in the 'switch' condition should be most impaired because weakly bound representation is thought to be more vulnerable to a confusing stimulus. Accuracy in the new condition should be unaffected, as it did not require bound representation to achieve a correct response. Accuracy in the 'same' condition should be impaired but not as much as in the 'switch' condition because an identical stimulus was presented a few seconds before. Reactivation of the identical memory trace was predicted to prevent dramatic decrease in accuracy. The methods allow us to capture the neural characteristics required for feature binding, by measuring brain activity when features are weakly bound.

Procedure

The study consisted of three sessions. In the first session, participants visited the laboratory for a medical check-up 1 week

prior to the anaesthesia experiment. They were also administered several psychometric batteries as well as pain sensation tasks.

In the second session, participants performed cognitive and nociception tasks under anaesthesia. The experiment was conducted in an operating room at Osaka University Medical Hospital. In the operating room, participants were instructed to lay on a surgical bed, and electrodes and other instruments for pulse monitoring were attached. Prior to the drug administration, we measured baseline task performance and electrophysiological activity. Participants performed the auditory-spatial working memory task, two verbal memory tasks and two pain sensation tasks. Results of the other tasks will be reported elsewhere. The auditory-spatial working memory task was always administered second in the series of tasks. Following the baseline measurement, drugs were administered with manipulating their concentration as described above, and task performance was measured under three sedative phases (i.e. deep, moderate and light). When all the tasks were performed, participants were moved to an examination room by wheelchair where they rested until their consciousness level was fully recovered.

The third session was held 1 week after the second session and consisted of participants receiving a follow-up medical check-up.

Electrophysiology measurement

All the recordings were performed at a temperature between 22 and 24°C. An EEG was recorded from electrodes placed on the Cz, Fz and F3, as referenced to linked earlobes. The earth electrode was placed on the nose bridge. An electrooculography electrode was placed near the right eye to detect unwanted blinking. We used the MEB-9400 EMG/EP system (NIHON KODEN Corporation, Tokyo, Japan) to record and analyse waveforms using a sensitivity of 20 μV/div, and a bandpass filter between 0.1 and 50 Hz. Impedance was tested at least twice during the experiment and was always kept below 5 kΩ.

Electrophysiological data were collected at a frequency of 500 Hz. ERPs started to be collected in response to a pulse signal delivered from a computer equipped with a stimulus presentation and pulse delivery software (Presentation, Neurobehavioral Systems, Inc., Albany, CA, USA).

Behavioural data analysis

Accuracy and reaction time were analysed using a three-way mixed analysis of variance (ANOVA) with factors of the drug type (two levels: propofol and midazolam), sedative phase (three levels: baseline, moderate and light) and task condition (three levels: 'same', 'switch' and 'new'). An alpha level of $P < 0.05$ was used as the statistical threshold. Shaffer's modified sequentially rejective Bonferroni procedure was performed for *post hoc* testing. Statistical analysis was performed using R (The R Foundation, Vienna, Austria).

Electrophysiology data analysis

ERP amplitude analysis

Electrophysiological data and associated log information were retrieved using NeuroNavi (NIHON KODEN Corporation, Tokyo, Japan), and baseline amplitude correction was performed using the same software. Further analysis was performed with the ERPLAB toolbox (http://erpinfo.org/erplab), integrated to EEGLAB (http://sccn.ucsd.edu/eeglab/), running on MATLAB (The MathWorks, Inc., Natick, MA, USA). As noted in the Results section, a large number of trial omissions were observed in the deep phase; therefore, we removed data in this phase from further analysis. We extracted the bin-based epoch ranging from −100 ms to 1000 ms for the first and second memorandum of each trial, and stimulus onset was defined as $t = 0$. The ERPs in response to a probe stimulus were not included in the analysis due to the small sample of trials in each experimental condition ('same' condition = 16 trials, 'switch' and 'new' conditions = 8 trials each, at each anaesthesia phase). Each bin-based epoch was coded according to the sedative phase (baseline, moderate and light) and behavioural outcome (correct, incorrect, or response omission). Artefact detection was applied with a moving window peak-to-peak threshold. Parameters for the detection were as follows: 100 μV for amplitude threshold, 200 ms for moving windows full width and 100 ms for window step. The ERPs over the threshold were removed from the analysis. The remaining ERPs were averaged at each condition (baseline, median and light) for each channel (Cz, Fz and F3). Only correct trials were included in the following statistical analysis ($M = 30.73$, $SD = 1.28$ for the baseline condition, $M = 24.2$, $SD = 5.23$ for the moderate anaesthesia and $M = 29.13$, $SD = 3.48$ for the light condition).

Time-frequency power analysis

Because time-frequency power analyses were performed for a preliminary purpose, we described the method in the Supplementary Text. All the data will be provided on request.

Results

Behavioural results

Participants showed a large number of trial omissions under the deep sedative state (median number = 13.9 of 32 trials, $SD = 9.94$) due to severe sedation. This number corresponds to 43% of the trials, suggesting that participants could not concentrate on the task. Therefore, we removed the behavioural and electrophysiological data in the deep phase from the analysis. For the remaining analysis, five participants (two from the propofol group and three from the midazolam group) were excluded due to a very low accuracy (more than 2 SDs away from the mean) or chance level performance in at least one of the three deep sedative phases. Thus, data from 15 participants were included in the statistical analysis.

