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Changes in measures of consciousness during anaesthesia of one hemisphere (Wada test)

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ABSTRACT

Background: In the Wada test, one hemisphere is selectively anaesthetised by unilateral intracarotid injection of a fast-acting anaesthetic agent. This gives a unique opportunity to observe the functions and physiological activity of one hemisphere while anaesthetising the other, allowing direct comparisons between brain states and hemispheres that are not possible in any other setting.

Aim: To test whether potential measures of consciousness would be affected by selective anaesthesia of one hemisphere, and reliably distinguish the states of the anesthetised and non-anesthetised hemispheres.

Methods: We analysed EEG data from 7 patients undergoing Wada-tests in preparation for neurosurgery and computed several measures reported to correlate with the state of consciousness: power spectral density, functional connectivity, and measures of signal diversity. These measures were compared between conditions (normal rest vs. unilateral anaesthesia) and hemispheres (injected vs. non-injected), and used with a support vector machine to classify the state and site of injection objectively from individual patient's recordings.

Results: Although brain function, assessed behaviourally, appeared to be substantially altered only on the injected side, we found large bilateral changes in power spectral density for all frequency bands tested, and functional connectivity changed significantly both between and within both hemispheres. Surprisingly, we found no statistically significant differences in the measures of signal diversity between hemispheres or states, for the group of 7 patients, although 4 of the individual patients showed a significant decrease in signal diversity on the injected side. Nevertheless, including signal diversity measures improved the classification results, indicating that these measures carry at least some non-redundant information about the condition and injection site. We propose that several of these results may be explained by conduction of activity, via the corpus callosum, from the injected to the contralateral hemisphere and vice versa, without substantially affecting the function of the receiving hemisphere, thus reflecting what we call "cross-state unreceptiveness".

1. Introduction

The nature of consciousness (conscious awareness) is widely regarded as one of the deepest unsolved problems in science (Chalmers, 1997; Crick, 1995; Dehaene, 2014; Koch, 2004; Llinás et al., 1998; Tononi and Edelman, 1998), and has been called "the major unsolved problem in biology" (Crick, 2004). Our limited understanding of conscious processing, and the severe difficulties connected with objectively assessing the presence or absence of consciousness, have wide-ranging theoretical and practical implications (Dehaene and Changeux, 2004; Monti et al., 2010; Tononi and Koch, 2015), including clinical and ethical problems regarding treatment of highly vulnerable patient groups, such as those suffering from disorders of consciousness (DOC) after brain injury (Giacino et al., 2014; Laureys et al., 2009; Owen et al., 2006, 2009; Schnakers et al., 2009;). Also for surgical anaesthesia, reliable methods for assessing states of consciousness are needed, as patients occasionally regain consciousness without this being detected by the clinicians (Ghoneim et al., 2009). Therefore, there is an urgent need for developing reliable methods for objectively assessing states of consciousness.

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In recent years, novel methods and theoretical advances have yielded promising results and opened up the field for scientific and clinical progress, including development of various measures of consciousness (Casali et al., 2013; Dehaene and Changeux, 2011; Seth et al., 2006; Storm et al., 2017). However, there is still a lack of reliable, well-tested, practical methods and measures for objective assessment of states of consciousness. This deficit is related to fundamental uncertainties regarding the theoretical framework for understanding consciousness. Although several such frameworks have been proposed and gained empirical support in recent years, e.g. (Mashour et al., 2020; Tononi et al., 2016), it is still very difficult to test these proposed measures and theories experimentally in a reasonably direct and reliable manner.

For example, when testing such measures in DOC patients, only between-group comparisons are normally possible, because these patients usually remain in essentially the same state for the duration of the study. Because of the heterogeneity of DOC patients, other variables may also influence the results. Furthermore, when general anaesthesia is used for within-subject tests of consciousness, factors that are specific to the various anaesthetics, but not directly related to the loss of consciousness, may strongly influence the results (Bonhomme et al., 2019; Hudetz and Mashour, 2016; Sanders et al., 2012). For example, many general anaesthetics have a muscle relaxing effect, which may significantly contaminate the EEG (Goncharova et al., 2003; Schuller et al., 2015). Thus, ideally, one needs an approach where measures of consciousness can be compared between conscious and unconscious brain states, while all other factors remain essentially the same. The Wada test, also called the etomidate speech and memory test, or eSAM; (Jones-Gotman et al., 2005) fills several of these test requirements.

In the Wada procedure (Wada and Rasmussen, 1960), large parts of one brain hemisphere are selectively anaesthetised by intracarotid injection of a fast-acting general anaesthetic (sodium amobarbital, or etomidate) into the internal carotid artery on one side, while the other hemisphere is not directly affected by the anaesthetic agent and appears to remain in an effectively awake state. This test is used prior to neurosurgery, typically in epilepsy or tumour patients, in order to determine hemispheric lateralisation of speech and memory, before ablative epilepsy surgery or tumour resection. Behavioural tests have shown that the patients undergoing the Wada test lose motor and sensory functions in the half of the body contralateral to the injection site, and any function specific to the anaesthetised parts of the injected hemisphere will be lost for the duration of the procedure (Hamberger and Hirsch, 1999; Loring et al., 2012; Tu et al., 2015; Wada and Rasmussen, 1960). E.g. typically speech will be impaired when the left side is injected, while if the injection is given to the right hemisphere, speech is typically not impaired and the patients are able to name and memorise objects presented to them, and the patient typically reports being fully awake and conscious. Thus, it seems that the non-anesthetised hemisphere maintains close to normal consciousness during the Wada test (Blackmon, 2018; Loring et al., 2012). This situation provides a unique opportunity for testing measures of consciousness as it offers a transient, rapidly reversible (within few minutes) anaesthesia of only “half the brain”. Thus, the Wada test allows for direct before-during-after comparisons of measures of consciousness, as well as testing of certain theoretical predictions of current theories of consciousness, which are not possible in any other known scenario, e.g. neither split-brain surgery (callosotomy) nor hemispherectomy (Kliemann et al., 2019; Sperry, 1961).

Hence, the Wada test offers a unique opportunity to test measures and theories of consciousness, by observing the functions and physiological wake activity of one hemisphere when suddenly removing the normal, wake activity in the other. Since the general anaesthetic is given locally to only one hemisphere, the patient is partly awake and able to provide reports about her/his content of consciousness during or after the procedure, and effects on speech, motor control, and other functions can be observed and tested directly. Furthermore, any change in EMG contamination of EEG due to direct muscle relaxing effects of the anaesthetic drug are avoided.

To prepare for the use of this procedure for testing theories of consciousness, it is important to determine which measures of conscious vs. unconscious brain states are sensitive enough to distinguish the different states of the anaesthetised and the awake hemisphere. In the current study, we focused on various proposed measures of consciousness based on spontaneous EEG patterns of activity distribution or connectivity (Chennu et al., 2017; Schartner et al., 2015). We analysed EEG data recorded during multiple Wada tests performed by intracarotid injection of the general anaesthetic etomidate. Specifically, we quantified the spectral power, functional connectivity, and signal diversity, in different frequency bands both before and during the Wada procedure. In general, we expected the low-frequency EEG power (delta, theta) to increase in the anaesthetised hemisphere (Tu et al., 2015), whereas interhemispheric connectivity was expected to decrease (Shahaf et al., 2016). We also hypothesised that the measures of signal diversity would indicate a loss of consciousness marked by a decrease in signal diversity in the anaesthetised hemisphere (ipsilateral) and be essentially unaffected in the un-anaesthetised (contralateral) hemisphere. However, we found substantial EEG changes in both the ipsi- and the contra-lateral hemisphere, including reduced functional connectivity within and between hemispheres. At the single patient level, a combination of complexity and power spectral features allowed classification of the condition and location of injection, reflecting differences between the hemispheres.

Some of the main results of this study were previously presented in abstract form (Halder et al., 2018).

2. Methods

2.1. Participants

After the institutional research and ethics board approval, we retrospectively reviewed the clinical EEG and neuropsychological data of seven patients (4 female, age range 27–52 years) who underwent etomidate Wada tests at the Toronto Western Hospital (Jones-Gotman et al., 2005, 2009). All patients suffered from medically refractory temporal lobe epilepsy and were on more than 3 antiepileptic drugs. None of them had tumors. One patient (Patient 3) had a previous left temporal lobectomy.

