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Alpha band frontal connectivity is a state-specific electroencephalographic correlate of unresponsiveness during exposure to dexmedetomidine and propofol

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Abstract

Background: Coherent alpha electroencephalogram (EEG) rhythms in the frontal cortex have been correlated with the hypnotic effects of propofol and dexmedetomidine, but less is known about frontal connectivity as a state-specific correlate of unresponsiveness as compared with long-range connectivity. We aimed to distinguish dose- and state-dependent effects of dexmedetomidine and propofol on EEG connectivity.

Methods: Forty-seven healthy males received either dexmedetomidine (n=23) or propofol (n=24) as target-controlled infusion with stepwise increments until loss of responsiveness (LOR). We attempted to arouse participants during constant dosing (return of responsiveness [ROR]), and the target concentration was then increased 50% to achieve presumed loss of consciousness. We collected 64-channel EEG data and prefrontal–frontal and anterior–posterior functional connectivity in the alpha band (8–14 Hz) was measured using coherence and weighted phase lag index (wPLI). Directed connectivity was measured with directed phase lag index (dPLI).

Results: Prefrontal–frontal EEG-based connectivity discriminated the states at the different drug concentrations. At ROR, prefrontal–frontal connectivity reversed to the level observed before LOR, indicating that connectivity changes were related to unresponsiveness rather than drug concentration. Unresponsiveness was associated with emergence of frontal-to-prefrontal dominance (dPLI: −0.13 to −0.40) in contrast to baseline (dPLI: 0.01–0.02). Coherence, wPLI, and dPLI had similar capability to discriminate the states that differed in terms of responsiveness and drug concentration. In contrast, anterior–posterior connectivity in the alpha band did not differentiate LOR and ROR.

Conclusions: Local prefrontal–frontal EEG-based connectivity reflects unresponsiveness induced by propofol or dexmedetomidine, suggesting its utility in monitoring the anaesthetised state with these agents.

Clinical trial registration: NCT01889004.

Keywords: anaesthesia; dexmedetomidine; directed connectivity; electroencephalogram; functional connectivity; propofol; responsiveness

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Editor's key points

- There are currently no EEG patterns that faithfully reflect anaesthetic-related transitions between responsiveness and unresponsiveness, and *vice versa*.
- Electroencephalogram-derived measures that are supposedly reflecting connectivity between brain regions were calculated.
- With propofol and dexmedetomidine, the transitions between responsiveness and unresponsiveness were associated with changes in functional and directed connectivity between frontal and prefrontal regions, assessed from electroencephalographic alpha waves (8–14 Hz).
- These findings enhance neurobiological understanding of state transitions and have translational implications for monitoring during sedation and general anaesthesia.

The role of frontal alpha oscillations as an EEG-based correlate of anaesthetic-induced unconsciousness has long been studied, but it is a topic of current controversy. Enhanced frontal alpha power has been correlated with anaesthetic-induced unresponsiveness with different anaesthetics in a concentration- and state-dependent manner.[1–3] Reduced or even reversed anterior–posterior feedback connectivity has been considered as a neural correlate of unresponsiveness,[4–12] although recent evidence has suggested that anterior–posterior functional connectivity in the alpha band during general anaesthesia is not stable, but rather fluctuates dynamically and non-randomly over time.[13,14] In terms of local connectivity within the anterior area, both dexmedetomidine and propofol induce frontal coherence in broad alpha band,[1,15,16] and increased frontal-to-prefrontal connectivity within the alpha–beta frequency band has been reported in moderate and deep propofol sedation.[17] Regardless of these converging findings on frontal alpha activity, which have been further corroborated by fMRI studies that showed changes in the activation and connectivity of the frontal lobe in response to anaesthetics,[18–20] connected consciousness can occur despite the presence of a prominent frontal alpha-delta EEG pattern during surgical anaesthesia.[21]

Despite this body of work, important questions remain unanswered. First, changes in alpha cortical connectivity patterns induced by dexmedetomidine, with distinct molecular mechanisms from propofol, are poorly understood. Second, although several studies have observed different connectivity patterns between anaesthetic-induced unresponsiveness and wakefulness,[4–10,12] mild and unresponsive sedation,[10,17,19] or unresponsive sedation and recovery period,[5,6,8,9] none of them have investigated unresponsiveness independently of drug concentration effect. Third, the roles of local and long-range connectivity measures as correlates of anaesthetic-induced unresponsiveness are still not completely understood.

To address these questions, we used individually titrated concentrations of dexmedetomidine and propofol to discriminate connectivity changes related to unresponsiveness from drug-induced effects: changes associated with an unresponsive state instead of the anaesthetic concentration are assumed to be reversed by a recovery of responsiveness even in the presence of continuous drug administration and independently of the drug mechanism. Using two measures for functional connectivity and one for directed connectivity, we compared the effect of anaesthetics on local frontal connectivity and long-range anterior–posterior connectivity. As far as we know, this is the first study to explore the effects of dexmedetomidine on EEG-based measures of connectivity other than coherence.[1,15]

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. The spontaneous EEG epochs analysed in the current study have previously been used in a study on spectral power and phase–amplitude coupling.[3] Event-related potential results and subjective experiences from the same experiment have also been reported elsewhere.[22,23] Detailed information on the participants, anaesthetic protocol, and EEG measurement of the experiment has been previously described.[3]

Participants

The participants were 47 healthy (American Society of Anesthesiologists physical status 1) 20- to 30-yr-old males. Only males were included because of the radiation exposure related to a subsequent positron emission tomography study. They were randomised to receive either dexmedetomidine (n=23) or propofol (n=24).

