BJA

British Journal of Anaesthesia, 121 (1): 270–280 (2018)

doi: 10.1016/j.bja.2018.04.032
Advance Access Publication Date: 23 May 2018
Research Article

Spoken words are processed during dexmedetomidine-induced unresponsiveness

R. E. Kallionpää[1,2,*], A. Scheinin[2,3], R. A. Kallionpää[4], N. Sandman[1], M. Kallioinen[2], R. Laitio[3], T. Laitio[3], K. Kaskinoro[3], T. Kuusela[5], A. Revonsuo[1,6], H. Scheinin[2,3,7] and K. Valli[1,2,6]

[1]Department of Psychology and Speech-Language Pathology, and Turku Brain and Mind Center, University of Turku, Turku, Finland, [2]Department of Perioperative Services, Intensive Care and Pain Medicine, Turku University Hospital, Turku, Finland, [3]Turku PET Centre, University of Turku and Turku University Hospital, Turku, Finland, [4]Institute of Biomedicine, University of Turku, Turku, Finland, [5]Department of Physics and Astronomy, University of Turku, Turku, Finland, [6]Department of Cognitive Neuroscience and Philosophy, School of Bioscience, University of Skövde, Skövde, Sweden and [7]Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku, Turku, Finland

*Corresponding author. E-mail: roosa.kallionpaa@utu.fi

Abstract

Background: Studying the effects of anaesthetic drugs on the processing of semantic stimuli could yield insights into how brain functions change in the transition from wakefulness to unresponsiveness. Here, we explored the N400 event-related potential during dexmedetomidine- and propofol-induced unresponsiveness.
Methods: Forty-seven healthy subjects were randomised to receive either dexmedetomidine (n=23) or propofol (n=24) in this open-label parallel-group study. Loss of responsiveness was achieved by stepwise increments of pseudo-steady-state plasma concentrations, and presumed loss of consciousness was induced using 1.5 times the concentration required for loss of responsiveness. Pre-recorded spoken sentences ending either with an expected (congruous) or an unexpected (incongruous) word were presented during unresponsiveness. The resulting electroencephalogram data were analysed for the presence of the N400 component, and for the N400 effect defined as the difference between the N400 components elicited by congruous and incongruous stimuli, in the time window 300–600 ms post-stimulus. Recognition of the presented stimuli was tested after recovery of responsiveness.
Results: The N400 effect was not observed during dexmedetomidine- or propofol-induced unresponsiveness. The N400 component, however, persisted during dexmedetomidine administration. The N400 component elicited by congruous stimuli during unresponsiveness in the dexmedetomidine group resembled the large component evoked by incongruous stimuli at the awake baseline. After recovery, no recognition of the stimuli heard during unresponsiveness occurred.
Conclusions: Dexmedetomidine and propofol disrupt the discrimination of congruous and incongruous spoken sentences, and recognition memory at loss of responsiveness. However, the processing of words is partially preserved during dexmedetomidine-induced unresponsiveness.
Clinical trial registration: NCT01889004.

Keywords: dexmedetomidine; event-related potentials; N400 evoked potential; propofol; semantics

Editorial decision: April 19, 2018; **Accepted:** April 30, 2018

Editor's key points

- Electroencephalographic N400 event-related potentials were used to assess semantic processing during dexmedetomidine- and propofol-induced unresponsiveness in healthy volunteers.
- Both dexmedetomidine and propofol disrupted discrimination of congruous and incongruous sentences at doses sufficient to induce unresponsiveness.
- The persistence of the N400 component indicates residual stimulus-dependent activity during dexmedetomidine-induced unresponsiveness.
- Thus, processing of words was partially preserved during dexmedetomidine-induced unresponsiveness.

Studying whether complex cognitive processing in response to semantic stimuli is preserved during anaesthetic-induced unresponsiveness could elucidate how brain functions change in the transition from wakefulness to unresponsiveness, and eventually to unconsciousness. Previous studies based on functional magnetic resonance imaging (fMRI) have shown brain activation related to auditory stimuli during propofol and dexmedetomidine sedation,[1–3] yet anaesthesia can disrupt higher-order processing of spoken words.[4] Based on fMRI, the discrimination between words and other sounds is preserved during propofol sedation,[1,5] although the processing of ambiguity of words is lost at low doses.[1] It is, however, poorly known how anaesthetics affect the processing of words in a context, which is the fundamental form of natural speech.

The electroencephalogram-based N400 event-related potential (ERP) could provide a method to study semantic processing during sedation.[6–9] The N400 ERP component is a negative deflection typically observed in the centroparietal electrodes 300–600 ms after a meaningful stimulus.[10,11] The N400 component is related to processing of meaning, and it is not confined to linguistic stimuli or a specific sensory modality.[7,12] The N400 component has been suggested to reflect the activation of semantic–conceptual representations, which comprises retrieving the representation of the stimulus and integrating it into the context.[7] Processing unexpected stimuli requires a more extensive search of representation in the semantic–conceptual network and produces a larger N400 component, whilst repetition of the same stimulus decreases the N400 amplitude.[13] The amplitude of the N400 component is inversely proportional to the expectancy of the stimulus,[10,14] and reflects the ease with which the stimulus is processed. The difference between the N400 components elicited by expected and unexpected stimuli is called the N400 effect. The N400 effect can be studied by comparing the N400 components evoked by related and unrelated pairs of stimuli, or sentences with expected and unexpected last words.

We studied semantic processing during dexmedetomidine- and propofol-induced unresponsiveness using the N400 component, which has not previously been reported in conjunction with anaesthetic drugs. The effects of the anaesthetics on N400 were examined in healthy subjects at doses individually determined based on loss of responsiveness (LOR). Our aim was to test the hypothesis that semantic processing can be preserved despite anaesthetic-induced unresponsiveness.

Methods

The study (ClinicalTrials.gov NCT01889004) was approved by the Ethics Committee of the Hospital District of Southwest Finland and the Finnish Medicines Agency Fimea. All subjects gave their written informed consent according to the Declaration of Helsinki. Spectral analysis of electroencephalogram[15] and subjective experiences[16] from the same study are reported elsewhere.

Subjects

The subjects were healthy (ASA physical status 1), fluent in Finnish language, and had normal hearing. Exclusion criteria included smoking, substance abuse, susceptibility for nausea, and history of psychiatric disorder. A total of 79 right-handed 20- to 30-yr-old male subjects were screened awake, and the 47 subjects with the most prominent visually identified N400 effect were enrolled to ensure that all subjects exhibited N400 in the absence of anaesthetics. The mean age of the subjects was 23.7 yr (range: 20–30 yr); they were on average 180 cm (range: 165–198 cm) tall and weighed 79.5 kg (range: 53–122 kg). The subjects were randomised to receive either dexmedetomidine (n=23) or propofol (n=24) in this open-label parallel-group study.