The Wada test was a part of presurgical evaluation of patients for epilepsy surgery. Epileptogenic foci were confirmed by EEG, and all patients showed some interictal spike activity before the Wada test. All patients underwent an extensive pre-surgical evaluation, comprising non-invasive and invasive investigations (where required), prior to surgical treatment of their medically refractory epilepsy. While hemispheric location of language dominance can usually be determined by fMRI, patients who are deemed to be at risk of dramatic postoperative memory dysfunction or those where language dominance could not be determined by fMRI, are referred for Wada procedure.

2.2. The intracarotid etomidate Wada procedure

All procedures were performed in a biplane angiography suite. Under local anaesthesia, the 5-French Berenstein diagnostic catheter (Merit Medical Systems, Inc. UT, USA; dead space of 1.2 cc) was placed in the cervical internal carotid artery, at the level of the anterior arch of C1 using a standard right femoral approach. The use of intravenous heparin was left to the discretion of the neuro-radiologist who did the procedure (dose usually between 2000 and 3000 units). Standard anteroposterior and lateral internal carotid artery angiograms were performed using contrast pump injection at a standard rate of 5 cc/s with total volumes of 10 cc (using a non-ionic contrast agent; Iohexol). Images were used to exclude persistent carotid-vertebral anastomosis, retrograde filling of the basilar artery through the posterior communicating artery (P Com), or significant cerebral cross flow.

Etomidate, an imidazole derivative, is a potent non-barbiturate hypnotic agent with no analgesic properties (Forman, 2011). Its anaesthetic

effects are mediated through the modulation of γ-amino butyric acid (GABA) A receptors (Forman, 2011). It has a rapid onset (30–60 s) and short duration of action (5–10 min) with minimal hemodynamic effects. Sodium amobarbital (sodium amytal) has been the standard drug used for the IAP for more than 50 years. Due to limited availability of sodium amobarbital, various other anaesthetic agents have been tried for this procedure (Patel et al., 2011). However, some of them had unpleasant side effects. Hence, etomidate has become a commonly used alternative to sodium amobarbital for the Wada test.

All seven patients were monitored with electrocardiogram, non-invasive blood pressure monitoring, pulse oximetry (SpO2), and continuous monitoring of 24-channel EEG. Immediately following angiography and ruling out significant cerebral cross flow, an anaesthesiologist administered etomidate and monitored the patient. A neuropsychologist performed the motor, language, and memory assessments during the procedure.

In all patients, 2 mg of Etomidate (2 mg/cc) was injected as a bolus over 30 s using a syringe driver infusion pump (Medfusion 3500, Smith Medical MD Inc., USA) followed by an infusion of 6 cc/h (12 mg/h) etomidate. The first injection was always ipsilateral to the epileptogenic hemisphere. After clinical and EEG confirmation of drug effect (ipsilateral slowing of the EEG and contralateral hemiplegia), language and memory tests were performed as described previously (Mariappan et al., 2013). In brief, after the onset of contralateral hemiplegia, adequate contact with the patient was verified by the execution of a simple verbal command or by observing the patient visually orienting or tracking stimuli. Then 10 objects to be remembered were shown before the infusion was stopped, followed by repeated sampling of language and motor functions. Once the patient recovered from the drug effect, both clinically and electrophysiologically, a yes-no recognition memory test of the 10 previously presented objects, and 10 novel objects, was performed. Patients were also tested with five pre-injection items to ensure that testing was valid. The duration of the clinical effects was recorded (patient 1: 17.0 min, patient 2: 5.0 min, patient 3: 12.5 min, patient 4: 5.2 min, patient 5: 8.5 min, patient 6: 8.8 min, patient 7: 5.5 min). If warranted by performance on the neuropsychological tests, a second test was then performed in a similar way on the other hemisphere at least 30 min after the first injection.

In our cohorts, seizure focus was identified to be on the left temporal lobe in all the patients and hence they had left sided Wada procedure. Of the seven datasets, in five datasets (patients 1, 2, 3, 5, 6) the drug was injected in the left hemisphere only. In the other two datasets (patients 4, 7), the drug was injected first on the left side, then in the right side, however, here we only considered recordings from the first injection (left hemisphere) for analysis, to avoid any possible contamination from late after-effects after the first injection.

2.3. EEG acquisition, preprocessing and analysis

2.3.1. EEG data acquisition

The EEG recordings were acquired using a 24 channel (Fp1, Fp2, Fz, Cz, Pz, FCz, FPz, F3, F7, F4, F8, T3, T5, T4, T6, C3, C4, P3, P4, A1, A2, O1, O2, and electrooculogram) sensor net from Natus Xltek (Natus DBA Excel-Tech Corp., Oakville, ON, Canada), with 0.1 Hz high-pass filter and a 50 Hz limit of the low-pass filter (the latter sometimes adjusted according to artefacts), sampled at 512 Hz. The ground electrode was placed on the forehead and reference electrodes behind the ears (non-linked).

2.3.2. Preprocessing

The data was preprocessed using Matlab 2016b. We resampled the data to 250 Hz (using a polyphase anti-aliasing filter), applied a 1 Hz highpass filter, suppressed line noise using an adaptive estimation with a sine wave (CleanLine function in EEGLAB; (Delorme and Makeig, 2004) and removed further artifacts using artefact subspace reconstruction (clean_asr function in EEGLAB; (Chang et al., 2018)). If channels were rejected we interpolated them using spherical interpolation. In total, three channels were interpolated due to artifacts: for patient 2 channel O1 and for patient 6 channels Fp1 and Fp2. Finally, the data were referenced using REST (Hu et al., 2018).

2.3.3. Analysis

After preprocessing, the data were further analysed using Python MNE v0.19 (Gramfort et al., 2013, 2014). The data was split into two sets: electrodes on the left hemisphere (A1, FP1, F7, T7, P7, O1, F3, C3, P3), and, electrodes on the right hemisphere (A2, FP2, F8, T8, P8, O2, F4, C4, P4). Electrodes along midline (Fz, Cz, Pz) were discarded. Then, the data was independently re-referenced: the left set to A1 and the right set to A2 (A1 and A2 were subsequently discarded). We defined a 120 s segment of EEG immediately before the injection as normal rest condition, discarded the first 30 s after the injection to let the effects of the drug stabilise, and defined the next 120 s as the Wada condition. The periods of both conditions were then segmented into regular epochs with a length that depended on the current analysis. Finally, all p-values obtained from statistical tests were adjusted using the Holm-Bonferroni procedure (Armstrong, 2014; Holm, 1979).

2.3.4. Power spectral density

We computed the PSD for every channel in our dataset using a multitaper method (MNE function psd_multitaper with the parameters bandwidth = None, adaptive = False, low bias = True, normalisation = “length”) from 1 to 45 Hz on epochs of 2 s length. The frequency bins of each channel were individually normalised with the median of that frequency bin of each channel for the whole segment (both conditions). For the statistical analysis, the data was averaged across time and frequency bins chosen according to canonical EEG bands (Delta: 1.0–3.5 Hz, Theta: 3.6–7.9 Hz, Alpha: 8.0–12.9 Hz, Beta: 13.0–25.9 Hz, Gamma: 26–45 Hz). We then compared the median across channels for these bins between left and right hemispheres, and between normal and Wada conditions, using repeated samples t-tests. Thus, we performed 20 tests (five bands, four comparisons per band) and adjusted the p-values using the Holm-Bonferroni procedure mentioned in Section 2.3.3.

2.3.5. Functional connectivity

We estimated the functional connectivity between sensors using estimates of the cross- and power spectral density to calculate the debiased weighted phase lag index (dwPLI) defined in (Stam et al., 2007; Vinck et al., 2011). This method is based on the expected value of the imaginary component of the cross spectrum and normalised by the expected value of the magnitude of the imaginary component spectrum. Volume conduction and volume-conducted uncorrelated noise should not affect the result (Stam et al., 2007). We calculated dwPLI between all electrodes for each of the canonical frequency bands and conditions using 5 s epochs. Since we were specifically interested in the connectivity within each of the two hemispheres and between the hemispheres, we compared the dwPLI values between conditions and connection sets (left, right, between hemispheres) using repeated samples t-tests. Furthermore, we performed this analysis for each canonical EEG rhythms individually (see above) and based on a broadband signal (1–45 Hz). Thus, we performed 18 tests (six bands, three comparisons per band) and adjusted the p-values using the Holm-Bonferroni procedure mentioned in Section 2.3.3.