Anaesthetic protocol

Briefly, an awake baseline EEG measurement with eyes closed was followed by target-controlled infusions of dexmedetomidine or propofol. The pseudo-steady-state plasma concentration of the anaesthetic was increased stepwise every 7 min until loss of responsiveness (LOR) (Fig 1). Loss of responsiveness was defined as a failure to respond to the 10 sentence stimuli (0/10) presented at each anaesthetic concentration. The pseudo-steady-state infusion was maintained ~25 min and stimuli to evoke event-related potentials were presented.[22] An attempt to arouse the participant was then made by calling him twice by name, with increasing volume, and shaking the shoulder to achieve return of responsiveness (ROR). If the participant woke up, he was interviewed[23] and then left unstimulated until a possible second LOR–ROR cycle. As we have reported, ROR was achieved in 18 dexmedetomidine and 10 propofol participants (78% and 42%, respectively).[3] In addition, most participants who woke up to ROR reported experiences from the preceding unresponsive period in the interview (dexmedetomidine 94%; propofol 80%).[23] Next, the target concentration was increased to 150% to reach a state assumed to represent loss of consciousness (LOC) before the drug infusion was ceased.

Electroencephalogram was recorded at 64 channels as described.[3] Stimulus-free 2 min epochs of EEG were segmented from six states for further analysis: baseline, last responsive sedation (SED), LOR, LOR_{late} (before the arousal attempt), ROR (after the interview), and LOC. The epochs were preprocessed[3] and cut to 110 s after removing artifactual signal segments. The preprocessed EEG signals were re-referenced to mastoid reference (averaged over TP9 and TP10).

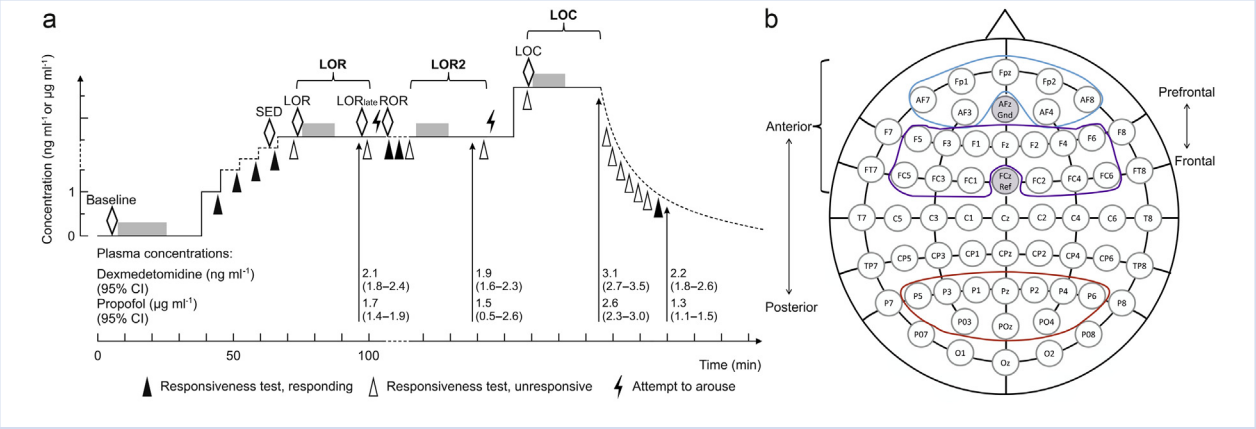

Fig 1. (a) Design of the experiment. The dosing of dexmedetomidine or propofol required to achieve loss of responsiveness (LOR) was individually determined by stepwise increments of plasma target concentrations and repeated testing of responsiveness. With dexmedetomidine, the starting target plasma concentration was 1.0 ng ml^{-1}, after which the target concentration was increased by 0.5 ng ml^{-1} and the following increments were 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 subsequent increments were 0.25 µg ml^{-1} each. The participant was attempted to be aroused from LOR to return of responsiveness (ROR), and then interviewed. After a second LOR–ROR cycle, the target concentration was increased 1.5-fold to induce presumed loss of consciousness (LOC). Event-related potential stimuli were presented in Baseline, LOR, and LOC, and they are marked with grey boxes.[22] Arrows show the timing of the blood samples used to determine the mean measured drug plasma concentrations.[3] The analysed EEG epochs are represented with diamonds. The LOR and LOR$_{late}$ epochs were both included to control the stability of the relatively long state. CI, confidence interval; SED, highest sedative concentration on which the participant was responsive. (b) Regions of interest between which coherence, weighted phase lag index, and directed phase lag index were calculated.

Connectivity measures

The undirected functional connectivity was estimated with magnitude squared coherence and weighted phase lag index (wPLI),[24] whose values range from 0 to 1. Higher values suggest stronger correlation (for coherence) or more consistent phase locking (for wPLI) between two signals. The directed connectivity was assessed with directed phase lag index (dPLI) that measures the asymmetry of lead/lag relationship of the phases of two signals with values ranging from −1 to 1, where the absolute value represents the strength of the phase locking and the sign indicates direction.[25] Both wPLI and dPLI are robust with respect to volume conduction and reference montages.[24,25] As coherence is sensitive to volume conduction effect, surface Laplacian transform was applied before the calculation of coherence.[26]

For implementation, the EEG epochs were divided into 2 s non-overlapping windows. For each window, the cross-spectral density was estimated using the multitaper method, with time–bandwidth product of 2 and number of tapers of 3,[27] and from these repetitions the averaged coherence, wPLI and dPLI values were estimated as a function of frequency, using the FieldTrip toolbox.[28] To mitigate the potential bias in the estimated measures, a series (n=20) of shuffled signal pairs were generated[29] and used to calculate the coherence, wPLI and dPLI values, the mean of which were subtracted from the raw connectivity values to achieve the final estimates of connectivity.