Stimuli and electroencephalogram recording

The stimuli were 620 Finnish auditory high-cloze-probability sentences. Twenty psychology students were asked to fill in the missing last word of the sentences using a word that first comes to their mind and fits the context. At least half of the participants had to complete each sentence with the same word (i.e. cloze probability $\geq$50% was required). Half of the sentences were randomly selected, and the expected (congruous) last word was replaced with an unexpected (incongruous) word, matched on inflection, word class, number of syllables, and word lemma frequency.[17] The resulting congruous and incongruous sentences did not differ in terms of last-word lemma frequency, the number of syllables in the last word, or sentence word count (Mann-Whitney U; P>0.05 for all). The stimuli were digitally recorded by a female native Finnish speaker (A.S.), and their amplitudes were normalised. There was a 1 s silence before and after the last word of each sentence. The sentence was followed by a response cue, which was a 100 ms sine sound, and 2.3 s for responding before the next sentence. The stimuli were organised into blocks with

50% incongruous sentences. Congruous and incongruous sentences were randomly ordered, and their presentation sequence was the same to all subjects. There were no significant differences between different blocks of stimuli in terms of last-word lemma frequency, the number of syllables in the last word, or sentence word count (Kruskal–Wallis H; $P>0.05$).

The stimuli and instructions were presented via headphones using the Presentation 17.0 software (Neurobehavioral Systems Inc., Berkeley, CA, USA). There was one stimulus block per condition, and the subjects heard each block only once (Fig. 1). Ten sentences (half incongruous) were used repeatedly to monitor the responsiveness of the subjects during the anaesthesia experiment. The responsiveness test was played at every drug concentration and whenever responsiveness was thought to have changed.

In the active baseline and in the anaesthesia experiment, the subjects were instructed to distinguish between congruous and incongruous sentences by pressing the right or left response handle after hearing the response cue. Handedness for responses was randomised: half of the subjects responded to congruous stimuli with the right hand and the other half with the left hand. Reminders to respond were given every time the subjects were awakened during the anaesthesia experiment and also before each responsiveness test. The subjects were told that their memory for the sentences would be tested at the end of the experiment.

Electroencephalogram was recorded at 64 channels according to the 10–10 electrode system using an active electrode cap (EasyCap GmbH, Herrsching, Germany), NeurOne 1.3.1.26 software (Mega Electronics Ltd., Kuopio, Finland), and Tesla #MRI 2013011 and #MRI 2013012 amplifiers (Mega Electronics Ltd.). The signal was referenced online to FCz and the ground electrode was AFz. The sampling rate was 1000 Hz with low-pass filter having a half-amplitude threshold of 360 Hz (transition band: 250–498 Hz) and high-pass filter of 0.16 Hz (6 dB octave^{-1}).

Awake baseline experiment

The baseline of N400 was first established in a separate session (Fig. 1), as auditory ERPs are known to display good test–retest reliability.[18] The subjects rested awake and eyes closed. The session started with 10 sentences for practice, followed by 150 stimuli with instructions to respond (active baseline). In the following passive baseline, the task was to listen to another block of 150 stimuli without responding.

Anaesthesia experiment

The subjects fasted overnight and refrained from using alcohol or medications for 48 h before the anaesthesia session that took place on a different day than the awake experiment. Before the initiation of drug administration, 150 sentences heard already in the awake experiment were used for practice. Dexmedetomidine or propofol was then administered as a target-controlled infusion, and vital parameters were monitored as described.[15] The pseudo-steady-state plasma concentration of the anaesthetic was increased stepwise every 7 min until LOR occurred (Fig. 1). LOR was defined as failure to answer any of the

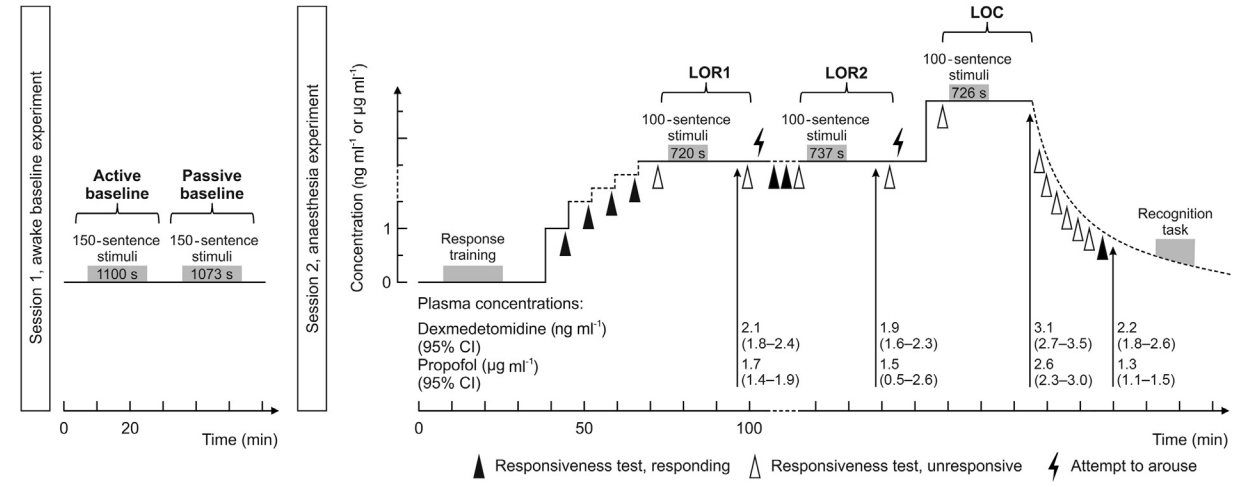

Fig 1. Design of the anaesthesia experiment. The dosing required to achieve loss of responsiveness (LOR1 and LOR2) was individually determined by stepwise increments of plasma target concentrations and repeated testing of responsiveness. The anaesthetics were administered as target-controlled infusion.[15] In the case of dexmedetomidine, the starting target plasma concentration was 1.0 ng ml^{-1}, after which the target concentration was first increased by 0.5 ng ml^{-1} and subsequently in steps of 0.25 ng ml^{-1}. For propofol, the starting target concentration was 1.0 µg ml^{-1}, the first increase was 0.5 µg ml^{-1}, and the following increments were 0.25 µg ml^{-1} each. The target concentration was then increased 1.5-fold to induce presumed loss of consciousness (LOC). All 47 subjects reached LOR1. The LOR2 was observed in 18 subjects receiving dexmedetomidine, but in only four subjects in the propofol group. The LOC was achieved in 23 subjects receiving dexmedetomidine and 22 subjects receiving propofol. One-hundred sentence stimuli were presented at each stage. Arrows show the timing of the blood samples used to determine the mean measured drug plasma concentrations, which are also reported elsewhere.[15] Eight subjects in the dexmedetomidine group and one subject in the propofol group were aroused 30 min after the discontinuation of the infusion, as they did not spontaneously regain responsiveness. In the dexmedetomidine group, the recognition task took place on average 26.7 min after the termination of the infusion and 7.6 min after the start of the interview. The respective numbers were 21.2 and 5.8 min in the propofol group. CI, confidence interval.