2.3.6. Measures of signal diversity

To quantify signal diversity, we used Lempel-Ziv complexity (LZc), which measures the compressibility of the data. The EEG time series are binarised by taking the absolute values of the Hilbert transformation and setting the samples to 1 if the amplitude is greater than the mean of the current time segment and to 0 otherwise. The compressibility of the sequence was then calculated by the Lempel-Ziv compression algorithm (Lempel and Ziv, 1976), and normalised by the compressibility of a scrambled version of the binarised original signal.

We computed single-channel LZc for each of the 16 channels individually using Python code provided by (Schartner et al., 2015). This measure we computed using segments of 6 s length using the broadband (0.1 - 45.6 Hz). For topographic plots we visualised the t-values between conditions. The *t*-tests were performed either on the median value of all epochs per participant (visualisation for all participants) or on the values per epoch (individual visualisations). For statistical comparisons and visualisation using boxplots, the data were z-scored and the median was computed over all channels. In this case, we performed four tests on all patients (four comparisons) and 28 tests on individual patients (four comparisons and seven patients), and adjusted the *p*-values using the Holm-Bonferroni procedure mentioned in Section 2.3.3.

In addition to LZc we applied two other measures of signal diversity to the spontaneous EEG, based on (Schartner et al., 2015): amplitude coalition entropy (ACE) and synchrony coalition entropy (SCE). The methods and results from these analyses are described in the **Supplement S2**.

2.3.7. Classification

To determine which measures could be used to differentiate between normal and Wada conditions, as well as the ipsi- from the contra-lateral hemisphere during the Wada condition, we trained a support vector machine (SVM) classifier using the Python sklearn package (Pedregosa et al., 2011). We used LZc, dwPLI in the delta band, dwPLI in the other bands, PSD in delta, PSD in the other bands, and a combination of all, to train the SVM. The features were extracted from 6 s epochs that overlapped by 3 s. This resulted in 40 segments before injection and 40 after injection per hemisphere per patient. The 30 s directly following the injection were also excluded from this analysis. All features were standardised by subtracting the mean and scaling to unit variance. The SVM was trained with a linear kernel, penalty parameter $C = 1$, $\gamma = 1$, and number of features $n = 5$. Ten-fold cross validation was performed individually for each patient. Accuracies were reported individually for each condition (rest/Wada) and hemisphere (ipsi/contra). For every patient, this resulted in two 2×2 confusion matrices for each combination showing the true and false classification rates for each comparison individually. The confusion matrices presented in the results were averaged over the individual matrices. Finally, we used recursive feature elimination to determine the most relevant three features for distinguishing the patients' state and the ipsi- and contra-lateral hemispheres.

3. Results

The unilateral injection of etomidate produced no apparent loss of general consciousness. While all patients developed contralateral hemiplegia, they were still oriented and communicating while under the effects of the anaesthetic, and were able to perform the language and memory tests. Epileptiform discharges were present at low frequencies of occurrence ($\lesssim$ 0.01 per s.) in the baseline EEG of all patients before the Wada test, and the frequency of such discharges (inter-ictal spikes and/or sharp waves) increased in all patients within ~40 s after the Wada test injection (mean 23.9 discharges in 120 s; SD 17.7 range 5–58; see **Fig. S8**). This type of increased epileptiform discharges is commonly seen with etomidate and some other general anaesthetic drugs, in particular at the onset of the drug effects, and is normally seen in the EEG from the injected hemisphere during Wada tests with etomidate (Modica et al., 1990a, 1990b; Voss et al., 2008). However, in patients 1–7, these discharges were modest in both amplitude and frequency of occurrence (~0.05 - 0.50 per s.; **Fig. S8**) and did not seem to correlate with or influence appreciably the measures used in this study (see below).

3.1. Effect on power spectral densities

Following injection of etomidate in the left cervical internal carotid artery, anesthetising the left hemisphere, the median value of the power spectral density (PSD) amplitudes of the EEG appeared to increase in both hemispheres. We visualised the data until 150 s after the injection (**Fig. 1A**) and the apparent changes remained stable for the entire period. Inspection of the spectrogram in **Fig. 1A** (right) indicated that the PSD increase in the hemisphere on the injected side seemed stronger than on the contralateral side. Visualisations of the topographies (**Fig. 1B**) showed similar increases in PSD amplitudes after the injection of the anaesthetic, in particular on the ipsilateral side. The median values of the PSD amplitude across patients (**Fig. 1C**) showed a trend to increase in the Wada condition in all canonical EEG frequency bands. This is in agreement with the initial, ~2–3 min increase in EEG amplitude previously observed with systemic induction of general anaesthesia with etomidate and other anesthetics during transition to unconsciousness (Kuizenga et al., 2001). However, in our data, the PSD increases were not restricted to the injected hemisphere (always left), but extended to the contralateral hemisphere (always right). The increases during Wada vs. normal condition were statistically significant ($p < .05$) both when comparing the delta band on the ipsilateral side after (Wada) vs. before injection ($t(12) = -4.9$, $p = .043$), when comparing the theta band on the ipsilateral side after vs. before injection ($t(12) = -10.5$, $p = .001$), when comparing the theta band on the contralateral side after vs. before injection ($t(12) = -13.8$, $p < .001$), when comparing the alpha band on the contralateral side after vs. before injection ($t(12) = -5.8$, $p = .02$), and when comparing the beta band on the contralateral side after vs. before injection ($t(12) = -7.9$, $p = .02$).

3.2. Effect on functional connectivity measures

The dwPLI functional connectivity values changed significantly after injection of the anaesthetic in the majority of the comparisons (**Fig. 2A**). The strength of broadband (0.1 - 45 Hz) functional connectivity both between the hemispheres (comparing electrodes on the left and the right side) and between electrodes within each of the hemispheres (**Fig. 2A** and **C** top left) was lower during the Wada condition than during the normal condition. This effect was significant for all three comparisons (between hemispheres: $t(126) = 9.0$, $p < .001$; left (injected): $t(54) = 5.3$, $p = < 0.001$; right (non-injected): $t(54) = 10.8$, $p < .001$) (**Fig. 2C** top left).

There was one important exception to the drop in connectivity strength: in the delta band, the functional connectivity strength increased after injection of the anaesthetic (**Fig. 2B** left and **C** top right). This effect was statistically significant both for the connectivity strengths between the two hemispheres ($t(126) = -4.8$, $p < .001$) and within the injected hemisphere ($t(54) = -3.0$, $p = .019$). Another, albeit small, increase occurred in the gamma band connectivity also between the left and right hemispheres. This change was significant ($t(126) = -3.7$, $p = 0.002$) (**Fig. 2B** right and **C** bottom right). In the remaining comparisons within the theta, alpha, beta, and gamma bands, the dwPLI connectivity values dropped, between as well as within the left and right hemispheres.

3.3. Effect on signal diversity of spontaneous EEG

At the group level, the Lempel-Ziv complexity signal diversity measure (LZc) decreased primarily on parietal/occipital locations in the injected hemisphere (**Fig. 3A**, top left). We also used two other signal diversity measures (ACE and SCE; see **Supplement S2**), but a similar, topographical analysis could not be performed for these, because they are calculated for the entire set of EEG electrodes. On the individual level, LZc signal diversity decreased significantly in the injected hemisphere for patients 1, 2 and 7, whereas LZc for patients 3, 4, 5 and 6 did not decrease noticeably in the injected hemisphere (**Fig. 3A**).

At the group level, the median (across channels) signal diversity values differed significantly between left and right hemispheres during the