The analysis was focused on anterior and posterior areas (Fig 1), where anaesthetic-induced changes have been reported.[4–12] Another analysis was conducted within the anterior area,[6,8,13,14,17] which was evenly divided into prefrontal and frontal areas (Fig 1). The coherence, wPLI and dPLI values between prefrontal and frontal or between anterior and posterior regions were calculated for each pair of channels in the two regions and then averaged. By definition, positive dPLI indicates dominant phase-lead relationship from front to back (anterior to posterior or prefrontal to frontal) and negative values from back to front (posterior to anterior or frontal to prefrontal). For statistical comparisons, the mean coherence, wPLI and dPLI were calculated in the alpha frequency band of 8–14 Hz. For comparison, the spectral power of alpha band was analysed as described previously,[3] limiting the analysis to the anterior area used in the current study.

Statistical analysis

Differences between the states were analysed using two-way repeated-measures analysis of variance with SAS/STAT, PROC MIXED (version 9.4; SAS Institute, Inc., Cary, NC, USA). State was set as a within factor and treatment (dexmedetomidine or propofol) as a between factor. We conducted two separate analyses: one comparing states with increasing anaesthetic concentration (baseline, SED, LOR, and LOC) and including all participants, and the other comparing epochs measured during the constant dosing (LOR, LOR$_{late}$, and ROR), including only participants in whom ROR was achieved. When the state-by-treatment interaction was significant, the *post hoc* tests for pairwise comparisons between states were performed separately for dexmedetomidine and propofol. If the interaction was not significant, the treatment groups were combined and the pairwise comparisons were adjusted for treatment. The *post hoc* tests were corrected using the Bonferroni method. Two-tailed P-values <0.05 were considered statistically significant.

Prediction probability (P_K) was used to assess how well the connectivity measures and anterior alpha power