Figure 2 (left panel) depicts the effect of sedation on the accuracy of the audio-spatial working memory task. In the 'same' condition, accuracy under sedation was lower than that at the baseline condition when collapsed across two drug agencies. More importantly, in the 'switch' condition, accuracy in the moderate condition was lower than that in the baseline and light conditions, when collapsed across the two drugs. A mixed ANOVA showed a significant interaction between factors of the sedative phase and experimental condition, $F(4, 52) = 2.83$, $P < 0.05$. Simple main effect analysis showed a significant main effect of sedation on the 'same' condition, $F(2, 26) = 5.73$, $P < 0.01$ and the 'switch' condition, $F(2, 26) = 9.58$, $P < 0.001$. *Post hoc* tests over the 'same' condition showed a significant difference between the baseline and moderate sedative phases ($P < 0.05$), while over the 'switch' condition significant differences between the baseline and moderate conditions ($P < 0.01$) and between the light and moderate conditions ($P < 0.05$) were observed. Although a significant main effect on the experimental condition was obtained under

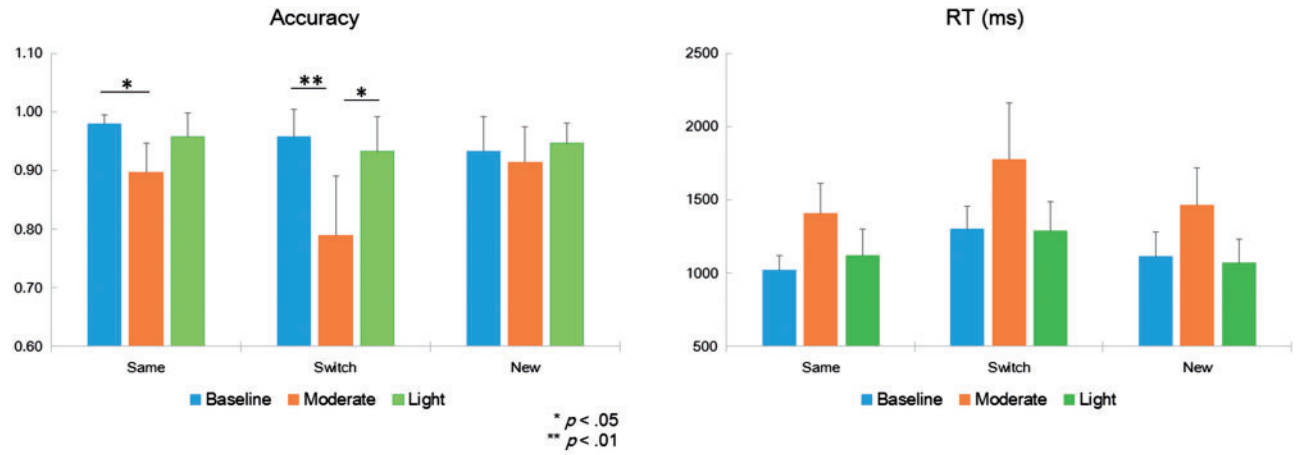

Figure 2. Performance of the audio-spatial working memory task under anaesthesia. Accuracy was modulated by the anaesthesia phase and trial type (left). Mean accuracy was lower under moderate sedation than at the drug-free baseline in the 'same' condition and for both baseline and light sedation in the 'switch' condition. The mean accuracy in the 'new' condition was equivalent across all three phases. Mean reaction time was slower under moderate sedation across the trial types, while mean reaction time in the 'switch' condition was slower across the sedative phases. Error bars represent 95% confidence interval.

moderate sedation, $F(2, 26) = 4.09$, $P < 0.05$, the other *post hoc* tests did not show significant differences across conditions ($P > 0.05$).

The mixed ANOVA also showed a significant interaction between the factors of the drug type and experimental condition, $F(2, 26) = 4.19$, $P < 0.05$, as summarized in Supplementary Table S1. The following simple main effect analysis showed a significant main effect of midazolam on the experimental conditions, $F(2, 12) = 5.17$, $P < 0.05$. However, *post hoc* testing did not detect significant differences in the accuracy across conditions ($P > 0.05$ for both).

The other finding was the main effect of the sedative phase, $F(2, 26) = 8.73$, $P < 0.005$, where accuracy in the moderate phase was lower than that in the baseline and light sedative phases ($P < 0.05$). Main effects were not significant either for the drug type, $F(1, 13) = 0.21$, $P > 0.05$, or for the experimental condition, $F(2, 26) = 2.60$, $P > 0.05$. A two-way interaction between factors of the drug type and sedative phase was not significant, $F(2, 26) = 0.01$, $P > 0.05$; similarly, a three-way interaction between factors of the drug type and sedative phase was not significant, $F(4, 52) = 0.47$, $P > 0.05$.

Reaction time results are also summarized in Fig. 2 (right) and Supplementary Table S1. Across the experimental conditions, the reaction time was slower under moderate sedation relative to the baseline and light sedation. Additionally, the reaction time in the 'switch' condition was slower than that in the 'same' and 'new' conditions across sedative phases. A mixed ANOVA showed a significant main effect of the sedative phase, $F(2, 26) = 23.77$, $P < 0.001$, and of the experimental condition, $F(2, 26) = 10.01$, $P < 0.001$. *Post hoc* tests over the sedative phase showed a significantly slower reaction time in the moderate sedative phase than in the baseline or light sedative phases ($P < 0.001$ for both). *Post hoc* tests over the experimental conditions showed significantly slower reaction time in the 'switch' condition than in the same or 'new' conditions ($P < 0.01$ for both).

A main effect of the drug type was not significant, $F(1, 13) = 0.07$, $P > 0.05$; neither were two-way interactions for the drug type × sedative phase, $F(2, 26) = 0.20$, $P > 0.05$; for the drug type × experimental condition, $F(2, 26) = 0.33$, $P > 0.05$; or for the sedative phase × experimental condition, $F(4, 52) = 0.65$, $P > 0.05$. A significant three-way interaction was also not observed, $F(4, 52) = 1.19$, $P > 0.05$.