stimuli (0/10) in the responsiveness test. Once the first LOR (LOR1) had been achieved, a block of 100 sentence stimuli was presented. The pseudo-steady-state infusion was maintained and an attempt to arouse the subject was made by addressing him twice by name, and then mildly shaking his shoulder. If the subject woke up, he was left unstimulated until a possible second LOR occurred (LOR2) to study those whose state was interchangeable between responsiveness and unresponsiveness, and to present new stimuli to confirm the observations from LOR1. Another 100 sentences were presented and an attempt was made to awaken the subject for an interview (reported in Radek and colleagues[16]).

Finally, the target concentration was increased by 50% to reach a state assumed to represent loss of consciousness (LOC), and 100 sentences were presented. The drug infusion was discontinued and the responsiveness test was repeated until recovery of responsiveness. If responsiveness did not spontaneously occur within 30 min, the subject was aroused. After regaining responsiveness, the subject was interviewed about possible experiences during drug administration, which ensured that he was awake and oriented to complete a recognition task.

Recognition task

At the end of the awake and anaesthesia experiments, recognition of the presented stimuli was tested. The recognition task consisted of 20% of the incongruous sentences heard during the respective experiment and the same number of novel incongruous stimuli. All five incongruous stimuli of the responsiveness test were also included in the recognition task of the anaesthesia experiment. The subject was asked to indicate within the 2.3 s after the response cue whether the sentences felt familiar or not (a 'know' response) using response handles.

Processing of the N400 ERP measurements

The electroencephalogram signal was pre-processed using MATLAB R2013b (MathWorks Inc., Natick, MA, USA) and EEGLAB 13_4_4b-toolbox (Swartz Center for Computational Neuroscience, University of California San Diego, La Jolla, CA, USA). The signal was down-sampled to 250 Hz and re-referenced to the mastoid average. Filtering was performed using half-amplitude passband of 0.5–20 Hz with non-causal Blackman-windowed sinc FIR filter with transition bandwidth of 1 Hz for high pass and 4 Hz for low pass (passband ripple 0.02%; stopband attenuation −74 dB).

The N400 component elicited by the first and last words of the sentence stimuli was analysed. The trials were segmented from −1000 to 1000 ms respective to the onset of the word. The baseline was corrected using the pre-stimulus period, −1000 to 0 ms. Noisy channels were visually identified and interpolated (mean: 0.23; standard deviation: 1.05 channels per subject in the anaesthesia experiment). Epochs with absolute value of amplitude exceeding 150 μV or amplitude changes greater than 100 μV in 80 ms were excluded using an automated algorithm. The median number of epochs excluded per stimulus block was 3 (range: 0–77) in the awake experiment and 9 (range: 0–100) in the anaesthesia experiment.

Statistical analysis

To analyse the N400 ERP component and effect, amplitude averages were computed in the presumed time window of the N400 component, 300–600 ms,[10] and in a corresponding control time window, −600 to −300 ms, from the pre-stimulus baseline. The control time window was chosen to account for baseline variation of the signal in segments of 300 ms. The amplitudes were averaged over trials for each subject within each stimulus block, separately for congruous and incongruous stimuli. Cases with data from less than 20 congruous or incongruous trials were excluded. The analysis was restricted to 13 centroparietal electrodes (Cz, C1, C2, C3, C4, Pz, P1, P2, P3, P4, CPz, CP1, and CP2). Linear mixed-effects regression was used for the statistical analysis. The regression models included random slopes for subject and channel to take into account the repeated measures within each subject and channel. In the case of non-convergence, the random slope for channel was reduced to random intercept. The N400 component was examined by comparing the N400 time window with the control time window, whereas the presence of the N400 effect was established by comparing data from congruous and incongruous trials. $P<0.05$ was considered as evidence of the N400 component or effect. The active awake baseline was compared with the unresponsive conditions, and the resulting P-values were multiplied by the number of comparisons (Bonferroni correction). The latency of the N400 component was determined as the most negative value observed in the time window 200–800 ms using the jackknife method.[19] The 13 centroparietal electrodes were combined by averaging for latency measurements.

Sentence recognition was analysed using discriminability measure d' and response bias criterion c.[20,21] Cases with missing recognition response to >50% of stimuli from a single condition were excluded. Two-tailed t-tests were used to determine whether the d' and c values differed significantly from zero indicating sentence recognition or response bias, respectively. The reaction time was measured from response cue to response, and values <100 ms were excluded. The natural logarithm of the reaction time was analysed using linear mixed-effects regression with random intercept for subject. All analyses were conducted using the R software (version 3.3.2, www.r-project.org) with lmerTest package (version 2.0–33, http://cran.r-project.org/package=lmerTest).

Results

N400 component and effect

In both active and passive awake baseline, the first words and incongruous last words of the sentences elicited an N400 component that was significantly more negative than the corresponding pre-stimulus control time window (Table 1; Fig. 2). Incongruous last words evoked a more negative N400 component than congruous last words ($P<0.001$) (i.e. the N400 effect was observed). The components elicited by the first and incongruous last words had latencies of 441 ms [95% confidence interval (CI): 371–511] and 382 ms (95% CI: 331–433) in the active baseline, respectively. The corresponding figures were 416 ms (95% CI: 400–432) and 414 ms (95% CI: 321–506) in the passive baseline.

A significant N400 component persisted in dexmedetomidine-induced unresponsiveness, yet no N400 effect between congruous and incongruous last words could be observed (Table 1; Figs 2–4). The N400 component elicited by the first words differed between active baseline, LOR1, LOR2, and LOC (condition main effect $F_{3,19.4}=4.1$; $P=0.021$), and was more negative in LOR1 and LOR2 than in the active awake

Table 1 Effect estimates and two-sided significance tests for the presence of the N400 component and N400 effect in awake baseline and different levels of drug-induced unresponsiveness. CI, confidence interval; LOC, presumed loss of consciousness; LOR1, first loss of responsiveness; LOR2, second loss of responsiveness; *n*, number of study subjects