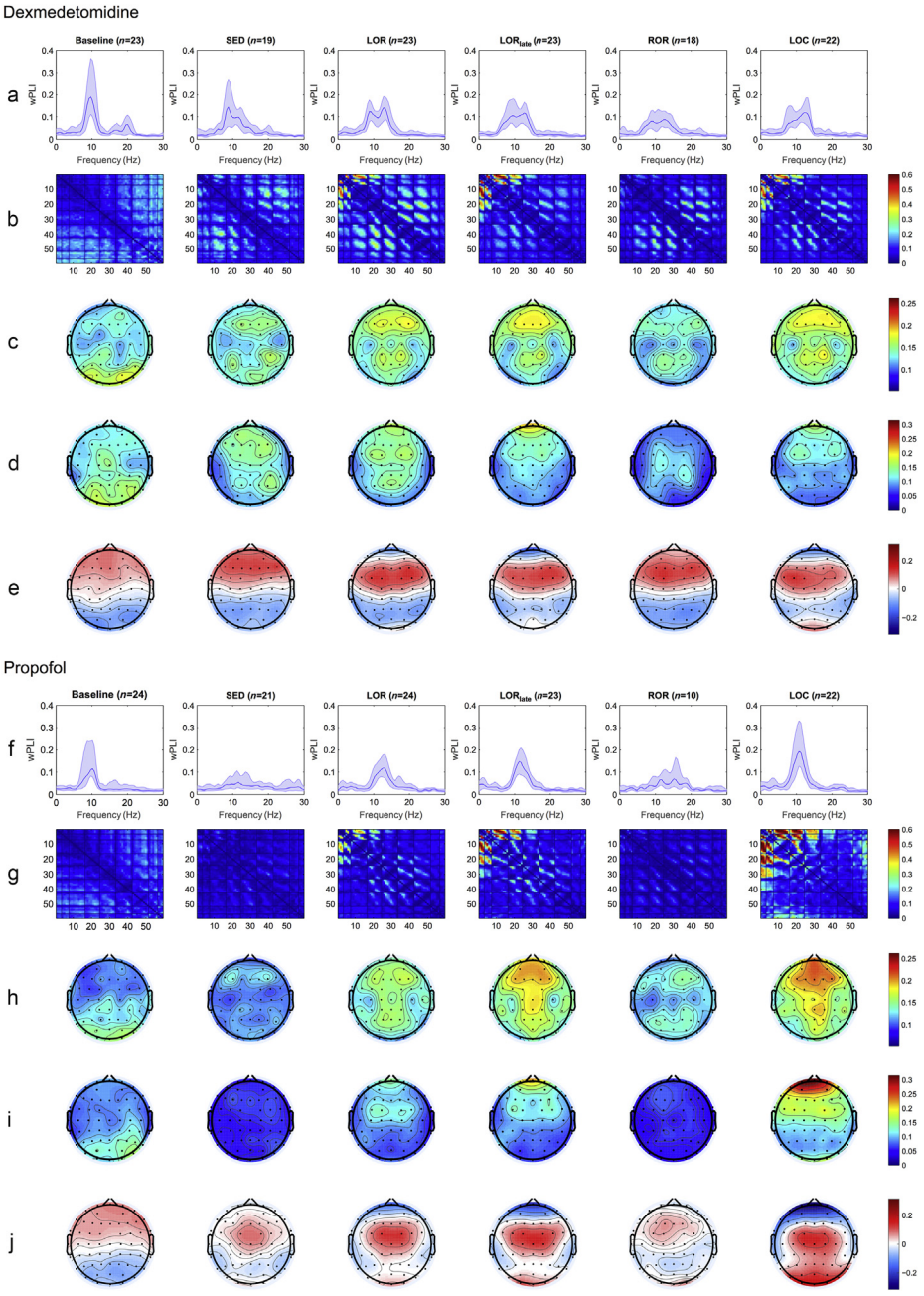

Fig 2. Spectral and topographic properties of cortical connectivity associated with dexmedetomidine (a–e) or propofol (f–j). (a) and (f) Mean weighted phase lag index (wPLI) across all electrode pairs by frequency, bold line shows the median, and shaded region indicates the 25% and 75% percentiles of the values from all the participants. (b) and (g) Group-level wPLI matrix (median across participants) in alpha band between all channel pairs (low numbers correspond to frontal electrodes). (c) and (h) Mean alpha band coherence in each channel averaged over all other channels. (d) and (i) Mean alpha band wPLI in each channel averaged over all other channels. (e) and (j) Mean alpha band directed phase lag index (dPLI) in each channel averaged over all other channels. The wPLI and dPLI results were calculated using mastoid average reference, whilst surface Laplacian transform was used in the coherence analysis to mitigate volume conduction effect, which highlights local spatial features, but attenuates spatially broad activities. LOC, loss of consciousness; LOR, loss of responsiveness; ROR, return of responsiveness; SED, highest responsive sedation.

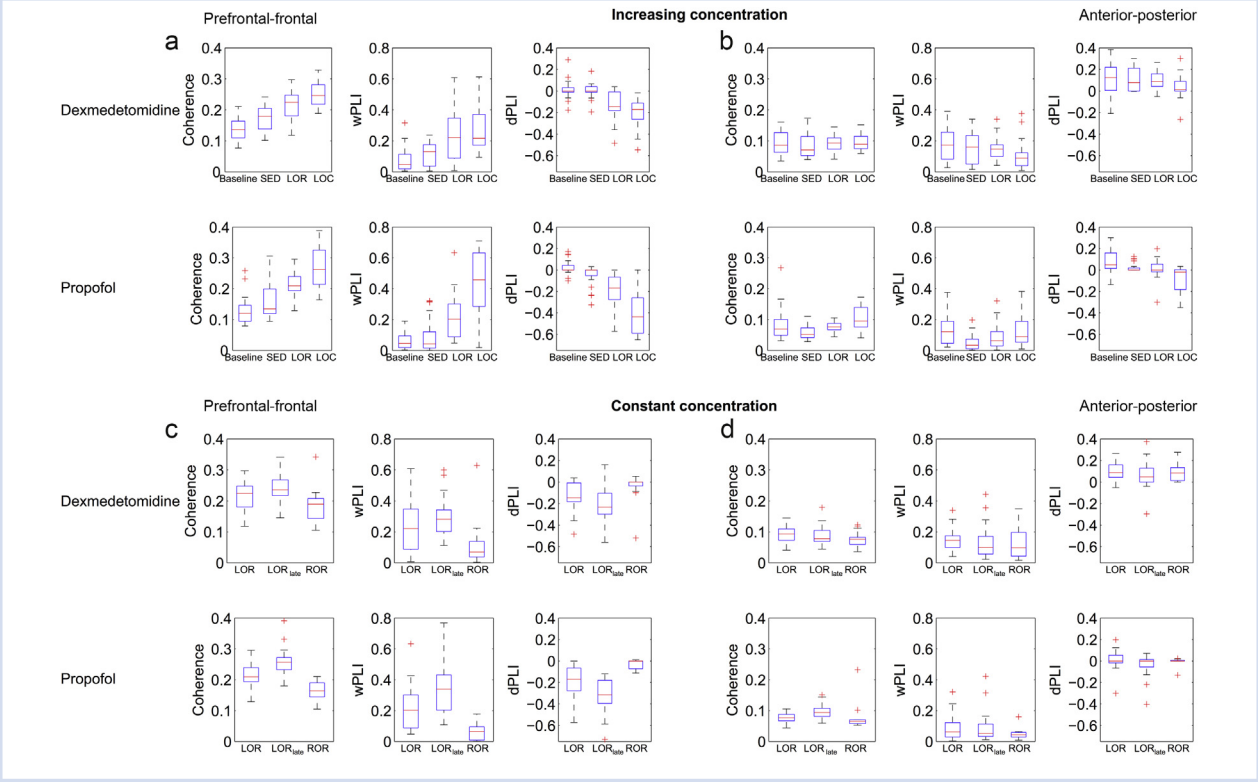

Fig 3. Connectivity values (coherence, weighted phase lag index [wPLI], and directed phase lag index [dPLI]) in the states with increasing (baseline, last sedation [SED], loss of responsiveness [LOR], and presumed loss of consciousness [LOC]) and constant (loss of responsiveness [LOR and LOR$_{late}$] and return of responsiveness [ROR]) drug concentration. (a) Increasing concentration, prefrontal–frontal region; (b) increasing concentration, anterior–posterior region; (c) constant concentration, prefrontal–frontal region; and (d) constant concentration, anterior–posterior region. The boxes of Tukey box plots show the lower and upper quartiles, the red horizontal line indicates the median, and the whiskers extend to extrema that are still within 1.5 inter-quartile range from the box.