Electrophysiological results

ERP amplitude results

Because the three-way interaction was not significant in the behavioural results, we combined the two drug groups into one anaesthesia group and examined the effects of the sedative phase and experimental conditions on the ERP amplitude of the three frontal channels (Cz, Fz and F3). The averaged ERP amplitude of each channel in each sedative phase is summarized in Fig. 3. For the statistical analysis, we resampled the data by simply averaging data every 50 ms. A two-way repeated ANOVA was performed for each channel with factors of the sedative phase (three levels: baseline, moderate and light) and time points (20 levels: from 0 to 1000 ms).

ERP amplitude to the first memorandum

In the Cz, ERP amplitudes in response to the first memorandum were found different among the three sedative phases (Fig. 3). The change in amplitude was smaller under moderate sedation relative to those at baseline or under light sedation. Specifically, under moderate sedation, the amplitude at 550 ms from event onset was significantly greater than only that at 350 ms, while for baseline, the amplitudes at 450 ms and 500 ms were significantly greater than those at 50–400 ms and 650–750 ms. For light sedation, the amplitudes at 450 ms and 500 ms were greater than those at 50–100 ms and 250–400 ms. The amplitude level at 900–950 ms under moderate sedation was greater than the same amplitude at baseline. Finally, the amplitude level at 100 ms and 450 ms under light sedation was lower than that at baseline. The amplitude level at 550 ms was also lower under light sedation than under moderate sedation. A three-way ANOVA showed a significant main effect of time, $F(19, 266) = 11.10$, $P < 0.001$, and a significant interaction between factors of the sedative phase and time, $F(38, 532) = 3.02$, $P < 0.001$. A main effect of the sedative phase was not significant, $F(2, 28) = 1.86$, $P > 0.05$. Statistical results on simple main effects and *post hoc* tests are summarized in Supplementary Table S2a and b.

In the Fz, an amplitude change was not found under moderate sedation when *post hoc* tests were performed (Fig. 3). On the other hand, at baseline, the amplitudes at 450 ms and 500 ms in response to the first memorandum were greater than those at

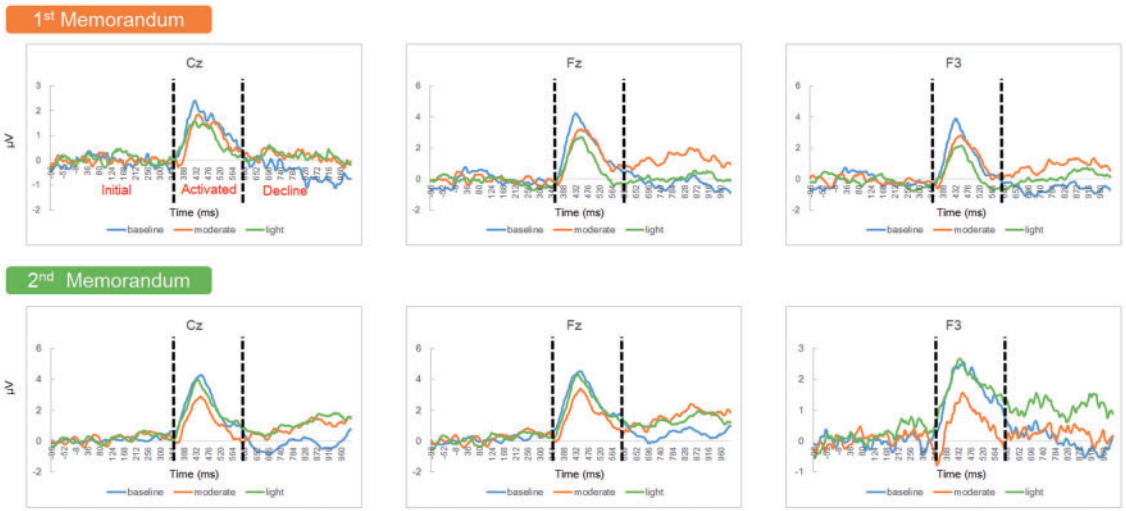

Figure 3. Time-course ERP amplitude averaged across the participants in response to the first (top) and second (bottom) memoranda in three frontal channel sites: Cz (left), Fz (middle) and F3 (right). The vertical axis represents the amplitude level and the horizontal axis represents time (ms).

50–100 ms, 200–400 ms and 600–650 ms. Under light sedation, the amplitudes at 450 ms and 500 ms were greater than those at 300–400 ms. Other findings include the amplitude at 100 ms being greater at baseline than under moderate or light sedation and the amplitude at 450 ms at baseline being greater than under light sedation. A three-way ANOVA showed a significant main effect of time, $F(19, 266) = 9.96$, $P < 0.001$, and a significant interaction between factors of the sedative phase and time, $F(38, 532) = 2.59$, $P < 0.001$. A main effect of the sedative phase was not significant, $F(2, 28) = 1.37$, $P > 0.05$. Statistical results on simple main effects and *post hoc* tests are summarized in Supplementary Table S3a and b.

Similar to the Cz, the F3 showed that moderate sedation produced a significant change in amplitude at 550 ms relative to 150 ms after the first memorandum, while at baseline, the amplitudes at 450 ms and 500 ms were greater than those at 300–400 ms and 600–750 ms (Fig. 3). Under light sedation, the amplitudes at 450 ms and 500 ms were greater than those at 150–200 ms and 300–400 ms. Regarding the effect of the sedative phase, the amplitude at 850 ms was greater under moderate sedation than at baseline. A three-way ANOVA showed a significant main effect of time, $F(19, 266) = 8.64$, $P < 0.001$, and a significant interaction between factors of the sedative phase and time, $F(38, 532) = 2.03$, $P < 0.001$. A main effect of the sedative phase was not significant, $F(2, 28) = 0.47$, $P > 0.05$. Statistical results on simple main effects and *post hoc* tests are summarized in Supplementary Table S4a and b.

ERP amplitude to the second memorandum

Similar to the ERP amplitude in response to the first memorandum, the pattern differed across sedative phases in response to the second memorandum. Again, changes in amplitude across an ERP were smaller under moderate sedation as compared to at baseline or under light sedation.