State	Stimulus	Time window	Dexmedetomidine						Propofol					
			n	Estimate	95% CI		P for N400 component	P for N400 effect	*n*	Estimate	95% CI		P for N400 component	P for N400 effect
Baseline, active	First word	Control	23	−0.04	−0.16	0.08	0.003		24	0.03	−0.05	0.11	<0.001	
		N400		−0.53	−0.81	−0.25				−0.81	−1.10	−0.52		
	Last word, congruous	Control	23	0.22	0.03	0.42	0.060	<0.001	24	0.01	−0.16	0.18	0.005	<0.001
		N400		0.66	0.26	1.05				0.75	0.30	1.20		
	Last word, incongruous	Control	23	−0.03	−0.19	0.13	<0.001		24	−0.06	−0.26	0.14	<0.001	
		N400		−2.03	−2.54	−1.52				−1.82	−2.31	−1.33		
Baseline, passive	First word	Control	23	0.02	−0.07	0.10	0.003		24	0.02	−0.07	0.11	<0.001	
		N400		−0.54	−0.84	−0.24				−0.71	−0.94	−0.49		
	Last word, congruous	Control	23	0.04	−0.16	0.23	0.020	<0.001	24	0.01	−0.10	0.12	0.102	<0.001
		N400		−0.40	−0.69	−0.11				−0.29	−0.64	0.06		
	Last word, incongruous	Control	23	0.03	−0.17	0.24	<0.001		24	−0.10	−0.22	0.01	<0.001	
		N400		−2.31	−2.78	−1.85				−1.96	−2.27	−1.65		
LOR1	First word	Control	22	−0.45	−0.91	0.01	0.016		22	−0.25	−0.44	−0.06	0.262	
		N400		−1.87	−2.89	−0.84				−0.63	−1.27	0.01		
	Last word, congruous	Control	22	0.36	−0.45	1.18	<0.001	0.149	21	−0.26	−0.74	0.21	0.366	0.706
		N400		−3.89	−5.38	−2.39				−0.86	−1.88	0.17		
	Last word, incongruous	Control	22	−0.39	−1.10	0.31	0.010		21	0.07	−0.32	0.46	0.017	
		N400		−2.49	−3.68	−1.29				−1.03	−1.72	−0.33		
LOR2	First word	Control	17	−0.20	−0.81	0.41	0.043		3	−0.20	−0.98	0.59	0.506	
		N400		−1.48	−2.25	−0.72				0.69	−2.22	3.61		
	Last word, congruous	Control	17	0.27	−0.25	0.79	0.010	0.575	3	0.96	−0.83	2.74	0.220	<0.001
		N400		−1.80	−3.39	−0.21				−1.26	−2.51	−0.01		
	Last word, incongruous	Control	17	0.18	−0.46	0.82	0.015		3	−0.41	−1.87	1.04	0.120	
		N400		−1.39	−2.28	−0.49				−2.72	−3.95	−1.48		
LOC	First word	Control	22	−0.15	−0.94	0.65	0.045		16	0.66	0.12	1.20	0.538	
		N400		−1.31	−2.24	−0.37				1.05	−0.05	2.15		
	Last word, congruous	Control	21	0.45	−0.27	1.17	0.022	0.116	17	−0.66	−1.57	0.25	0.032	0.355
		N400		−1.04	−2.04	−0.03				1.27	−0.25	2.79		
	Last word, incongruous	Control	21	0.74	−0.29	1.77	0.007		17	0.32	−0.34	0.97	0.957	
		N400		−1.50	−2.42	−0.58				0.27	−1.38	1.92		

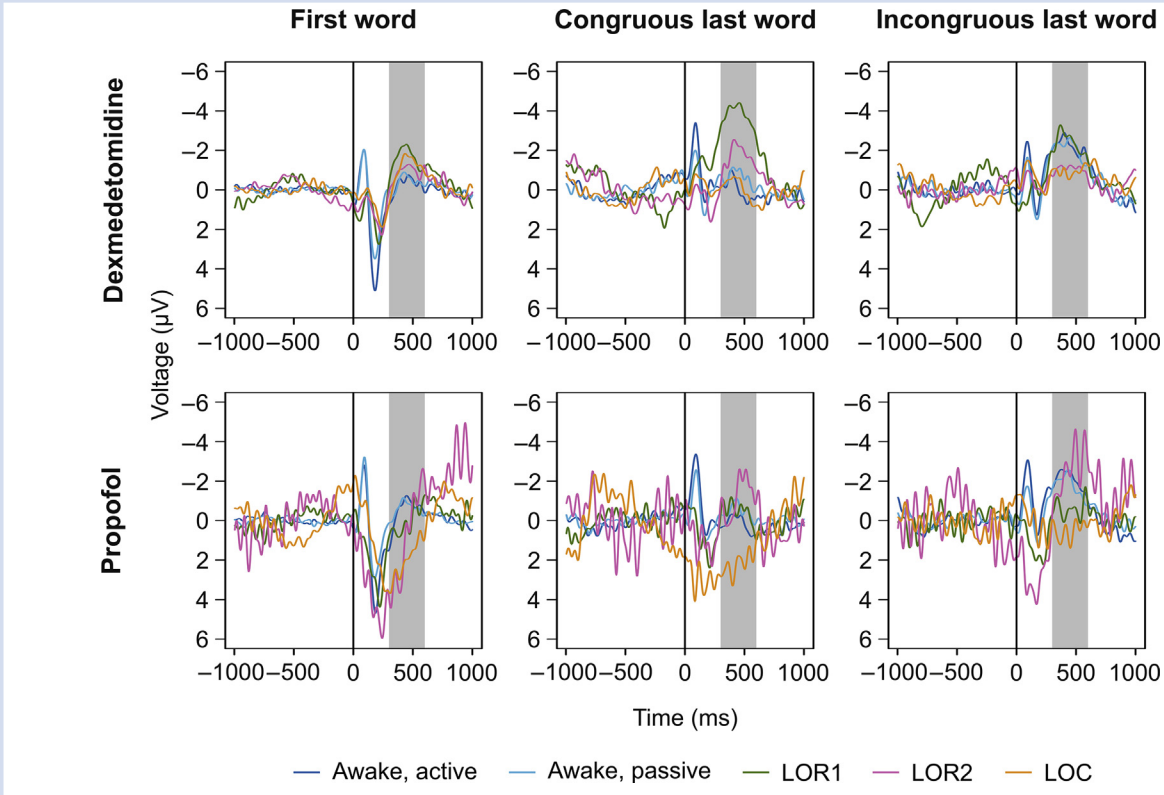

Fig 2. N400 components elicited by the first and last words of sentences in active and passive awake baseline, loss of responsiveness (LOR1 and LOR2), and presumed loss of consciousness (LOC). Data from C4 electrode are shown. The number of subjects in each condition is shown in Table 1.

baseline ($t_{21.8}$=2.7; P=0.043 and $t_{16.9}$=2.6; P=0.052, respectively). This was also the case for the N400 component evoked by congruous last words (condition main effect: $F_{3,18.5}$=11.2; P<0.001) in LOR1, LOR2, and LOC ($t_{23.2}$=5.8, P<0.001; $t_{16.8}$=3.3, P=0.014; and $t_{28.3}$=2.9, P=0.019, respectively). The N400 component observed during dexmedetomidine-induced unresponsiveness resembled the large N400 component associated with incongruous last words in the awake state regardless of the stimulus type (Figs 2 and 3). The N400 component elicited by incongruous words did not differ between awake baseline and dexmedetomidine administration ($F_{3,17.6}$=1.3; P=0.316). The latency of the N400 component varied from 360 ms (95% CI: 134–586) observed for congruous last words in LOC to 462 ms (95% CI: 200–724) seen with incongruous last words in LOR2.