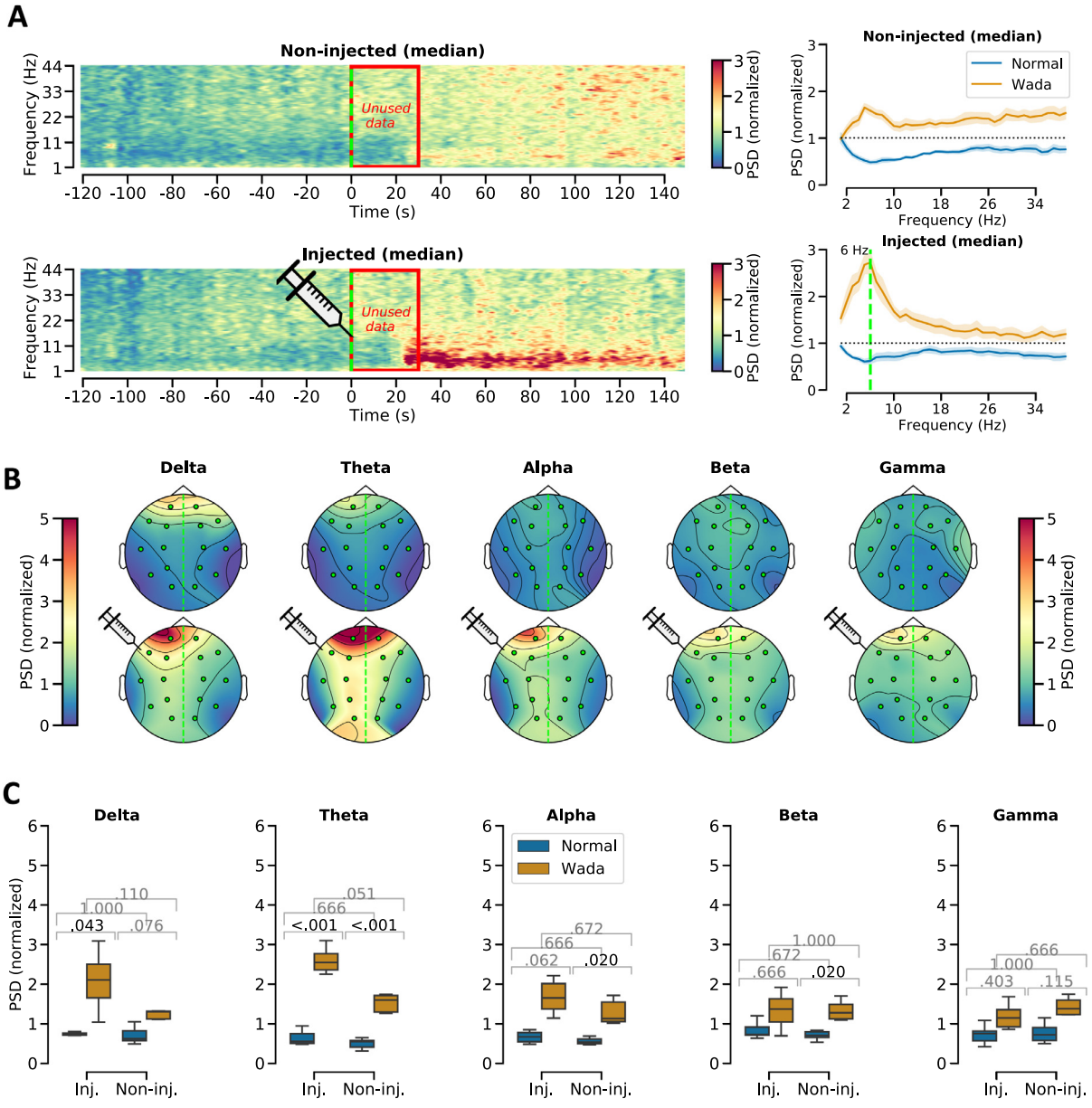

Fig. 1. Group level changes in electroencephalogram (EEG) power spectrum following injection of anaesthetic in the left hemisphere (Wada test). (**A,** left) Median power spectral density (PSD) over time for electrodes on the injected, left hemisphere (ipsilateral) and on the non-injected, right hemisphere (contralateral). The time of injection is marked with a vertical green line in A, left panel. The red box indicates the time period for which the data that was discarded to let the effect of the drug stabilise. (**A**, right) Median PSDs averaged over time are shown on the right-hand side. Blue line: pre-injection normal condition. Red line: Wada condition after the injection. The vertical green line indicates the frequency (6 Hz) of the peak amplitude on the injected side during Wada. The apparent reduction in the normalised PSD around ~6 Hz in the normal condition (blue line) is due to the normalisation of the data. A dotted black line shows the normalised median amplitude of the PSD. (**B**) Topographic visualisations of the PSD for each canonical frequency band of the EEG (top row: rest, bottom row: Wada). The vertical green lines in panel B indicate the midline. (**C**) Comparison of the PSD values for each canonical EEG band. Blue bars reflect normal condition, orange bars reflect Wada condition. In each plot, the *p*-values for every *t*-test (corrected for multiple comparisons) are noted. Statistically significant differences ($p < .05$) are printed in black font. The black bars indicate the median, the extent of the boxes the interquartile range, whereas the whiskers extend to the rest of the distribution (excluding anything outside 1.5 times the interquartile range). Syringes indicate the time point of injection (A) and hemisphere of injection (A, B).

Wada condition (t(12) = −3.5, p = .048; Fig. 3**B**, top left). At the individual level, however, there was significant inter-patient variability in the apparent effect of the Wada-intervention on the values and spatial distributions of LZc. Although the LZ values on the left hemisphere (injected) dropped significantly from wakefulness to the Wada condition for 4 patients (patient 1 (t(76) = −16.3, p = <0.001), patient 2 (t(76) = −13.5, p = <0.001), patient 4 (t(76) = −4.4, p = <0.001), and patient 7 (t(76) = −11, p = <0.001), we found significantly increased LZc values during the Wada condition for two patients (patient 3 (t(76) = 6.2, p = <0.001) and patient 5 (t(76) = 4.9, p = <0.001)). For patient 6 there was no significant change (t(76) = 1.8, p = .387). The more consistent effect of the Wada intervention among individual patients, however, was the difference in LZc values between hemispheres. For example, LZc was lower in the injected than non-injected hemisphere for 6 of the7 patients tested (patient 1 (t(76) = −21.1, p = <0.001), patient 2 (t(76) = −14.2, p = <0.001), patient 4 (t(76) = −7.9, p = <0.001), patient 5 (t(76) = −7.6, p = <0.001), patient 6 (t(76) = −13.8, p = <0.001), and patient 7 (t(76) = −18.2, p = <0.001)). Only for patient 3, which is