In the Cz, the amplitude under moderate sedation was different at 450–500 ms compared to at 150 ms and 600 ms in response to the second memorandum (Fig. 3). On the other hand, the amplitude at baseline from 450–500 ms was different from those at 50 ms, 150–400 ms and 600–700 ms. Under light sedation, differences were found between 450–500 ms compared to 50–400 ms and 650 ms following presentation of the second memorandum. Regarding an effect of the sedation, the amplitude at 950 ms was greater under light sedation relative to at baseline. A three-way ANOVA showed a significant main effect of time, $F(19, 266) = 13.76$, $P < 0.001$, and a significant interaction between factors of the sedative phase and time, $F(38, 532) = 1.91$, $P < 0.005$. A main effect of the sedative phase was not significant, $F(2, 28) = 0.73$, $P > 0.05$. Statistical results on simple main effects and *post hoc* tests are summarized in Supplementary Table S5a and b.

In the Fz, a change in amplitude was not observed under moderate sedation in *post hoc* tests (Fig. 3). At baseline, the amplitude at 450–500 ms was greater than that at 50 ms, 200–400 ms and 550–650 ms. For light sedation, differences in amplitude were detected between 450–500 ms and 250–400 ms. A three-way ANOVA showed a significant main effect of time, $F(19, 266) = 11.78$, $P < 0.001$, and a significant interaction between factors of the sedative phase and time, $F(38, 532) = 1.45$, $P < 0.05$. A main effect of the sedative phase was not significant, $F(2, 28) = 0.10$, $P > 0.05$. Statistical results on simple main effects and *post hoc* tests are summarized in Supplementary Table S6.

F3 was the only exception to the pattern described above, because an amplitude change across ERPs was not detected under either moderate or light sedation (Fig. 3). On the other hand, at baseline, the amplitude at 450–550 ms was greater than that at 100–150 ms, 250–400 ms and 650–700 ms following presentation of the second memorandum. A three-way ANOVA showed a significant main effect of time, $F(19, 266) = 7.55$, $P < 0.001$, and a significant interaction between factors of the sedative phase and time, $F(38, 532) = 1.56$, $P < 0.05$. A main effect of the sedative phase was not significant, $F(2, 28) = 0.25$, $P > 0.05$. Statistical results on simple main effects and *post hoc* tests are summarized in Supplementary Table S7.

ERPs phase results

As expected, the audio-spatial working memory task elicited P3 components, which lasted for approximately 250 ms from a time-point of 350–600 ms. Subsequently, we separated the individual ERPs of each participant into three task phases: an initial phase (0–350 ms), activated phase (350–600 ms) and decline