Propofol-induced unresponsiveness generally resulted in an indistinct N400 component observed during LOR1 and LOR2 (Fig. 2). The N400 component was mostly not statistically significant and completely disappeared in LOC (Table 1).

Recognition of stimuli

Incongruous sentences heard during the awake baseline experiment were recognised at an average rate of 87% in the active awake baseline and 71% in the passive awake baseline, whilst the respective false-alarm rates were 7.7% and 13%. The reaction times were significantly shorter for sentences correctly identified as novel or familiar than for misidentified sentences (Table 2), yet no difference in reaction times was observed between familiar and novel sentences.

Both propofol and dexmedetomidine abolished sentence recognition in terms of d' (P<0.001). Twenty-one per cent of the incongruous sentences heard during dexmedetomidine- and propofol-induced unresponsiveness were reported as familiar, whilst the corresponding false-alarm rate was 18%. Furthermore, the difference in reaction times between sentences correctly identified and misidentified disappeared when testing stimuli heard during unresponsiveness (Table 2). The response bias criterion c showed a conservative bias (i.e. tendency to report stimuli as novel when unsure).

The five incongruous sentences used in responsiveness testing were reported as familiar more often than novel sentences with recognition rates of 32% in the dexmedetomidine group and 45% in the propofol group. During the whole experiment, the subjects had heard the responsiveness test a median 3 (range: 0–11) times in responsive state and 11 (range: 2–27) times in unresponsive state. No correlation was observed between sentence recognition and the number of responsiveness tests heard in either responsive or unresponsive state.

Discussion

The results show a persistent N400 component during dexmedetomidine-induced unresponsiveness. However,

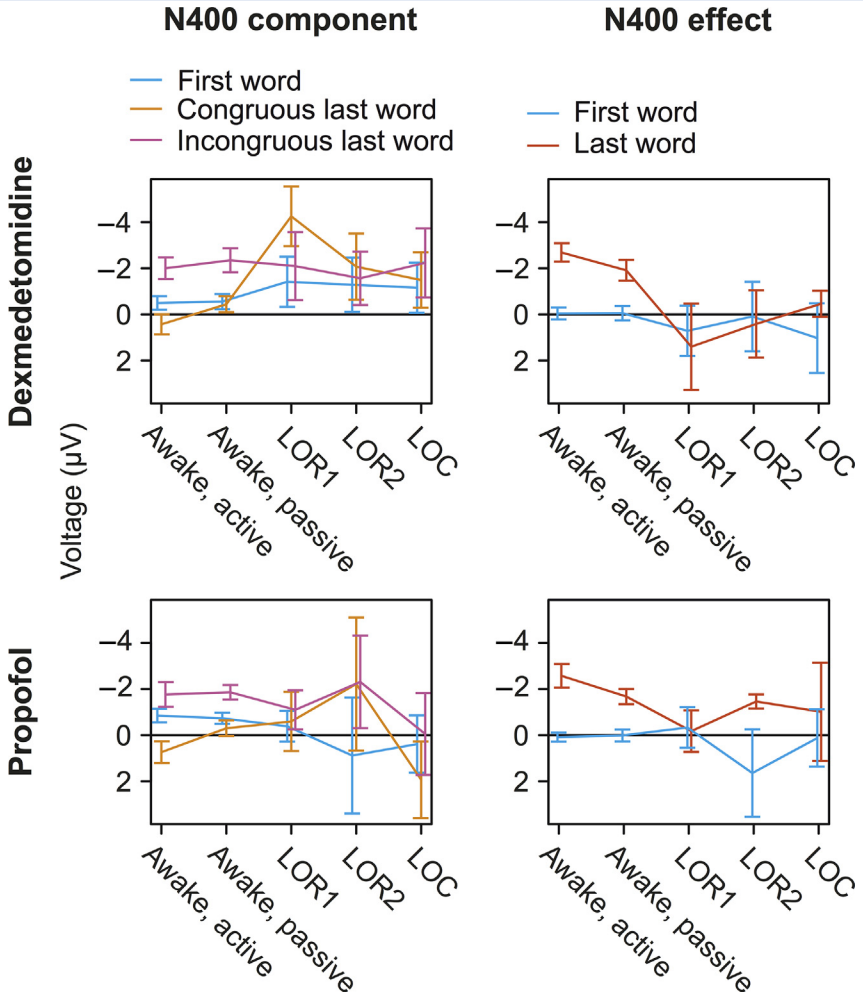

Fig 3. Estimates of the N400 component and effect and their 95% confidence intervals at awake baseline, loss of responsiveness (LOR1 and LOR2), and presumed loss of consciousness (LOC). The estimates combine data from 13 centroparietal electrodes. The number of subjects in each condition is shown in Table 1.

discrimination of congruous and incongruous words (N400 effect) was lost at LOR. The time window of the N400 component consistently showed negativity during propofol-induced unresponsiveness, suggesting the presence of the N400 component. However, the difference to control time window was only occasionally significant, and the averaged signal showed high variation and no consistent N400 waveform, limiting our ability to draw conclusions on the N400 during propofol-induced unresponsiveness.

The persistence of the N400 component indicates stimulus-dependent activity during dexmedetomidine-induced unresponsiveness. A late ERP component, such as the N400, is expected to reflect high-order cognitive processing, although the depth of processing associated with the N400 is still disputed. Whilst the N400 seems to associate with semantic processing in the awake state, it is unclear whether it represents similar processes during unresponsiveness. Nevertheless, the N400 correlates with positive prognosis in disorders of consciousness, which suggests that it reflects processes relevant to consciousness.[8,22] The current study indicated negativity in the centroparietal area at 300–600 ms post-stimulus both awake and during unresponsiveness, but also frontal negativity in the active baseline, possibly associated with responding and attention, and posterior negativity during LOC. Therefore, the stimulus-dependent processing observed during anaesthetic-induced unresponsiveness might not be fully analogous with the N400 observed awake, yet the results suggest that at least some parts of semantic processing can be evoked during unresponsiveness.