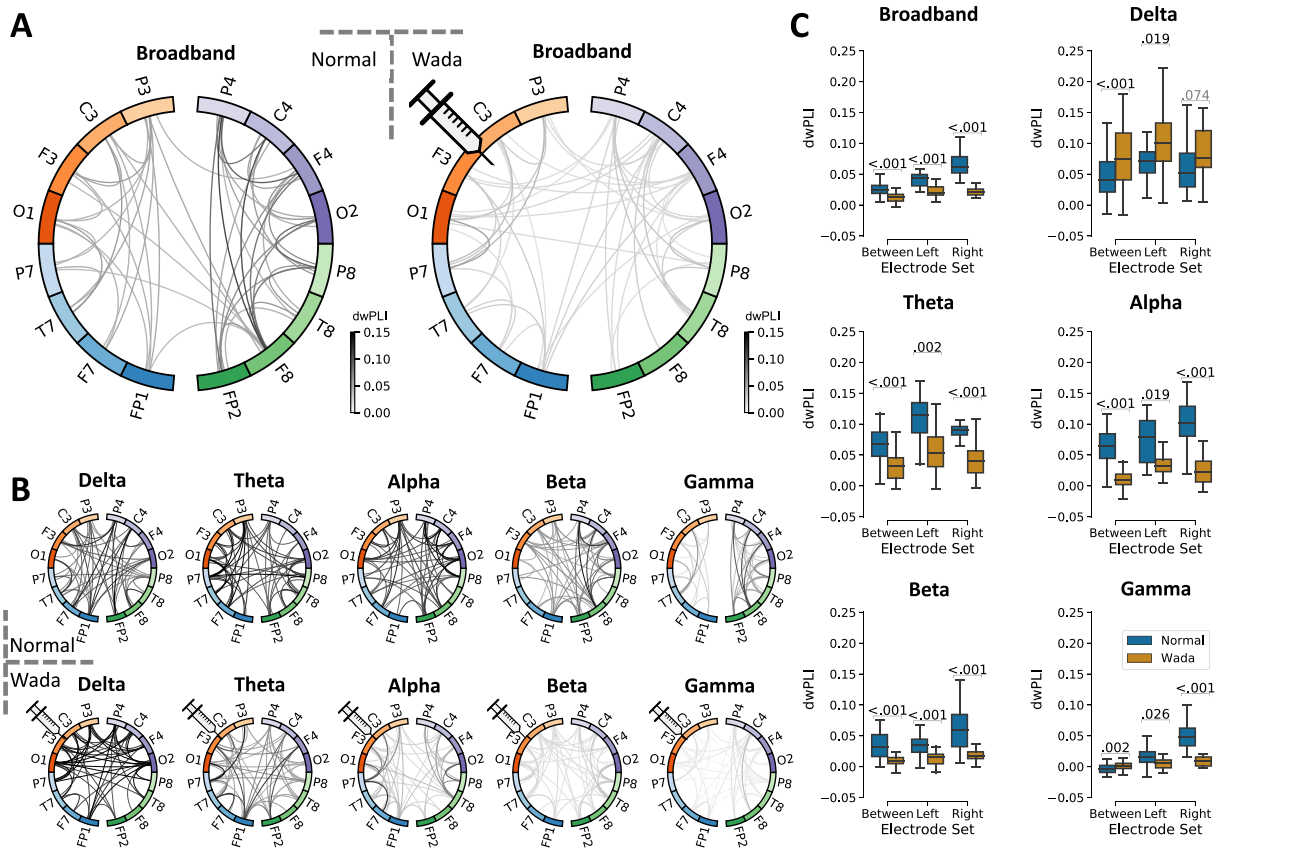

Fig. 2. Functional Connectivity values (using the debiased weighted phase locking index to quantify connectivity) between electrodes on the left hemisphere (blue/orange) and right hemisphere (purple/green). Strength of connections between electrodes is indicated by the colour of the connecting line (stronger connections have a darker colour). (**A**) Connectivity values averaged over time and participants, in the frequency range from 1 to 45 Hz (broadband), with pre-injection normal condition in the left circular plot and Wada condition after injection on the right circular plot. The connectivity values were calculated for five-second epochs. In each circular plot the 50 strongest connections were drawn. (**B**) Additionally, one circular plot was generated for each canonical frequency band (delta, theta, alpha, beta, gamma). (**C**) Comparison of the intra-hemispheric connectivity values (left (injected) and right hemisphere) as well as the inter-hemispheric connectivity values (between). Blue bars represent the normal condition, orange bars represent the Wada condition. The black bars indicate the median, the extent of the boxes indicate the inner quartiles, whereas the whiskers extend to the range of the distribution (excluding anything outside 1.5 times the interquartile range). The syringe indicates the hemisphere of injection.

the only patient that had a previous left temporal lobectomy, the signal diversity score was higher in the injected vs. non-injected hemisphere ($t(76) = 3.4$, $p = .01$; Fig. 3**B**).

3.4. *Classification of patient state and injected hemisphere*

Despite the large inter-patient variability (Fig. 3), classification of the state of the patients with LZc resulted in 70% correctly detected normal segments and 69% correctly detected Wada condition segments. Using dwPLI features in the delta range, we obtained lower classification rates for the Wada condition (rest 63%, Wada 46%) and using dwPLI features in the higher bands low classification rates for both states (rest 54%, Wada 53%). PSD features from the delta band had higher accuracies (rest 69%, Wada 64%) and higher bands of the PSD had the highest true classification rates of all individual features for both states (rest 82%, Wada 88%). When using all seven features for classification, we obtained the highest true classification rates overall (rest 92%, Wada 93%) for the state of the patient (Fig. 4**A**).

The highest accuracies for classifying the contra-lateral hemisphere were obtained using the single channel LZ complexity (ipsi 88%, contra 85%). Using dwPLI features in the delta range, we again obtained lower classification rates for the Wada condition (rest 53%, Wada 55%) and using dwPLI features in the higher bands low classification rates for both states (rest 64%, Wada 56%). PSD features from the delta band again resulted in higher accuracies (rest 66%, Wada 80%) and which can also be observed using higher bands of the PSD (rest 64%, Wada 83%). Again, overall rates were highest for the combined feature set (ipsi 92%, contra 92%; Fig. 4**B**).

We used recursive feature elimination to determine which were most relevant for the classification. Overall, the higher frequency bands of PSD (12 out of 14) and LZs (11 out of 14) were selected most often (Table 1). The PSD values were most often selected for classifying the state of the patient, while signal diversity measures were more often used for classifying the hemisphere of injection.

Table 1
Number of times each feature was selected during recursive feature elimination. The maximum number that could have been reached was seven times. The features were grouped according to their type and were determined for the detection of the state (rest vs. Wada) and the hemisphere (ipsi vs. contra) separately.

	Complexity	Connectivity		Spectral	
	LZc	dwPLI δ	dwPLI > δ	PSD δ	PSD > δ
State	4	2	2	6	7
Hemisphere	7	1	4	4	5

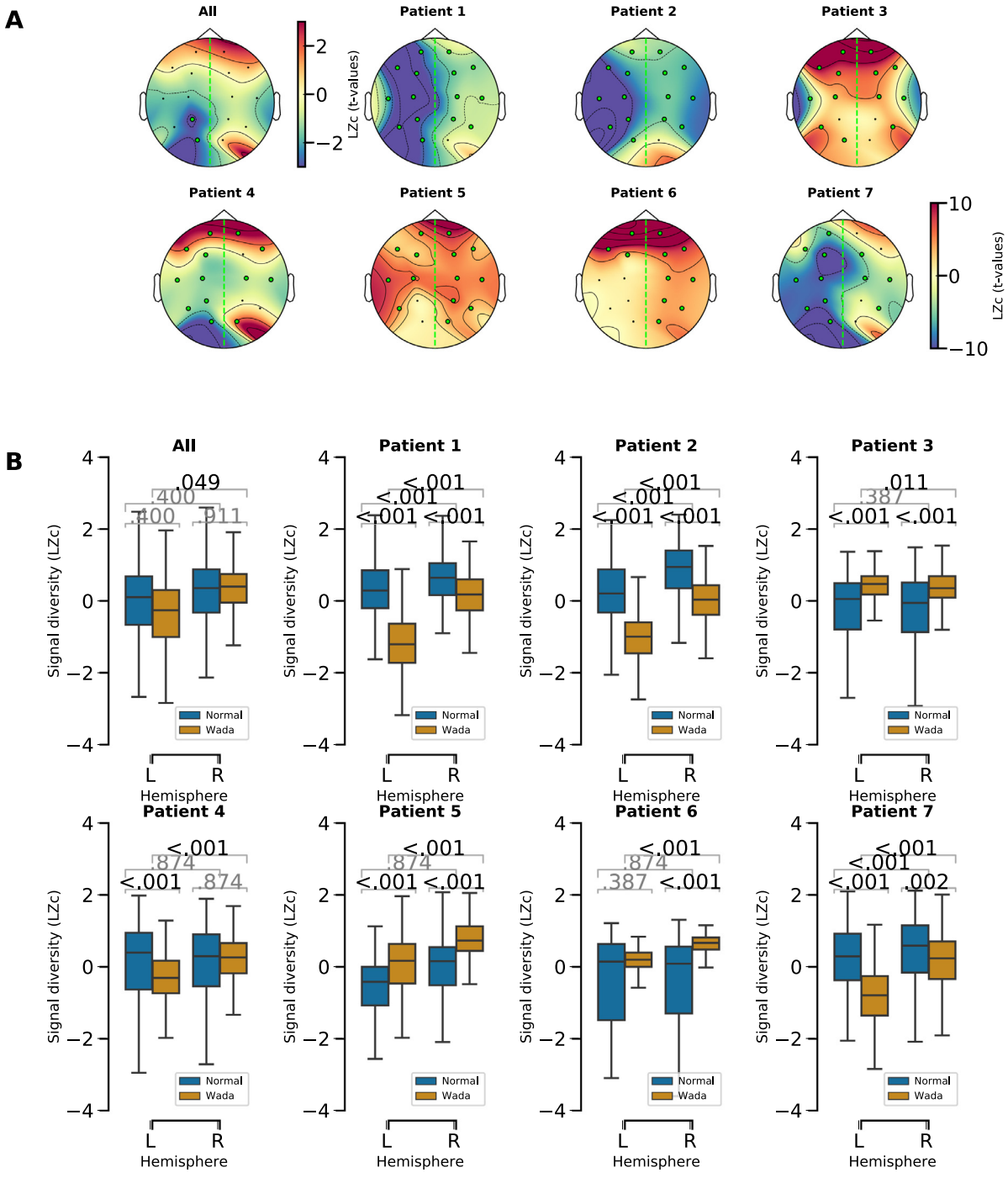

Fig. 3. Effects of the anaesthetic on LZc signal diversity values. (**A**) Changes from wakefulness to Wada in signal diversity (Lempel-Ziv complexity; LZc) visualised topographically. Red indicates an increase and blue a decrease during Wada relative to baseline LZc values immediately before the injection. (**B**) Distribution of signal diversity values during rest (blue) and Wada (orange) on left (L) and right (R) hemisphere. We used the median over all channels of the z-scored signal diversity values and the variance is shown across epochs.The black bars indicate the median, the extent of the boxes indicate the inner quartiles, whereas the whiskers extend to the rest of the distribution (excluding anything outside 1.5 times the interquartile range). The *p*-values were calculated using repeated measure *t*-tests and adjusted using the Holm-Bonferroni procedure.