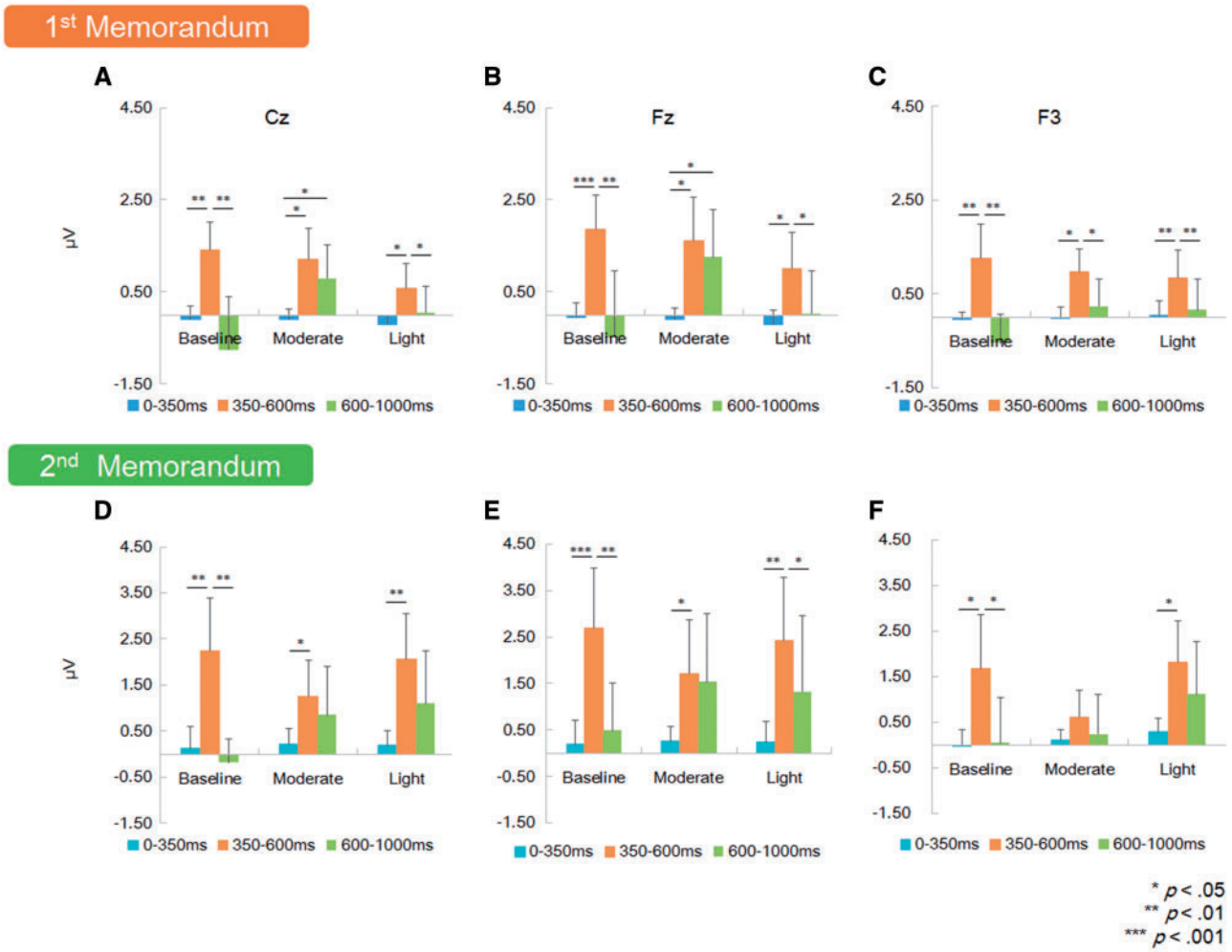

Figure 4. Averaged ERP amplitude across three time phases in response to the first (top) and second (bottom) memoranda in three frontal channel sites: Cz (left), Fz (middle) and F3 (right). Under the baseline and light sedative phases, ERPs in the activated phase (350–600 ms) were greater than those in the initial (0–350 ms) and decline phases (600–1000 ms). However, under moderate sedation, ERPs in the activated phase showed a similar magnitude to those in the decline phase. A similar pattern was obtained in response to the second memorandum. Error bars represent 95% confidence interval.

phase (600–1000 ms). This analysis was performed to interpret the amplitude data in a simpler way; however, it should be noted that the phase separation is arbitrary and requires future investigation.

ERP phase amplitude to the first memorandum

In response to the first memorandum, EPR amplitudes in the Cz were greater in the activated phase than in the initial and decline phases at baseline and under light sedation ($P < 0.05$) (Fig. 4A). On the other hand, under moderate sedation, ERP amplitudes were greater in the activated and decline phases than in the initial phase ($P < 0.05$) (Fig. 4A). Furthermore, ERP amplitudes in the decline phase were greater under moderate sedation than at baseline ($P < 0.05$). A repeated measures ANOVA showed a significant main effect of the task phase, $F(2, 28) = 10.74$, $P < 0.001$, and a task phase × sedative phase interaction, $F(4, 56) = 5.57$, $P < 0.001$. A main effect of the sedative phase was not significant, $F(2, 28) = 1.59$, $P > 0.05$. Statistical results of *post hoc* tests are summarized in the Supplementary Text.

Similarly, in the Fz, EPR amplitudes in response to the first memorandum were greater in the activated phase than in the initial and decline phases at baseline and under light sedation ($P < 0.05$) (Fig. 4B). On the other hand, under moderate sedation, ERP amplitudes were greater in the activated and decline phases than in the initial phase ($P < 0.05$) (Fig. 4B). A repeated measures ANOVA showed a significant main effect of the task phase, $F(2, 28) = 10.69$, $P < 0.001$, and a task phase × sedative phase interaction, $F(4, 56) = 4.28$, $P < 0.001$. A main effect of the sedative phase was not significant, $F(2, 28) = 1.21$, $P > 0.05$.

In the F3, ERP amplitudes in response to the first memorandum were greater in the activated phase than in the initial and decline phases; however, the patterns of the response differed across the sedative phases. At baseline and under light sedation, the difference in amplitude was greatest between the activated and decline phases ($P < 0.05$), while it was greatest between the activated and baseline phases under moderate sedation ($P < 0.05$) (Fig. 4C). A repeated measures ANOVA showed a significant main effect of the task phase, $F(2, 28) = 11.11$, $P < 0.001$, and a task phase × sedative phase interaction, $F(4, 56) = 4.30$, $P < 0.001$. A main

effect of the sedative phase was not significant, $F(2, 28) = 0.22$, $P > 0.05$.

ERP phase amplitude to the second memorandum

In response to the second memorandum, ERP amplitudes in the Cz were greater in the activated phase than in the initial or decline phases at baseline and under light sedation ($P < 0.05$) (Fig. 4D). On the other hand, under moderate sedation, ERP amplitudes were greater in the activated phase than in the initial phase ($P < 0.05$), but were the same as in the decline phase ($P > 0.05$) (Fig. 4D). A repeated measures ANOVA showed a significant main effect of task phase, $F(2, 28) = 16.29$, $P < 0.001$, and a task phase × sedative phase interaction, $F(4, 56) = 3.54$, $P < 0.05$. A main effect of the sedative phase was not significant, $F(2, 28) = 0.58$, $P > 0.05$.

Similarly, in the Fz, ERP amplitudes in response to the second memorandum were greater in the activated phase than in the initial or decline phases at baseline and under light sedation ($P < 0.05$) (Fig. 4E). On the other hand, under moderate sedation, ERP amplitudes were greater in the activated phase than in the initial phase ($P < 0.05$) but were the same as in the decline phase ($P < 0.05$) (Fig. 4E). A repeated measures ANOVA showed a significant main effect of task phase, $F(2, 28) = 15.32$, $P < 0.001$, and trend toward a significant task phase × sedative phase interaction, $F(4, 56) = 2.33$, $P = 0.067$. A main effect of the sedative phase was not significant, $F(2, 28) = 0.06$, $P > 0.05$.

In the F3, ERP amplitudes in response to the second memorandum were greater in the activated phase than in the initial and decline phases at the baseline condition ($P < 0.05$) (Fig. 4F). ERP amplitudes were greater in the activated phase than in the initial phase ($P < 0.05$) but were the same as in the decline phase under light sedation ($P > 0.05$). ERP amplitudes did not differ across time phases under moderate sedation ($P > 0.05$) (Fig. 4F). A repeated measures ANOVA showed a significant main effect of the task phase, $F(2, 28) = 10.77$, $P < 0.001$, and trend toward a significant task phase × sedative phase interaction, $F(4, 56) = 2.38$, $P = 0.063$. A main effect of the sedation was not significant, $F(2, 28) = 1.51$, $P > 0.05$.

Time-frequency analysis results

As the results of event-related oscillation power are preliminary, we reported them in the Supplementary Files.