differentiated LOR and ROR, and the four states with increasing drug concentration (baseline, SED, LOR, and LOC).[30,31] Prediction probability is the multi-class generalisation of the non-parametric estimation of area under the receiver operating characteristic curve (AUC), and it is based on weighting AUC for distinct pairs of states by normalised products of state probabilities. If P_K is 1.0, then the measure predicts the observed state correctly, and P_K=0.5 corresponds to the chance level. P_K and its standard error were estimated using the jackknife method with PKMACRO Microsoft Excel macro.[30] The P_K values were compared to the random level 0.5 and between different measures using Bonferroni-corrected paired *t*-tests with PKDMACRO.[30]

Results

Spectral and topographic characteristics of connectivity

Both dexmedetomidine and propofol produced frequency-dependent changes in averaged wPLI connectivity, predominantly in the alpha band (8–14 Hz) (Fig 2). We therefore focused on the alpha frequency band that has also been associated with anaesthesia-induced unresponsiveness.[8,9,32–35] Topographic analysis of alpha connectivity as assessed by coherence, wPLI, and dPLI showed state-dependent changes especially in the anterior areas (Fig 2), as expected based on the literature.[6,8,13,14,17] The visualisations of wPLI and dPLI calculated using surface Laplacian transformation showed a similar pattern as mastoid-referenced wPLI and dPLI (data not shown).

Concentration- and state-dependent changes in alpha connectivity

When states with different anaesthetic concentrations (baseline, SED, LOR, and LOC) were compared, the prefrontal–frontal connectivity differed between the states based on coherence, wPLI, and dPLI (P<0.001; Fig 3; Table 1). With both drugs, coherence and wPLI strongly increased with the increasing concentration. This may be explained by the switch of the prefrontal–frontal phase lead–lag relationship in baseline to frontal-to-prefrontal pattern in drug-induced states as observed in the dPLI analysis. The dominant direction of connectivity differed already between baseline and responsive sedation in the propofol group (P=0.041), and the effect strengthened with the increasing dose and the LOR in both treatment groups (P<0.001 for sedation *vs* unresponsive states).

For the anterior–posterior connectivity, coherence (P=0.002), wPLI (P=0.051), and dPLI (P<0.001) separated the states associated with different anaesthetic concentrations

Table 1 Coherence, weighted phase lag index (wPLI), and directed phase lag index (dPLI) at states with increasing drug concentrations (baseline, highest responsive sedation [SED], loss of responsiveness [LOR], and loss of consciousness [LOC]). Estimated mean values from the statistical model. SE, standard error. The pairwise *P*-values show the difference to the reference state (Ref.) of each column and are Bonferroni corrected.

		Mean	SE	*P* for pairwise comparisons		
				Comparison with baseline	Comparison with SED	Comparison with LOR
Prefrontal–frontal coherence (state $P<0.001$; treatment $P=0.716$; state*treatment $P=0.406$)						
Dexmedetomidine+propofol, treatment adjusted	Baseline	0.1339	0.0057	Ref.		
	SED	0.1671	0.0082	0.007	Ref.	
	LOR	0.2133	0.0063	<0.001	<0.001	Ref.
	LOC	0.2593	0.0080	<0.001	<0.001	<0.001
Prefrontal–frontal wPLI (state $P<0.001$; treatment $P=0.192$; state*treatment $P=0.004$)						
Dexmedetomidine	Baseline	0.0762	0.0141	Ref.		
	SED	0.1155	0.0218	0.7674	Ref.	
	LOR	0.2270	0.0306	<0.001	0.020	Ref.
	LOC	0.2634	0.0379	<0.001	0.002	1
Propofol	Baseline	0.0634	0.0138	Ref.		
	SED	0.0894	0.0208	1	Ref.	
	LOR	0.2207	0.0299	<0.001	0.003	Ref.
	LOC	0.4377	0.0377	<0.001	<0.001	<0.001
Prefrontal–frontal dPLI (state $P<0.001$; treatment $P=0.003$; state*treatment $P=0.005$)						
Dexmedetomidine	Baseline	0.0130	0.0158	Ref.		
	SED	0.0043	0.0202	1	Ref.	
	LOR	−0.1336	0.0292	0.001	<0.001	Ref.
	LOC	−0.1955	0.0371	<0.001	<0.001	0.691
Propofol	Baseline	0.0213	0.0154	Ref.		
	SED	−0.0448	0.0193	0.041	Ref.	
	LOR	−0.1828	0.0286	<0.001	<0.001	Ref.
	LOC	−0.4026	0.0369	<0.001	<0.001	<0.001
Anterior–posterior coherence (state $P=0.002$; treatment $P=0.075$; state*treatment $P=0.064$)						
Dexmedetomidine+propofol, treatment adjusted	Baseline	0.0888	0.0070	Ref.		
	SED	0.0721	0.0048	0.174	Ref.	
	LOR	0.0847	0.0030	1	0.112	Ref.
	LOC	0.0994	0.0048	1	0.002	0.030
Anterior–posterior wPLI (state $P=0.051$; treatment $P=0.002$; state*treatment $P=0.035$)						
Dexmedetomidine	Baseline	0.1805	0.0220	Ref.		
	SED	0.1585	0.0191	1	Ref.	
	LOR	0.1514	0.0160	1	1	Ref.
	LOC	0.1110	0.0207	0.142	0.662	0.608
Propofol	Baseline	0.1383	0.0215	Ref.		
	SED	0.0490	0.0182	0.015	Ref.	
	LOR	0.0875	0.0156	0.227	0.439	Ref.
	LOC	0.1241	0.0207	1	0.069	0.805
Anterior–posterior dPLI (state $P<0.001$; treatment $P<0.001$; state*treatment $P=0.266$)						
Dexmedetomidine+propofol, treatment adjusted	Baseline	0.0903	0.0184	Ref.		
	SED	0.0643	0.0126	1	Ref.	
	LOR	0.0560	0.0125	0.926	1	Ref.
	LOC	−0.0216	0.0167	<0.001	<0.001	<0.001