4. Discussion

We investigated the effects of the etomidate Wada test on measures of consciousness calculated from EEG data. In these Wada tests, the short-acting anaesthetic drug etomidate was injected into the internal carotid artery on one side, to transiently anaesthetise only one brain hemisphere. Therefore, one might expect that these measures would have changed only for the injected hemisphere (ipsi-lateral), and remained essentially unchanged for the hemisphere contra-lateral to the injection, which should not be directly affected by the injected anaesthetic. However, to the contrary, we consistently observed, for both hemispheres, an increase in power spectral density (PSD) in all frequency bands. Furthermore, we found a significant decrease in connectivity strength between and within the hemispheres, in all frequency

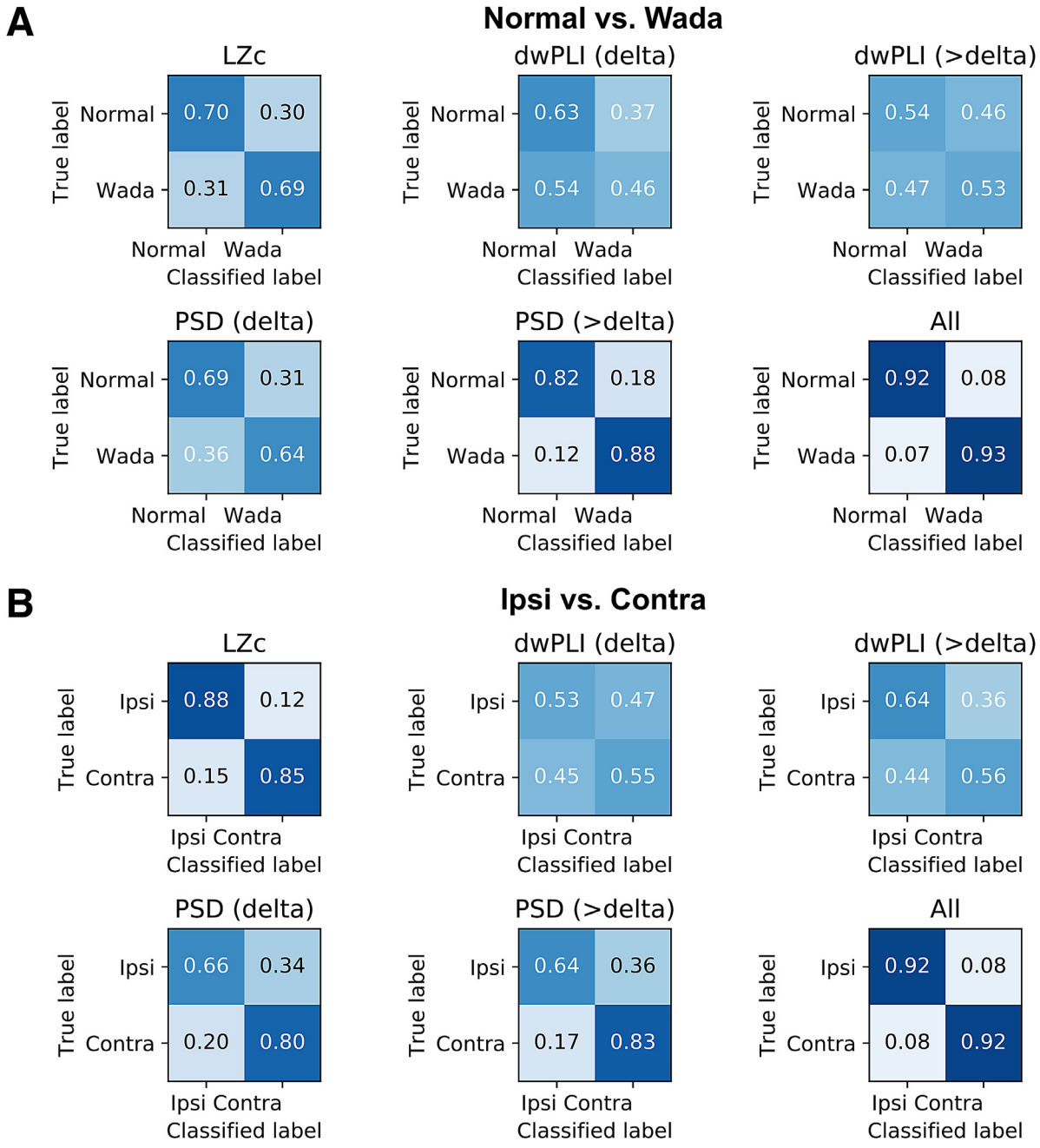

Fig. 4. Confusion matrices for classification of patient state and injected hemisphere. (**A**) Classification of the patients' states using data from both hemispheres. One confusion matrix is shown for each of the five features and the combination of all. (**B**) Classification of the injected hemisphere using data from during the procedure. All features were calculated on the basis of 6 s long EEG segments. The values shown in the plots are the ratios with which the classifier determined the label correctly or incorrectly.

bands except delta, for which the functional connectivity strength increased significantly. Interestingly, while we found no significant change in either hemisphere for the signal diversity measure LZc between the baseline (pre-test) and Wada test conditions, LZc was significantly lower in the injected than non-injected hemisphere in the Wada condition.

4.1. Why were bilateral changes in EEG observed, although the injection of anaesthetic was always unilateral?

Several previous EEG studies of Wada tests have also found bilateral changes in EEG, although the injection of anaesthetic was unilateral (see for example Gotman et al., 1992; Hong et al., 2000; Jones-Gotman et al., 2005; Shahaf et al., 2016). Hence, it seems likely that the Wada-test-induced bilateral changes in median values of EEG-derived measures that we observed here, i.e. changes in both the ipsilateral (injected) and contralateral (non-injected) hemispheres, reflected real bilateral changes in brain activity, although most of the apparent differences in signal diversity were not statistically significant in our data. Thus, it seems unlikely that the consistently bilateral changes seen both in our data and in those of others were accidental. That we also found the effective connectivity to be significantly affected on both sides (**Fig. 2**), supports the conclusion that both hemispheres were affected.

What could be the reason for these bilateral changes in EEG, following the unilateral injection of anaesthetic? There seem to be at least 6 possibilities:

(1) The EEG reference used may cause apparent, artefactual "spreading" off EEG activity to the contralateral hemisphere

The choice of reference has a large effect on the topographical character of the EEG signal. Thus, it is conceivable that at least some of the observed contralateral slowing of the EEG after the injection is an artefact caused by the particular location and type of reference used. To minimise the probability that this was the case, we chose to re-reference the signal using the REST technique (Yao, 2001) which approximates referencing the signal to a point at infinity. Furthermore, when studying signal properties of individual hemispheres, we further re-referenced the signal to the most lateral channel in the data (A1 for left side channels, A2 for right side channels), to minimise the probability of the common signal in the reference electrode bleeding into the recorded signal on both sides. Finally, earlier work has used different choices for referencing, such as local bipolar references or double-banana montage, and still reported seeing bilateral slowing of the signal (Hong et al., 2000). Hence, it is unlikely that the EEG reference used was the cause of the apparent spreading of EEG activity to the contralateral hemisphere.

(2) Volume conduction of EEG signals to EEG electrodes on the contralateral hemisphere

This also seems unlikely to be the main explanation, because we detected no obvious gradient in slow EEG activity from the midline to the lateral parts of the contralateral hemisphere, as would be expected if the slow activity simply spread by volume conduction from the injected to the contralateral hemisphere. Furthermore, a previous study using intracranial EEG (with stereotactically implanted depth electrodes in the temporal lobe) also found that the EEG of the contralateral hemisphere was affected by a unilateral carotid injection of amobarbital, albeit for a shorter duration and with smaller amplitudes of the contralateral slow waves (Gotman et al., 1992). It seems highly unlikely that implanted electrodes in the temporal lobe, i.e. far from the injected hemisphere, can pick up any significant, volume-conducted electrical signals from the contralateral hemisphere. Besides, Gotman et al. (1992) found local differences in the duration of the drug-induced delta activity between the anterior and middle hippocampus, which seems incompatible with significant volume conduction of EEG signals over larger distances, between the hemispheres.