Based on studies with awake subjects, the N400 has been suggested to index the effort of retrieving the representation of a semantic stimulus.[12] Normally, the N400 component diminishes towards the end of the sentence as the context builds up.[14] In the current study, loss of the N400 effect during dexmedetomidine administration was attributable to the increased negativity of the N400 component elicited by congruous words, whilst the component resulting from incongruous stimuli remained unchanged. We speculate that all words trigger maximal semantic processing to retrieve the representation of the word in dexmedetomidine-induced

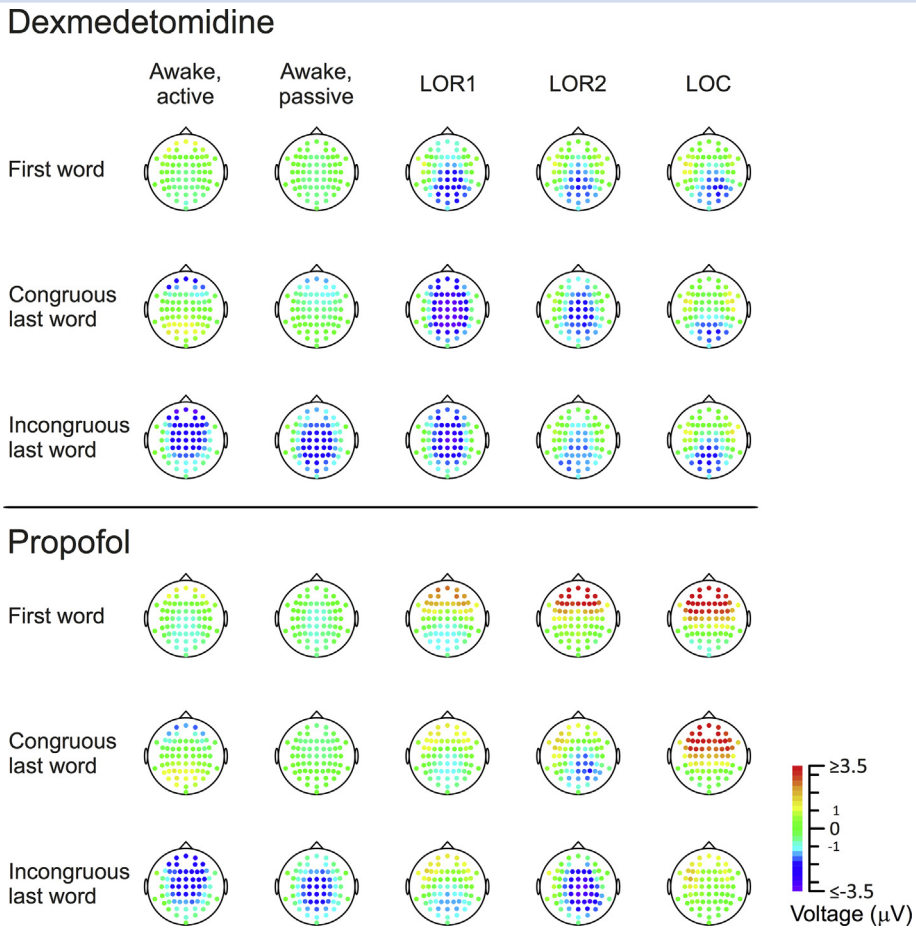

Fig 4. Topographical maps showing mean voltage 300–600 ms post-stimulus in active and passive awake baseline, loss of responsiveness (LOR1 and LOR2), and presumed loss of consciousness (LOC) in the dexmedetomidine and propofol groups. The number of subjects in each condition is shown in Table 1. Note that the scale is restricted to −3.5 to 3.5 μV to improve readability, and values smaller or larger than these, respectively, have been collapsed into each end of the scale. The topographical maps show negativity consistent with the N400 component in the centroparietal area both awake and during dexmedetomidine-induced unresponsiveness, yet its amplitude is small in the case of the first words and congruous last words during awake baseline.

unresponsiveness, possibly because of loss of context or expectations. This might be caused by the inability to integrate spoken words into sentences or to remember the beginning of the sentence. Dexmedetomidine has previously been found to decrease memory encoding,[23,24] yet the disrupted processing of the preceding sentence context cannot explain the increased negativity observed for the first words of the sentences.

Stimulus-locked phase resetting of the delta and theta frequency bands of electroencephalogram has been suggested to contribute to the N400 component.[25,26] These frequencies overlap the wavelength of the N400, and dexmedetomidine and propofol are both known to increase delta power, yet their effect on theta is not equally clear.[15,27,28] Dexmedetomidine-induced increase in the power of the delta band might contribute to the increase of the N400 component evoked by first words and congruous last words if stimulus-locked phase resetting occurs. However, the N400 component evoked by incongruous last words showed no additional negativity during unresponsiveness, suggesting that the increase of the component is not solely attributable to drug-induced delta. The N400 has been previously reported during N2 sleep[29–31] and in some patients with disorders of consciousness.[8,9,22] These conditions also show an increase in delta power compared with the awake state,[32,33] yet the increase in the negativity of the N400 component seems to be a unique effect of dexmedetomidine-induced unresponsiveness.

This is apparently the first report on the N400 ERP component during anaesthetic-induced unresponsiveness, and also adds to the sparse data on the effects of dexmedetomidine-induced unresponsiveness on semantic processing. The inability to discriminate congruous and incongruous words during drug-induced unresponsiveness is consistent with the previous observation that propofol sedation prevents the processing of ambiguity.[1] Previous fMRI studies on propofol sedation have shown the activation of superior[3] and middle[1] temporal gyri in response to sentence stimuli, whilst the anaesthetic suppresses the activation of inferior frontal

Table 2 Recognition of the stimuli heard in the different conditions during the experiment. c, response bias criterion; CI, confidence interval; d′, discriminability measure; LOC, presumed loss of consciousness; LOR1, first loss of responsiveness; LOR2, second loss of responsiveness. *After exclusion of cases with missing response to >50% of stimuli from a single condition