(Fig 3; Table 1). The dPLI analysis suggested that the direction of phase lead–lag relationship turned from anterior–posterior pattern in baseline to posterior-to-anterior dominance in LOC. Coherence and wPLI showed a transient but mostly non-significant decrease in sedation and LOR in the propofol group, but the values in LOC were similar to baseline (Fig 3).

When states with constant drug concentration (LOR, LOR_{late}, and ROR) were studied, the prefrontal–frontal connectivity measures separated ROR from LOR and LOR_{late} (Fig 3; Table 2). Notably, in ROR, the phase lead–lag relationship measured with dPLI reverted from frontal-to-prefrontal dominance during unresponsiveness to a level comparable with the responsive states preceding LOR. The differences between LOR and LOR_{late} epochs indicated that the unresponsive period was not completely stable despite the pseudo-steady-state target concentration; the connectivity values shifted towards LOC values, with an increase in absolute connectivity values from LOR to LOR_{late}.

As opposed to prefrontal–frontal connectivity measures, there were no major differences between states achieved during constant dosing in the anterior–posterior connectivity in either dexmedetomidine or propofol group (Fig 3; Table 2). Importantly, anterior–posterior connectivity measured with coherence, wPLI, or dPLI did not differentiate ROR from LOR and LOR_{late}, which suggests that there was no effect of responsiveness when the drug concentration remained constant. The levels of the wPLI and dPLI measures observed during the administration of the anaesthetics differed between dexmedetomidine and propofol ($P=0.012$ for wPLI; $P<0.001$ for dPLI).

Table 2 Coherence, weighted phase lag index (wPLI) and directed phase lag index (dPLI) at states with constant drug concentration (loss of responsiveness [LOR and LOR_{late}] and return of responsiveness [ROR]). Estimated mean values from the statistical model. SE, standard error. The pairwise *P*-values show the difference to the reference state (Ref.) of each column and are Bonferroni corrected.

		Mean	SE	*P* for pairwise comparisons	
				Comparison with LOR	Comparison with LOR_{late}
Prefrontal–frontal coherence (state $P<0.001$; treatment $P=0.930$; state*treatment $P=0.247$)					
Dexmedetomidine+propofol, treatment adjusted	LOR	0.2133	0.0063	Ref.	
	LOR_{late}	0.2475	0.0070	<0.001	Ref.
	ROR	0.1795	0.0093	0.004	<0.001
Prefrontal–frontal wPLI (state $P<0.001$; treatment $P=0.910$; state*treatment $P=0.095$)					
Dexmedetomidine+propofol, treatment adjusted	LOR	0.2238	0.0214	Ref.	
	LOR_{late}	0.3213	0.0217	<0.001	Ref.
	ROR	0.1009	0.0225	<0.001	<0.001
Prefrontal–frontal dPLI (state $P<0.001$; treatment $P=0.171$; state*treatment $P=0.017$)					
Dexmedetomidine	LOR	−0.1336	0.0292	Ref.	
	LOR_{late}	−0.2096	0.0347	0.038	Ref.
	ROR	−0.0618	0.0244	0.050	<0.001
Propofol	LOR	−0.1828	0.0286	Ref.	
	LOR_{late}	−0.3237	0.0344	<0.001	Ref.
	ROR	−0.0448	0.0301	<0.001	<0.001
Anterior–posterior coherence (state $P=0.063$; treatment $P=0.903$; state*treatment $P=0.008$)					
Dexmedetomidine	LOR	0.0925	0.0043	Ref.	
	LOR_{late}	0.0883	0.0056	1	Ref.
	ROR	0.0770	0.0088	0.302	0.649
Propofol	LOR	0.0770	0.0042	Ref.	
	LOR_{late}	0.0978	0.0055	0.001	Ref.
	ROR	0.0854	0.0116	1	0.900
Anterior–posterior wPLI (state $P=0.139$; treatment $P=0.012$; state*treatment $P=0.859$)					
Dexmedetomidine+propofol, treatment adjusted	LOR	0.1195	0.0112	Ref.	
	LOR_{late}	0.1118	0.0150	1	Ref.
	ROR	0.0893	0.0146	0.148	0.399
Anterior–posterior dPLI (state $P=0.003$; treatment $P\leq0.001$; state*treatment $P=0.830$)					
Dexmedetomidine+propofol, treatment adjusted	LOR	0.0560	0.0125	Ref.	
	LOR_{late}	0.0141	0.0171	0.003	Ref.
	ROR	0.0290	0.0130	0.181	1

The coherence, wPLI, and dPLI were tested also in prefrontal–posterior and frontal–posterior areas to ensure that there is no bias caused by broad anterior area, and the results were very similar to anterior–posterior results (data not shown).