Discussion

The present study investigated the neural mechanism of conscious stimulus perception from a convergent perspective of feature binding and LOC under general anaesthesia. By manipulating the concentration level of the anaesthetic agent in the brain, we found that a moderate sedative state, which was determined by simulated Ce of 50% needed for LOC, selectively impaired audio-spatial binding in working memory, while such selective impairment disappeared in a light sedative state. Under a deep sedative state, general task performance was impaired by severe sedation. As predicted, a P3 component was detected in response to memoranda from the frontal electrodes; however, its amplitude did not differ significantly across the three different sedative phases. Interestingly, P3 recovery latency was significantly slower in a moderate sedative state. Importantly, slow recovery was found in both the first and second memoranda. These results conversely indicate that the neural mechanism supporting efficient recovery of the P3 component may be underlying audio-spatial feature binding.

Attenuation of feature binding in a moderate sedative state

The behavioural results showed several important characteristic effects of anaesthesia. Regarding accuracy, it was decreased in the moderate sedative state both in the 'same' and 'switch' conditions, but not in the 'new' condition. This result is quite important because binding judgment is selectively impaired, but general cognitive function is preserved. If that were not the case, performance in the 'new' condition under moderate sedation should have been impaired to the same extent as in the 'same' and 'switch' conditions. Moreover, performance in the 'switch' condition was lower under moderate sedation than at baseline or under light sedation, while performance in the 'same' condition was lower under moderate sedation than at baseline only. Although both the 'same' and 'switch' conditions required bound representations to make an old–new judgment, a more robust representation would be demanded in the 'switch' condition. This demand is because the probe stimulus reactivated an encoded item that had been presented several seconds before in the 'same' condition, while such reactivation did not occur in the 'switch' condition. These patterns of the sedative effect on the task indicate that moderate sedation induced by general anaesthesia selectively impairs feature binding in working memory. Interestingly, the drugs showed their effects when moderate sedation was induced, but general task performance was disrupted under deep sedation. This result may indicate that general anaesthetic agents produce a neurophysiological change in the central nervous system, when their dose reaches a level to induce moderate sedation, consequently disrupting feature binding.

Regarding the reaction times, we made two important observations. The first was that switching across features consumes more cognitive resources, which was reflected by the slower reaction time across the sedative phases in the 'switch' condition. One possible process consuming cognitive resources in the 'switch' condition is the two-matching requirement. While each feature was encoded in the working memory, the participants were required to match frequency first and then space. Another process is the response competition at recognition, as each feature of a probe triggered a 'yes' response because they had been presented at encoding even if a 'no' response was correct. The second observation is that moderate sedation slowed the reaction time across three different probes possibly by affecting general motor movement or cognitive processes such as probe encoding.

Sustained P3 component as the electrophysiological underpinning of feature unbinding

The electrophysiological activity at the frontal electrodes showed a characteristic pattern when feature binding was disrupted under moderate sedation. Although the P3 components were similarly observed across baseline and two sedative conditions, the subsequent decline of electrophysiological activity differed. At baseline and under the light sedative state, the amplitude decreased to the pre-activated level range (0–350 ms). On the other hand, under moderate sedation, it only slightly declined, but not to the same extent seen at baseline and under light sedation. Sustained P3 components have been previously reported. Raine and Venables (1988) found that prisoners with psychopathic tendencies showed a delayed recovery of the P3 component in response to a target stimulus in the continuous performance task. Moreover, the authors inferred that the slow

recovery was due to a bizarre interaction between frontal negativity and posterior positivity. Based on this, the sustained P3 components observed in the present study may also reflect an impaired fronto-parietal interaction.

A sustained P3 component at the central electrodes has been also reported when a global rule was violated, while a local rule violation failed to produce the effect (Bekinschtein *et al.* 2009). The sustained P3 can be used as an indicator of consciousness because unawareness of the global rule violation extinguished the P3 component. Moreover, patients in a vegetative state failed to show the sustained P3 components associated with global rule violation, while they showed normal mismatch negativity component coupled with the local rule violation. Interestingly, the few patients who showed sustained P3 components regained their conscious state within 3–4 days following the study (Faugeras *et al.* 2011). Although not explicitly compared, the patients appeared to show a greater P3 sustainment in comparison to healthy controls (Fig. Ca and Cb in Faugeras *et al.* 2011). Longer sustainment of the P3 component under moderate sedation observed in the present study may be comparable to the P3 components of the patients in a vegetative state who recovered their consciousness afterwards. In other words, longer sustainment of the P3 may be a critical sign of attenuated conscious processing. Taken together, the moderate sedation induced by the general anaesthetics, which disrupted audio-spatial binding, produced a sustained P3 component, which may need to be inhibited for conscious processing.

Possible neural mechanism mediating audio-spatial feature binding

Previous studies using general anaesthesia have reported an increase in low-frequency oscillations in the frontal sites, and a decrease in high-frequency bands across the whole brain during LOC (John *et al.* 2001; Purdon *et al.* 2013). Those studies also found hyper-synchronization in the frontal regions during LOC. John *et al.* (2001) suggested that the distinctive changes in oscillation and coherence in the frontal cortex dedifferentiated and disorganized the cooperative system in the brain. The slow recovery observed in the present study might be one form of such a disorganized state of the brain system. Supp *et al.* (2011) found a similar hyper-coherence in the frontal cortex, arguing that a shift in dynamics of the thalamo-cortical loops contributed to the hyper-synchronized alpha activity in the frontal cortex under LOC. The sustained P3 component in the present study may also reflect such a shift in the thalamo-cortical loops by affecting the GABAergic cortical interneurons.