Presentation of stimuli (encoding)	n*	Response rate in recognition (%)*	d′ (95% CI)	P for difference of d′ to 0	c (95% CI)	P for difference of c to 0	Reaction time, correctly identified stimuli (ms, 95% CI)	Reaction time, incorrectly identified stimuli (ms, 95% CI)	P for difference in reaction times
Dexmedetomidine									
Baseline, active	23	100.0	2.5 (2.3–2.7)	<0.001	0.1 (0.0–0.2)	0.241	405 (353–465)	465 (400–540)	<0.001
Baseline, passive	23	99.6	1.7 (1.4–2.0)	<0.001	0.3 (0.1–0.4)	<0.001	417 (355–489)	459 (389–542)	<0.001
LOR1	20	82.1	0.1 (−0.2 to 0.4)	0.440	0.9 (0.6–1.2)	<0.001	582 (502–673)	612 (528–710)	0.382
LOR2	13	83.1	0.0 (−0.3 to 0.3)	0.943	0.9 (0.6–1.2)	<0.001	640 (519–790)	604 (491–744)	0.420
LOC	21	75.4	0.1 (−0.1 to 0.4)	0.253	0.8 (0.5–1.1)	<0.001	615 (533–710)	584 (505–676)	0.395
Responsiveness test	21	83.8	0.7 (0.3–1.1)	0.001	0.8 (0.5–1.1)	<0.001	526 (402–686)	610 (491–758)	0.310
Propofol									
Baseline, active	24	100.0	2.5 (2.3–2.8)	<0.001	0.1 (0.0–0.3)	0.109	436 (396–480)	550 (491–617)	<0.001
Baseline, passive	24	100.0	1.6 (1.3–1.8)	<0.001	0.3 (0.1–0.4)	0.004	443 (403–487)	520 (470–576)	<0.001
LOR1	23	95.9	0.1 (0.0–0.3)	0.124	0.7 (0.5–1.0)	<0.001	568 (487–664)	617 (527–722)	0.102
LOR2	4	96.3	−0.4 (−1.0 to 0.1)	0.094	1.0 (0.0–2.0)	0.047	551 (383–794)	523 (365–750)	0.601
LOC	22	96.1	0.1 (0.0–0.3)	0.129	1.0 (0.7–1.3)	<0.001	637 (544–746)	568 (484–666)	0.012
Responsiveness test	23	95.7	0.8 (0.4–1.2)	<0.001	0.5 (0.2–0.8)	0.001	619 (493–773)	614 (504–753)	0.950

gyrus[1,3,4] and its connections with the auditory cortex.[4] All these areas are associated with the N400,[12,34] suggesting partial impairment of processes linked to N400 in drug-induced unresponsiveness, as indeed was found.

The association of the N400 and attention is under debate, yet the N400 effect that is observed awake is known to be enhanced by top-down processes, such as directed attention and following task instructions.[35–37] Attention has been suggested to be a prerequisite of the N400 effect, whilst it might not be necessary for the N400 component.[38] Although maintaining top-down attention might not be possible during drug-induced unresponsiveness, spoken stimuli are expected to evoke bottom-up stimulus-driven attention. The N400 effect typically cannot be observed in all awake individuals,[22] indicating only partial sensitivity at single-subject level. In the current study, the anaesthetic-related delta activity, overlapping the wavelength of the N400, increased the variation in signal amplitudes even in the absence of stimuli. This prevented us from performing single-subject analyses, which would be required for the diagnostic use of N400. In addition, observing the N400 is not sufficient evidence of consciousness or awareness.[9] Future studies should explore the N400 component in responsive sedation accompanied by placebo group to elucidate further the disruption of semantic processing.

No signs of recognising the sentence stimuli heard during unresponsiveness were observed after recovery of responsiveness, unlike for emotional sounds presented in the same experiment at the same drug concentrations.[16] The reaction times further confirmed the memory impairment. The sentences presented in the responsiveness test were recognised above chance level, but less frequently than in the awake baseline. Thus, responsive sedation impaired, but did not completely prevent explicit memory for the stimuli.

Previous studies examining the recognition of stimuli presented during anaesthesia have demonstrated implicit memory in the absence of explicit memory.[39,40] The current study design was not optimal for testing sentence recognition: the subjects were not instructed to respond as quickly as possible. In addition, the recognition test took place soon after recovery of responsiveness, and the residual anaesthetic concentrations could have confounded the results.[41,42] It is, therefore, impossible to determine if the results were attributable to disruption of memory encoding, storage, or retrieval.

Conclusions

The current results demonstrate that dexmedetomidine and propofol disrupt the discrimination of congruous and incongruous sentences already at doses sufficient to induce unresponsiveness. However, the processing of words is partially preserved during dexmedetomidine-induced unresponsiveness.

Authors' contributions

Principal investigator: H.S.
Study design/planning: R.E.K., A.S., N.S., R.L., T.L., K.K., A.R., H.S., K.V.
Subject recruitment: A.S.
Experiment conduct: R.E.K, A.S., M.K., R.L., T.L., K.K., H.S., K.V.
Data analysis: R.E.K., R.A.K., T.K.
Drafting paper: R.E.K., A.S., R.A.K.
Revising paper: all authors.

Acknowledgements

The authors thank I. Dahlblom and L. Vierikko for their assistance during the anaesthesia sessions, and M. Karvonen, V. Moberg, and O. Saariluoma for assistance with electroencephalogram recordings. The authors are also very grateful to M. Tallgren for the opportunity to perform this study in the Intensive Care Unit of Turku University Hospital.

Declaration of interest

T.L. is a paid consultant at NeuroproteXeon Ltd.

Funding

Academy of Finland (266467 and 266434); Jane and Aatos Erkko Foundation; VSSHP-EVO (13323); Doctoral Programme of Clinical Investigation, University of Turku Graduate School to A.S.; Signe and Ane Gyllenberg Foundation to K.V.