Ability of alpha connectivity measures to discriminate states

To assess the performance of alpha cortical connectivity as potential indicator of depth of anaesthesia, prediction probability (P_K) values were calculated to compare the connectivity measures with each other and with anterior alpha power values.[3] Anterior alpha power and prefrontal–frontal wPLI and dPLI discriminated LOR and ROR states and states with increasing concentration (baseline, SED, LOR, and LOC), as indicated by P_K values (Table 3). There were no significant differences in the P_K values between different measures in LOR–ROR comparison with either of the two drugs (dexmedetomidine P_K 0.71–0.80; propofol P_K 0.79–0.88), or in baseline–SED–LOR–LOC comparison with propofol (P_K 0.81–0.86). However, in the dexmedetomidine group, the connectivity measures had significantly higher P_K values (0.76–0.83) than the alpha spectral power (0.61) when states with increasing drug concentration were analysed. The correlation between anterior alpha power and connectivity was fairly high across the different drug concentrations, especially in the propofol group (Supplementary Fig 1).

In contrast to the prefrontal–frontal measures, anterior–posterior connectivity did not discriminate LOR and ROR (P_K 0.53–0.67; *P*-values 0.176–1 for comparison with the chance level) (Supplementary Table 1). In the propofol group, coherence and dPLI differentiated states with increasing concentration above chance level ($P=0.006$ and $P<0.001$, respectively). Similar to prefrontal–frontal measures, anterior–posterior connectivity correlated with anterior alpha power (Supplementary Fig 2).

Discussion

In the current study, dexmedetomidine and propofol were titrated individually to different behavioural endpoints based on LOR, and their effects on EEG connectivity patterns were explored. Despite distinct molecular mechanisms of action, both drugs induced changes related to increasing drug concentrations in prefrontal–frontal functional connectivity and reversion of the prefrontal–frontal directed connectivity in alpha frequency band, suggesting suppression of feedback connectivity in the most anterior leads. Upon ROR, the connectivity returned to a level comparable with the preceding responsive states despite constant drug infusion, indicating that the connectivity changes were related to unresponsiveness rather than the drug concentration itself. In contrast,

Table 3 Prediction probability (P_K) of anterior alpha power, and prefrontal–frontal coherence, weighted phase lag index (wPLI), and directed phase lag index (dPLI). P_K values are compared with the chance level (0.5) and with the P_K values of other measures. *P*-values of paired *t*-tests are Bonferroni corrected. LOC, loss of consciousness; LOR, loss of responsiveness; ROR, return of responsiveness; SE, standard error; SED, highest responsive sedation.

		LOR–ROR					Baseline–SED–LOR–LOC				
		P_K (SE)	*P* (*vs* 0.5)	*P* (comparison with power)	*P* (comparison with coherence)	*P* (comparison with wPLI)	P_K (SE)	*P* (*vs* 0.5)	*P* (comparison with power)	*P* (comparison with coherence)	*P* (comparison with wPLI)
Dexmedetomidine	Power	0.80 (0.07)	<0.001	Ref.			0.61 (0.04)	0.019	Ref.		
	Coherence	0.71 (0.08)	0.065	1	Ref.		0.83 (0.02)	<0.001	<0.001	Ref.	
	wPLI	0.74 (0.08)	0.029	1	1	Ref.	0.76 (0.03)	<0.001	0.019	0.069	Ref.
	dPLI	0.77 (0.08)	0.005	1	1	1	0.80 (0.03)	<0.001	<0.001	1	1
Propofol	Power	0.79 (0.09)	0.009	Ref.			0.83 (0.02)	<0.001	Ref.		
	Coherence	0.83 (0.08)	<0.001	1	Ref.		0.83 (0.03)	<0.001	1	Ref.	
	wPLI	0.85 (0.07)	<0.001	1	1	Ref.	0.81 (0.03)	<0.001	1	1	Ref.
	dPLI	0.88 (0.06)	<0.001	1	1	1	0.86 (0.03)	<0.001	1	1	0.339

anterior–posterior connectivity in the alpha band was not associated with unresponsiveness. These results suggest that prefrontal–frontal alpha connectivity could provide a means to differentiate brain states of sedated patients independent of the anaesthetic drugs used. This is further supported by evidence that ketamine anaesthesia, which has a mechanism distinct from both propofol and dexmedetomidine, is associated with a suppression of prefrontal–frontal dPLI in the alpha bandwidth.[8] To our knowledge, these are the first data on the effects of dexmedetomidine on directed connectivity, and the results may be relevant for its clinical use in ICU settings.

We focused on the alpha frequency band (8–14 Hz), where the largest anaesthetic-induced changes in connectivity were observed (Fig 2) and with which anaesthetic-induced unresponsiveness has previously been associated.[8,9,32–35] Both propofol and dexmedetomidine increase alpha power, especially in the anterior channels, but the power of frontal alpha induced by propofol is several-fold higher than that of dexmedetomidine.[1,3] Unlike dexmedetomidine, propofol also shows the stereotypical phase–amplitude coupling between the phase of slow waves and the power of the alpha band.[3,16,36] In addition, propofol is known to induce alpha hypercoherence, which leads to disconnection of the frontal cortex from the other parts of the brain.[19,20,35,37,38] Although frontal alpha-delta pattern can occur independent of the state of responsiveness in surgical anaesthesia,[21] our study demonstrates that alpha band connectivity is indeed linked to the state of responsiveness instead of mere drug concentration with both dexmedetomidine and propofol.