(3) Cross-flow of anaesthetic from the injected side to the non-injected side

This seems unlikely to be the main explanation because our angiography results indicated that there was no significant cerebral cross-flow from the injected side to the other hemisphere in any of the Wada tests included in this study (see Methods Section 2.2, above). Other studies using angiography control for Wada tests support this conclusion (Gotman et al., 1992; Hong et al., 2000; Jones-Gotman et al., 2005; Shahaf et al., 2016), although angiographic cross-filling were observed in a minority of cases in some of those studies. Thus, Hong et al. (2000) monitored the amobarbital distribution using intracarotid single photon emission computed tomography and correlated the perfusion with contra-lateral EEG, and concluded that contra-lateral effects were produced only indirectly from the ipsilateral hemisphere during the procedure.

(4) Recirculation of the anaesthetic via systemic circulation

This is also very unlikely, because the recirculated drug, after passing through systemic circulation, is diluted to a very low concentration, giving no measurable clinical or electrographic effects (Perria et al., 1961).

(5) Deafferentation-induced slow activity in the contralateral hemisphere, due to loss of input

Convergent evidence indicates that slow EEG activity (delta, slow waves) can be caused by complete or partial deafferentation of parts of the cerebral cortex (Gloor et al., 1977; Timofeev et al., 2000). This has been suggested as a likely cause of the slow activity observed in the contralateral hemisphere during Wada tests, as well as local slow delta activity within parts of the hippocampus that were not directly affected by the injected anaesthetic. Thus, Gotman et al. (1992) wrote: "Since much of the hemisphere around it is directly affected, the middle hippocampus may be functionally isolated; it can no longer communicate with the anesthetised structures around it, and therefore it is also impaired and generates slow waves." This may be a plausible mechanism for relatively small parts of a hemisphere such as the middle hippocampus, which may become "functionally isolated" if surrounded by "anaesthetised" cortical tissue, in a somewhat similar manner as the isolated cortical slabs studied by Timofeev et al. (2000). However, this deafferentation mechanism seems unlikely to cause slow activity in an entire "isolated" hemisphere, since it is known, e.g. from split-brain and hemispherectomy patients, that an isolated hemisphere alone can maintain a fairly normal awake, conscious state, which is incompatible with a state dominated by slow wave activity, even if it is separated from the other hemisphere (Gazzaniga, 2005; Sperry, 1961). Furthermore, it is likely that a major cause of the slow wave activity in the undercut cortical slabs studied by Timofeev et al. (2000), is the loss of ascending input from subcortical structures, including fibres from the ascending activation systems, rather than mere loss of callosal/commissural or other cortico-cortical input from the contra- or ipsi-lateral hemisphere, which is likely to dominate in the Wada test.

(6) Conduction of slow activity, via corpus callosum axons, from the injected hemisphere to the contralateral hemisphere

It seems likely that when deep anaesthesia induces an increase in slow activity in the cortex of one hemisphere, this slow activity will be conducted via action potentials in callosal and other commissural axons to the contralateral hemisphere, and impose a similarly slow activity there. We suggest that such externally imposed slow activity, originating in one hemisphere and actively propagated to the other hemisphere via callosal fibres, can cause measurable slow EEG activity in the latter hemisphere, by being essentially "added" to the intrinsic activity there. Thus, the EEG signals are thought to mainly reflect postsynaptic currents along pyramidal cell dendrites due to synchronous neural inputs rather than local spiking activity. We further suggest that such externally imposed slow activity does not strongly affect the local capacity for information processing in the receiving hemisphere, in contrast to the hemisphere where the slow activity originates, e.g. due to local anaesthesia of that hemisphere only.

Thus, we suggest that the presence of observable slow-wave cortical activity by itself does not cause loss of consciousness; rather it is the loss of capacity for normal high-frequency, desynchronised information processing that occurs when it is replaced by slow activity in the cortex where it originates that disrupts the processing associated with consciousness in that part of the cortex. Thus, the "addition" of some incoming slow activity that can be detected in EEG, is in itself relatively harmless and with little consequence, as long as it does not disrupt the capacity to process information-rich, high-frequency activity with which consciousness is associated (Tononi and Edelman, 1998). This hypothesis would explain why the hemisphere that passively receives imposed slow activity maintains its awake, conscious state, although the other hemisphere, where the slow activity originates, is fully anaesthetised. This situation may be reminiscent of that of dolphins and other cc, cetaceans, when they have slow-wave sleep in one hemisphere while the other is awake (Rattenborg et al., 2000) - although the corpus callosum is relatively small in some dolphin species (Tarpley and Ridgway, 1994).

This concept may be called "cross-state unreceptiveness" (CSUR) (or "cross-state unresponsiveness/ refractoriness"). According to this idea, when a part of the cortex is in one particular state (e.g. awake) it is essentially not receptive to input from another part that is in a quite different state (e.g. anesthetised, or sleep). Thus, the awake cortex presumably cannot receive and make use of input from a quite different state, because of certain cellular and network properties that are characteristic of each state, e.g. determined by neuromodulators or drugs/anesthetics.

Thus, the sleep activity is "rejected" by the awake cortex, and therefore does not interfere with its intrinsic, awake information processing. However, importantly, even the "rejected" signals will likely be observable with scalp EEG, as they are still likely to reach the contralateral hemisphere and create postsynaptic potentials contributing to the EEG signals there (Buzsáki et al., 2012). Likewise, a sleeping or anesthetised part of the cortex presumably cannot receive and make use of input from an awake part of the cortex. E.g., the sleeping hemisphere of a dolphin can therefore continue its sleep state and sleep functions undisturbed by the barrage of input from the awake hemisphere, just as the awake hemisphere can maintain its awake state and wake, conscious processing, without being inhibited by the slow wave activity transmitted via commissural fibres from the other, sleeping hemisphere. Thus, this CSUR principle is likely to work both ways, and may also help explain how other forms of local sleep can occur in parts of the cortex (Murphy et al., 2011; Vyazovskiy et al., 2011). This situation may be compared to a radio receiver tuned to a certain frequency: in an antenna one can measure a variety of frequencies that are received from various stations, but further processing and output of the radio is restricted to the narrow frequency band to which it is tuned.

4.2. Power spectral density (PSD)

Previous studies of Wada tests also found increases in PSD in both ipsi- and contralateral hemispheres after unilateral injection of the anaesthetic, specifically in the alpha and delta bands (Jones-Gotman et al., 2005; Shahaf et al., 2016). Generally, widespread increase in delta and theta power is associated with the loss of consciousness (Gugino et al., 2001). However, as discussed above, it seems unlikely that the slow oscillations contralaterally to the injection have the same mechanistic causes as slow waves typically associated with unconsciousness. While slow waves in sleep and anaesthesia are often thought to be due to local cortical intrinsic and synaptic mechanisms, thalamocortical projections have been found to influence and even entrain cortical slow waves (David et al., 2013). However, according to the preprocedure angiographic inspection, the injected etomidate should not affect the thalamus or the contra-lateral hemisphere directly, suggesting that the observed contra-lateral low-frequency power increase is neither local nor of thalamic in origin. It should also be noted that the participants of the current study underwent the procedure in preparation for epilepsy surgery. Consequently, it is conceivable that some changes in the spectral composition of the EEG may have been related to epileptic discharges and not the loss of consciousness of one hemisphere (Englot et al., 2010). From a methodological perspective, using a parametric estimation of the PSD (as described e.g. in Haller et al., 2018) instead of pre-defined canonical frequency bands (Newson and Thiagarajan, 2018) may provide additional insights into the changes in each hemisphere.

4.3. Connectivity

We investigated changes in functional connectivity, quantified by debiased weighted phase lag index (dwPLI; see Methods Section 2.3.5) within and between the hemispheres. Overall, the functional connectivity increased in the delta band but dropped in all other frequency bands, except the connectivity between hemispheres in the gamma band. The increased functional connectivity in the delta band may be related to transition to slow-wave activity during anaesthesia, perhaps reflecting a selective increase in low frequency network activity, receptivity, and resonance (Alkire et al., 2008; Wang, 2010). The difference in the observed behaviour between the delta and the other bands was not consistent with findings in studies using amplitude-based connectivity metrics (however, others have also reported reduction in delta band connectivity, see Shahaf et al. (2016)). Using synchronisation likelihood as an estimate of connectivity, Douw et al. (2009) found that the within-hemisphere connectivity increased in delta and theta bands ipsilaterally, while it dropped in the contralateral hemisphere. Furthermore, the authors found increases in connectivity in the beta band, which is also not consistent with our results. As both Douw et al. (2009) and Shahaf et al. (2016) calculated connectivity based on amplitude and phase correlations whereas our dwPLI measure was based primarily on phase correlations, we assume the difference between our results was based on changes in amplitude. We also found that amplitudes tend to increase after injection of the anaesthetic, in agreement with the initial increase in EEG power previously observed during induction of general anaesthesia with etomidate, which was proposed to reflect early effects on 'systems that cause EEG activation' (Kuizenga et al., 2001).