References

1. Davis MH, Coleman MR, Absalom AR, et al. Dissociating speech perception and comprehension at reduced levels of awareness. *Proc Natl Acad Sci U S A* 2007; **104**: 16032–7
2. Frölich MA, Banks C, Ness TJ. The effect of sedation on cortical activation: a randomized study comparing the effects of sedation with midazolam, propofol, and dexmedetomidine on auditory processing. *Anesth Analg* 2017; **124**: 1603–10
3. Heinke W, Fiebach CJ, Schwarzbauer C, Meyer M, Olthoff D, Alter K. Sequential effects of propofol on functional brain activation induced by auditory language processing: an event-related functional magnetic resonance imaging study. *Br J Anaesth* 2004; **92**: 641–50
4. Liu X, Lauer KK, Ward BD, Rao SM, Li S, Hudetz AG. Propofol disrupts functional interactions between sensory and high-order processing of auditory verbal memory. *Hum Brain Mapp* 2012; **33**: 2487–98
5. Adapa RM, Davis MH, Stamatakis EA, Absalom AR, Menon DK. Neural correlates of successful semantic processing during propofol sedation. *Hum Brain Mapp* 2014; **35**: 2935–49
6. Geukes S, Huster RJ, Wollbrink A, Junghöfer M, Zwitserlood P, Dobel C. A large N400 but no BOLD effect — comparing source activations of semantic priming in simultaneous EEG-fMRI. *PloS One* 2013; **8**. e84029
7. Kutas M, Federmeier KD. Thirty years and counting: finding meaning in the N400 component of the event-related brain potential (ERP). *Annu Rev Psychol* 2011; **62**: 621–47
8. Steppacher I, Eickhoff S, Jordanov T, Kaps M, Witzke W, Kissler J. N400 predicts recovery from disorders of consciousness. *Ann Neurol* 2013; **73**: 594–602
9. Beukema S, Gonzalez-Lara LE, Finoia P, et al. A hierarchy of event-related potential markers of auditory processing in disorders of consciousness. *Neuroimage Clin* 2016; **12**: 359–71
10. Kutas M, Hillyard SA. Reading senseless sentences: brain potentials reflect semantic incongruity. *Science* 1980; **207**: 203–5
11. Kutas M, Hillyard SA. An electrophysiological probe of incidental semantic association. *J Cogn Neurosci* 1989; **1**: 38–49
12. Lau EF, Phillips C, Poeppel D. A cortical network for semantics: (de)constructing the N400. *Nat Rev Neurosci* 2008; **9**: 920–33
13. Van Petten C, Kutas M, Kluender R, Mitchiner M, McIsaac H. Fractionating the word repetition effect with event-related potentials. *J Cogn Neurosci* 1991; **3**: 131–50
14. Van Petten C, Kutas M. Interactions between sentence context and word frequency in event-related brain potentials. *Mem Cognit* 1990; **18**: 380–93
15. Scheinin A, Kallionpää RE, Li D, et al. Differentiating drug-related and state-related effects of dexmedetomidine and propofol on the electroencephalogram. *Anesthesiology* 2018. https://doi.org/10.1097/ALN.0000000000002192. Advance Access published on April 10
16. Radek L, Kallionpää RE, Karvonen M, et al. Dreaming and awareness during dexmedetomidine- and propofol-induced unresponsiveness. *Br J Anaesth* 2018; **121**: 260–9
17. Laine M, Virtanen P. *Wordmill, lexical search program*. Turku, Finland: Centre for Cognitive Neuroscience, University of Turku; 1999
18. Walhovd KB, Fjell AM. One-year test–retest reliability of auditory ERPs in young and old adults. *Int J Psychophysiol* 2002; **46**: 29–40
19. Miller J, Patterson T, Ulrich R. Jackknife-based method for measuring LRP onset latency differences. *Psychophysiology* 1998; **35**: 99–115
20. Wickens TD. *Elementary signal detection theory*. 1st ed. New York, NY, USA: Oxford University Press; 2002
21. Hautus MJ. Corrections for extreme proportions and their biasing effects on estimated values of *d'*. *Behav Res Methods* 1995; **27**: 46–51
22. Rohaut B, Faugeras F, Chausson N, et al. Probing ERP correlates of verbal semantic processing in patients with impaired consciousness. *Neuropsychologia* 2015; **66**: 279–92
23. Hayama HR, Drumheller KM, Mastromonaco M, Reist C, Cahill LF, Alkire MT. Event-related functional magnetic resonance imaging of a low dose of dexmedetomidine that impairs long-term memory. *Anesthesiology* 2012; **117**: 981–95
24. Pryor KO, Reinsel RA, Mehta M, Li Y, Wixted JT, Veselis RA. Visual P2–N2 complex and arousal at the time of encoding predict the time domain characteristics of amnesia for multiple intravenous anesthetic drugs in humans. *Anesthesiology* 2010; **113**: 313–26
25. Steele VR, Bernat EM, van den Broek P, Collins PF, Patrick CJ, Marsolek CJ. Separable processes before, during, and after the N400 elicited by previously inferred and new information: evidence from time–frequency decompositions. *Brain Res* 2013; **1492**: 92–107
26. Fell J, Dietl T, Grunwald T, et al. Neural bases of cognitive ERPs: more than phase reset. *J Cogn Neurosci* 2004; **16**: 1595–604
27. Purdon PL, Sampson A, Pavone KJ, Brown EN. Clinical electroencephalography for anesthesiologists: part I: background and basic signatures. *Anesthesiology* 2015; **123**: 937–60
28. Akeju O, Kim S, Vazquez R, et al. Spatiotemporal dynamics of dexmedetomidine-induced electroencephalogram oscillations. *PloS One* 2016: 11. e0163431
29. Brualla J, Romero MF, Serrano M, Valdizan JR. Auditory event-related potentials to semantic priming during sleep. *Electroencephalogr Clin Neurophysiol* 1998; **108**: 283–90
30. Perrin F, Bastuji H, Garcia-Larrea L. Detection of verbal discordances during sleep. *Neuroreport* 2002; **13**: 1345–9

31. Ibáñez A, López V, Cornejo C. ERPs and contextual semantic discrimination: degrees of congruence in wakefulness and sleep. *Brain Lang* 2006; **98**: 264–75
32. Sitt JD, King J, El Karoui I, et al. Large scale screening of neural signatures of consciousness in patients in a vegetative or minimally conscious state. *Brain* 2014; **137**: 2258–70
33. Prerau MJ, Brown RE, Bianchi MT, Ellenbogen JM, Purdon PL. Sleep neurophysiological dynamics through the lens of multitaper spectral analysis. *Physiology* 2017; **32**: 60–92
34. Van Petten C, Luka BJ. Neural localization of semantic context effects in electromagnetic and hemodynamic studies. *Brain Lang* 2006; **97**: 279–93
35. Deacon D, Shelley-Tremblay J. How automatically is meaning accessed: a review of the effects of attention on semantic processing. *Front Biosci* 2000; **5**: 82–94
36. Erlbeck H, Kubler A, Kotchoubey B, Veser S. Task instructions modulate the attentional mode affecting the auditory MMN and the semantic N400. *Front Hum Neurosci* 2014; **8**: 654
37. Cruse D, Beukema S, Chennu S, Malins JG, Owen AM, McRae K. The reliability of the N400 in single subjects: implications for patients with disorders of consciousness. *Neuroimage Clin* 2014; **4**: 788–99
38. Bentin S, Kutas M, Hillyard SA. Semantic processing and memory for attended and unattended words in dichotic listening: behavioral and electrophysiological evidence. *J Exp Psychol Hum Percept Perform* 1995; **21**: 54–67
39. Deeprose C, Andrade J, Varma S, Edwards N. Unconscious learning during surgery with propofol anaesthesia. *Br J Anaesth* 2004; **92**: 171–7
40. Lubke GH, Kerssens C, Phaf H, Sebel PS. Dependence of explicit and implicit memory on hypnotic state in trauma patients. *Anesthesiology* 1999; **90**: 670–80
41. Hall JE, Uhrich TD, Barney JA, Arain SR, Ebert TJ. Sedative, amnestic, and analgesic properties of small-dose dexmedetomidine infusions. *Anesth Analg* 2000; **90**: 699–705
42. Veselis RA, Reinsel RA, Feshchenko VA, Wronski M. The comparative amnestic effects of midazolam, propofol, thiopental, and fentanyl at equisedative concentrations. *Anesthesiology* 1997; **87**: 749–64

Handling editor: H.C. Hemmings Jr