Several studies have provided evidence to support the hypothesis that a loss of feedback connection from the prefrontal cortex to the posterior parietal cortex is a critical source of LOC under general anaesthesia (Ferrarelli *et al.* 2010; Ku *et al.* 2011; Lee *et al.* 2013). Studies on feature binding have reported that the posterior parietal cortex is the core neural structure for feature binding, possibly via spatial attention (Cohen and Ivry 1991; Friedman-Hill *et al.* 1995; Shafritz *et al.* 2002). Although still speculative, the sustained P3 may impair the normal feedback signal to the posterior parietal cortex, preventing this area from integrating independent features into a coherent representation. We suggest that the emergence and normal recovery of the P3 component in the frontal regions play a critical role in integrating features for conscious awareness through efficient feedback connection with the posterior parietal cortex. This hypothesis seems to fit well the global workspace theory of consciousness (Baars 2005) and cognitive unbinding theory (Mashour 2004, 2013).

Possible effects of general anaesthesia on neural oscillatory power

The effects of anaesthesia on oscillatory power were not consistent between the first and second memoranda, and they differed across channels. Furthermore, as the number of trials in this study was inadequate, the results should be interpreted as preliminary.

There were two main preliminary findings. The first is that, under the moderate and light sedations, we found an increase in the beta-band oscillation in the Cz in response to the first memorandum, while such increase was not detected in the alpha- and gamma-bands. Selective increase in beta-band was due to the anaesthetic level that sedated participants but was not sufficient to induce LOC. A similar finding was reported in a previous study, where propofol enhanced beta-band oscillation before LOC, while the enhancement of alpha-band oscillation appeared simultaneously with LOC (Purdon *et al.* 2013). The second is that moderate sedation prevented the alpha-band oscillation from increasing from the initial task phase to the middle one at Fz in the second memorandum. Although statistical analyses failed to show consistent results in the other channels, the heat-map (Supplementary Fig. S3 top-left) showed a similar pattern in the Cz. An enhancement of the task-related alpha oscillation observed in the present study may also be related to working memory function (Jensen *et al.* 2002). Importantly, the oscillation was attenuated under moderate sedation in response to the second memorandum, but not in response to the first. It is possible that an interaction of memory load and anaesthesia contributes to the attenuation of alpha-band oscillation. In particular, the memory load following the first memorandum may consume neural resources to process further information, especially under moderate sedation. Depleted neural resources during the second memorandum may be indicated by reduced event-related oscillation at the alpha band. However, those interpretations are still speculative, due to the small sample size and number of trials, and thus further investigations are mandatory.

Limitations

The most critical limitation of the present study is the small number of trials ($n = 32$ at maximum) in each condition, which was measured from only three channels of the frontal site. The number is less likely to be suitable especially for EEG data, indicating that the present results are tentative and should be interpreted cautiously. Moreover, the oscillatory power results are of poor quality, and they should be interpreted as preliminary. The present study was implemented as a part of a sequence of experiments that included two other memory tasks and two nociceptive tasks. Moreover, those tasks were administered at four different sedative phases (i.e. baseline, deep, moderate and light), which necessitated approximately 6 h to complete the experiment for each participant. This circumstance restricted the number of trials administered in each participant, which weakened the statistical power to detect an anaesthesia effect. Therefore, a future replication is inevitable to elucidate the electrophysiological change induced by the anaesthetic agents. It is also essential for future studies to focus on a single hypothesis with a sufficient number of experimental trials. Another significant concern was that we could not identify the neural source

of the sustained P3. We believe that neurophysiological changes in the fronto-parietal or thalamo-cortical network underlie the sustained P3 component, as suggested previously (see Mashour 2013). However, a recording from the three frontal channels does not allow us to argue regarding the precise source for the ERP components, and further investigations are required, for example, by using a simultaneous recording of EEG and fMRI (Purdon *et al*. 2009).

Conclusion

The present study challenged the problem of conscious stimulus perception from an integrative perspective that covered feature binding and anaesthesia-induced LOC. The results showed that moderate sedation induced by general anaesthetics impaired audio-spatial feature binding. The impairment was associated with sustained P3 components from the frontal channels. Although the precise neural mechanism for the ERP component is still unknown, it would be possible that disorganization of the thalamo-cortical loop or fronto-parietal network is plausible candidates. Further assessment of the conscious state under moderate sedation might offer important insights into the neurophysiological basis of conscious stimulus perception.

Supplementary data

Supplementary data is available at *NCONSC Journal* online.

Acknowledgements

We thank Kaori Endo for support in data collection and arrangement of the study. We also appreciate Naoyuki Osaka for his comments on an early version of the manuscript.

Funding

This study was supported by a grant from the Japan Society for the Promotion of Science to M.O.[23240046] and the Global Center of Excellence Program, Center of Future Engineering based on Understanding of Cognitive Brain, for Osaka University.

References

Alkire MT, Haier RJ, Fallon JH editors. 1998. *Toward the Neurobiology of Consciousness: Using Brain Imaging and Anesthesia to Investigate the Anatomy of Consciousness*. Cambridge, MA: MIT Press.

Alkire MT, Hudetz AG, Tononi G. Consciousness and anesthesia. *Science* 2008;**322**:876–80.

Baars BJ. Global workspace theory of consciousness: toward a cognitive neuroscience of human experience. *Prog Brain Res* 2005;**150**:45–53.

Bekinschtein TA, Dehaene S, Rohaut B *et al*. Neural signature of the conscious processing of auditory regularities. *Proc Natl Acad Sci USA* 2009;**106**:1672–7.

Brown EN, Purdon PL, Van Dort CJ. General anesthesia and altered states of arousal: a systems neuroscience analysis. *Annu Rev Neurosci* 2011;**34**:601–28.

Changeux JP. Conscious processing: implications for general anesthesia. *Curr Opin Anaesthesiol* 2012;**25**:397–404.

Ching S, Cimenser A, Purdon PL *et al*. Thalamocortical model for a propofol-induced alpha-rhythm associated with loss of consciousness. *Proc Natl Acad Sci USA* 2010;**107**:22665–70.