Our results on directed connectivity show that the net direction of local connectivity was reversed by anaesthetics, which can be interpreted as loss of feedback connectivity in the anterior channels. By distinguishing between state- and concentration-related effects, our results extend the previous observations that both dexmedetomidine and propofol induce increased frontal alpha coherence.[1] Furthermore, higher and more stereotypical prefrontal–frontal functional connectivity of alpha–beta band has been reported in unresponsive propofol sedation compared with responsive states, with frontal channels leading prefrontal electrodes.[17] Whilst our study focused on the discrimination of responsive and unresponsive states, the observed changes in prefrontal–frontal connectivity further strengthened from LOR to LOC. Prefrontal–frontal connectivity may thus reflect the participant's state also with doses higher than those required for the LOR, and a threshold value representing LOC may potentially exist. The clinical applications of prefrontal–frontal measures are facilitated by the ease of accessing the required electrodes in the operating theatre. Whilst many of the most prominent changes in connectivity and spectral power co-occur in the same frequency band, as seen in the current study and also reported previously,[8,17] the connectivity measures utilised in the present study differentiated the increasing drug concentrations better than alpha power in the dexmedetomidine group.

We demonstrated that local prefrontal–frontal connectivity is a more relevant marker of unresponsiveness than long-range measures in the alpha frequency band. The disruption of anterior–posterior feedback connectivity and long-distance corticocortical networks of the waking state in general has previously been suggested as the key mechanism of anaesthetic-induced unresponsiveness based on fMRI and EEG data.[7,10–12,39] Moreover, the anaesthetic-induced decrease of frontal-to-parietal EEG connectivity has been observed to

correlate with decrease in the functional connectivity of the anterior default mode network and thalamocortical networks measured with fMRI.[4,12] However, recent evidence has suggested that the connectivity patterns fluctuate and frontoparietal connectivity does not strictly correlate with unresponsiveness in humans or rodents.[13,14,40] The current results speak in favour of local frontal connectivity measures and are in line with a study in non-human primates, where the repertoire of functional connectivity was shown to be constrained to structurally connected regions during anaesthesia and an increase in functional correlation within prefrontal areas was observed.[41] We focused on the broad alpha frequency band, and future studies should explore whether the long-range connectivity in frequencies outside the alpha bandwidth might reflect the behavioural state during constant dosing. Nevertheless, based on Fig 2, most of the overall wPLI changes between 0 and 30 Hz occurred in the alpha frequency range in the present study. Even in the alpha band, the hypothesis of anterior–posterior connectivity as a marker of consciousness cannot be completely abandoned, as we observed reversed net direction of anterior–posterior connectivity during presumed LOC.

In this study, the experimental setting where two different anaesthetics were individually and carefully titrated in healthy participants allowed us to separate the effects of state and drug concentration without confounding effects of surgery, pain, or polypharmacy. Two different measures of functional connectivity and one for directed connectivity support the robustness of the results. The conclusions are also supported by observing similar effects using two drugs acting through different molecular mechanisms: dexmedetomidine is an α_2-receptor agonist and propofol acts mainly through the enhancement of gamma-aminobutyric acid system. Comparing connectivity in drug-induced unresponsiveness and natural sleep could provide further confirmation of the current conclusions and provide insight into the mechanisms involved. Responsiveness to sentence stimuli is composed of several components: motor function, connectedness, comprehension, and willingness to follow instructions. Dissecting the phenomena behind the observed changes in prefrontal–frontal connectivity could be a subject for future studies.

Despite the strengths of the experimental setting, our results are based on group-level analysis, and alpha band connectivity did not serve as a reliable single-subject indicator of unresponsiveness. The use of averaged connectivity values in 2-min-long EEG epochs loses the temporal resolution required to capture the dynamic nature of connectivity.[13] For clinical use, connectivity measures would need to be calculated in shorter epochs (e.g. 10 s) than in the present research-oriented setting. The sensor-based analysis that was restricted to two different electrode combinations is compatible with the clinical setting, where frontal channels are easily accessed, but did not provide information on the actual signal sources or underlying networks. If the participant did not wake up to ROR, we did not record EEG comparable with ROR, but directly proceeded with dose increment. Therefore, we were not able to compare the connectivity between the aroused participants and those who failed to be aroused, and thus could not control for the effect of the arousal attempt itself.

We were able to separate electroencephalographic effects associated with unresponsiveness from those directly associated with dexmedetomidine or propofol administration. Despite the different molecular mechanisms of the drugs and the differences in their effects on the alpha spectral power, both drugs increased the functional alpha connectivity and shifted the baseline prefrontal–frontal direction of alpha connectivity to the frontal-to-prefrontal direction. Return of responsiveness during constant drug infusion reversed the effects on the prefrontal–frontal alpha connectivity, indicating that the changes are related to the state of responsiveness. Thus, prefrontal–frontal alpha connectivity could help differentiate brain states of anaesthetised patients independently of the anaesthetic drug used.

Authors' contributions

Study design/planning: REK, KV, AS, JL, AM, AR, HS, GAM, DL
Principal investigation: HS
Experimentation: REK, KV, AS, JL, AM, HS
Data analysis: REK, DL
Statistical analysis: TV
Drafting of paper: REK, DL
Revising of paper: all authors

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2020.05.068.

Declarations of interest

The authors declare that they have no conflicts of interest.

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