Cohen A, Ivry RB. Density effects in conjunction search: evidence for a coarse location mechanism of feature integration. *J Exp Psychol Hum Percept Perform* 1991;**17**:891–901.

Desimone R, Duncan J. Neural mechanisms of selective visual attention. *Annu Rev Neurosci* 1995;**18**:193–222.

Donchin E, Coles MGH. Is the P300 component a manifestation of context updating? *Behav Brain Sci* 1988;**11**:355–72.

Faugeras F, Rohaut B, Weiss N *et al*. Probing consciousness with event-related potentials in the vegetative state. *Neurology* 2011;**77**:264–8.

Ferrarelli F, Massimini M, Sarasso S *et al*. Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. *Proc Natl Acad Sci USA* 2010;**107**:2681–6.

Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat Rev Neurosci* 2008;**9**:370–86.

Friedman-Hill SR, Robertson LC, Treisman A. Parietal contributions to visual feature binding: evidence from a patient with bilateral lesions. *Science* 1995;**269**:853–5.

Fries P. Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu Rev Neurosci* 2009;**32**:209–24.

Gray JM, Kenny GN. Development of the technology for 'Diprifusor' TCI systems. *Anaesthesia* 1998;**53 Suppl 1**:22–7.

Hall MD, Pastore RE, Acker BE *et al*. Evidence for auditory feature integration with spatially distributed items. *Percept Psychophys* 2000;**62**:1243–57.

Hemmings HC Jr, Akabas MH, Goldstein PA *et al*. Emerging molecular mechanisms of general anesthetic action. *Trends Pharmacol Sci* 2005;**26**:503–10.

Jensen O, Gelfand J, Kounios J *et al*. Oscillations in the alpha band (9-12 Hz) increase with memory load during retention in a short-term memory task. *Cereb Cortex* 2002;**12**:877–82.

John ER, Prichep LS, Kox W *et al*. Invariant reversible QEEG effects of anesthetics. *Conscious Cogn* 2001;**10**:165–83.

Kahneman D, Treisman A, Gibbs BJ. The reviewing of object files: object-specific integration of information. *Cogn Psychol* 1992;**24**:175–219.

Kang H, Nakae A, Ito H *et al*. Effects of sedation on subjective perception of pain intensity and autonomic nervous responses to pain: a preliminary study. *PLoS One* 2017;**12**:e0183635.

Kanwisher NG. Repetition blindness: type recognition without token individuation. *Cognition* 1987;**27**:117–43.

Ku SW, Lee U, Noh GJ *et al*. Preferential inhibition of frontal-to-parietal feedback connectivity is a neurophysiologic correlate of general anesthesia in surgical patients. *PLoS One* 2011;**6**:e25155.

Lee U, Ku S, Noh G *et al*. Disruption of frontal-parietal communication by ketamine, propofol, and sevoflurane. *Anesthesiology* 2013;**118**:1264–75.

Lewis LD, Weiner VS, Mukamel EA *et al*. Rapid fragmentation of neuronal networks at the onset of propofol-induced unconsciousness. *Proc Natl Acad Sci USA* 2012;**109**:E3377–86.

Mashour GA. Consciousness unbound: toward a paradigm of general anesthesia. *Anesthesiology* 2004;**100**:428–33.

Mashour GA. Cognitive unbinding: a neuroscientific paradigm of general anesthesia and related states of unconsciousness. *Neurosci Biobehav Rev* 2013;**37**:2751–9.

Mayr S, Buchner A, Moller M *et al*. Spatial and identity negative priming in audition: evidence of feature binding in auditory spatial memory. *Atten Percept Psychophys* 2011;**73**:1710–32.

Palva S, Palva JM. New vistas for alpha-frequency band oscillations. *Trends Neurosci* 2007;**30**:150–8.

Palva S, Palva JM. Functional roles of alpha-band phase synchronization in local and large-scale cortical networks. *Front Psychol* 2011;**2**:204.

Purdon PL, Pierce ET, Bonmassar G *et al*. Simultaneous electroencephalography and functional magnetic resonance imaging of general anesthesia. *Ann N Y Acad Sci* 2009;**1157**:61–70.

Purdon PL, Pierce ET, Mukamel EA *et al*. Electroencephalogram signatures of loss and recovery of consciousness from propofol. *Proc Natl Acad Sci USA* 2013;**110**:E1142–51.

Purdon PL, Sampson A, Pavone KJ *et al*. Clinical electroencephalography for anesthesiologists: Part I: background and basic signatures. *Anesthesiology* 2015;**123**:937–60.

Raine A, Venables PH. Enhanced P3 evoked potentials and longer P3 recovery times in psychopaths. *Psychophysiology* 1988;**25**: 30–8.

Shafritz KM, Gore JC, Marois R. The role of the parietal cortex in visual feature binding. *Proc Natl Acad Sci USA* 2002;**99**:10917–22.

Singer W. Consciousness and the binding problem. *Ann N Y Acad Sci* 2001;**929**:123–46.

Supp GG, Siegel M, Hipp JF *et al*. Cortical hypersynchrony predicts breakdown of sensory processing during loss of consciousness. *Curr Biol* 2011;**21**:1988–93.

Veselis RA, Reinsel RA, Beattie BJ *et al*. Midazolam changes cerebral blood flow in discrete brain regions: an H2(15)O positron emission tomography study. *Anesthesiology* 1997;**87**: 1106–17.

Zimmer HD, Mecklinger A, Lindenberger U. Levels of binding: types, mechanisms, and functions of binding in remembering. In: Zimmer HD, Mecklinger A, Lindenberger U (eds), *Handbook of Binding and Memory: Perspectives from Cognitive Neuroscience*. Oxford: Oxford University Press, 2006, 3–22.