4.4. Signal diversity

In line with findings in earlier publications (Schartner et al., 2015), and inspired by theoretical relations between brain complexity and consciousness (Carhart-Harris et al., 2014; Tononi and Edelman, 1998), we had expected to find that the signal diversity measures would show a marked decrease in the hemisphere that was anaesthetised, compared to the contralateral, awake hemisphere. This expectation was based on an assumption that almost half of the cerebral cortex and forebrain (the hemisphere directly affected by the anaesthetic) would be in an anaesthetised state and therefore no longer had the capacity to support consciousness, while the contralateral hemisphere would be largely unaffected by the anaesthetic and therefore maintain its capacity to support consciousness. Furthermore, clinical reports of apparent hemineglect-like symptoms in patients undergoing the Wada-test indicates that the patients' conscious experience is altered in a way that would be consistent with one hemisphere not contributing to experience in a normal way.

Using standard implementation of LZc, we found that signal diversity was significantly lower in the injected than the non-injected hemisphere in the Wada condition, but there was no significant difference in the measure between hemispheres in the awake condition or between conditions in either hemisphere (**Fig. 3**). This was partially in line with what was expected, although large inter-individual variability complicates the interpretation of these results. While the participant was awake behaviourally during the Wada test, the anesthetised hemisphere appeared to be in a state of reduced complexity. Thus, the patient's overall state of consciousness may have been largely unaltered, while the contents of their experience would presumably be dominated by properties of the uninjected hemisphere. Although the broadband LZc did not change significantly after injection of etomidate, it appeared to drop bilaterally at high frequencies (mainly beta and gamma bands) for epochs longer than 4 s, while it appeared largely unchanged for lower frequencies (see **Supplementary Fig. 2**). The apparent reduction in high frequency signal diversity in the injected, anaesthetised hemisphere, seems to agree with previous, convergent evidence that high frequency cortical activity is an essential feature of awake brain states and conscious processing (Koch et al., 2016; Llinás et al., 1998; Mashour and Hudetz, 2018; Singer, 2011; Tononi and Edelman, 1998). Furthermore, while the observed reduction in high frequency signal diversity also in the contra-lateral, awake hemisphere (panel B in **Supplementary Fig. 2**) may seem surprising at first, it may be related to cross-callosal signal propagation from the anaesthetised hemisphere (see point (6) in paragraph 4.1, above). Again, we suggest that "cross-state unreceptiveness" may explain why the non-injected hemisphere can maintain its awake, conscious state and processing, in spite of an apparent reduction in signal diversity/complexity, presumably because this reduction only reflects a change in input, rather than a change in intrinsic processing capability. This explanation is supported in part by the observation that the drop in LZc at high frequencies is lower in the non-injected than in the injected hemisphere.

Since the implementations of pre-processing and LZc in this work are necessarily different from that applied in earlier work, it is hard to compare our frequency profiles directly to these earlier studies. However, given that large parts of one hemisphere is under the effect of an

anaesthetic in the Wada condition, the lack of a significant decrease in LZc may seem surprising in view of changes that have been found during general anaesthesia with propofol (Schartner et al., 2015) and nREM sleep (Schartner et al., 2017). However, as discussed above (see 4.1, point (6)), the surprisingly unaffected signal diversity in the injected hemisphere, may not reflect the actual change in its internal state. If complex signals (originating in the unaffected hemisphere) are superimposed on simpler signals (originating in the injected hemisphere) through callosal interaction, the purely observational signal diversity measures may be expected to yield similar results for both hemispheres. Thus, these measures may not be able to distinguish mechanistic differences between the hemispheres that are important for understanding how they contribute to the patient's conscious experiences. It may also be argued that since patients undergoing Wada seem to remain largely conscious throughout the procedure (they are responsive, and can name and remember objects shown to them when the non-dominant hemisphere is anaesthetised, and afterwards they report having been conscious throughout the procedure, and can recall much of what happened), the lack of a clear reduction in signal diversity may seem roughly compatible with the general idea that consciousness is related to the brain's capacity for complex activity (Tononi and Edelman, 1998), although the underlying causes are likely to be more nuanced. Still, the unexpectedly modest and frequency-dependant effects of the Wada test on complexity measures are interesting and should be studied further.

In particular, a possible reason why the complexity measures used here did not change as expected in the Wada condition is related to the fact that these measures are derived from observational data (spontaneous EEG activity) as opposed to interventional data. Given that the observed activity and derived signal diversity measures in both hemispheres may not be due to changes in the local neural mechanisms, but rather reflect effects of the callosal input from contralateral hemisphere (e.g. slow waves imposed on the non-injected hemisphere, and complex activity imposed on the injected hemisphere), the apparent changes in complexity (or lack thereof) may not correctly reflect the mechanistic complexity in that hemisphere. Since the capacity for consciousness is more likely to depend on the actual mechanistic capacities of the brain or hemisphere, rather than its apparent capacities inferred from spontaneous EEG activity, perturbational methods may be required to properly probe the relevant properties of the brain/hemisphere (Casali et al., 2013; Massimini et al., 2009).

4.5. Differences between individual patients

While our analyses uncovered several statistically significant differences between conditions and hemispheres, caution should be taken in the interpretation of these results. One reason for this is the large variability in effects of the Wada intervention on the LZc values obtained (see **Fig. 3**). Not only was there no consistent drop in signal diversity across patients when going from wakefulness to the Wada condition (as expected from previous studies of effects of anesthetics on brain signal diversity, e.g. Abásolo et al., 2015; Ferenets et al., 2007; Schartner et al., 2015; Hudetz et al., 2016), but two of the 7 patients (patients 3 and 5) surprisingly showed an apparent increase in LZc during the Wada test. Actually, other measures of signal diversity (ACE, SCE; not shown) and power spectra also showed large inter-patient variability, suggesting large variability in the underlying data between patients. For example, while patient 2, after the injection, showed little to no change in the non-injected hemisphere and increased low frequency power in the injected hemisphere, patient 6 showed strong activation in high frequencies in the non-injected hemisphere, but showed little to no increase in lower frequencies.

What may have caused this high variability? First, although all the patients suffered from epilepsy with epileptic foci in the left temporal lobe, the group was necessarily heterogeneous, probably including variable pathological changes after years of severe epilepsy. Thus, one of the two patients showing a surprising increase in LZc (#3) even had a previous left temporal lobectomy. Furthermore, all the patients were on personalised antiepileptic medication which may have caused individual differences in the response to etomidate injection. The patients may also have responded differently to the drug for other reasons, but since there were no notes from the clinicians indicating abnormal responses, this seems less likely. Also, since the measures we used were normalised to baseline values immediately before the test, it is possible that differences in the patients' physiological states in the time leading up to the intervention may have affected the values. For example, some patients may have been tense and nervous before the test, whilst others may have been calm or even drowsy. This might have affected the baseline values in a different way for each and led to large inter-individual differences in our results. In addition, possible EMG contamination from tense muscles (Goncharova et al., 2003; Schuller et al., 2015), or other variable noise in the EEG signal, may have contributed to the variability. This possibility seems to be supported by the observation that the overall EEG quality seemed to be best for patients 1, 2, and 7, which also showed the clearest, left-sided drop in LZc (**Fig. 3**). Finally, there may even be larger variability in responses to etomidate within the normal population than is usually appreciated. Regardless of the causes of the variability, it is important to interpret the results with caution.

4.6. Classification at a single patient level

It should be noted that the significance of the measures was calculated at the group level and the group size ($N = 7$) was rather small. Thus, we additionally attempted to determine the condition (Wada vs. rest) and injection site (left vs. right) at an individual level using six-second segments of EEG (40 segments per condition/site). Broadband complexity measures and PSD above delta were the best single variables for determining condition and injection site. This indicates that there were indeed differences that might not have been captured by simple statistical tests at the group level. In fact, the recursive feature elimination suggested that LZc together with both PSD bands were the most important features for classification of the state of individual patients - as well as the site of injection. Connectivity features were the least used and also provided the lowest classification rates of all individual features. Considering that we used 6-second EEG segments in this analysis, it is probable that the signal-to-noise ratio (SNR) of the dwPLI for the individual segments was too low to be a useful feature for the classifier. More sophisticated metrics calculated on the connectivity networks might increase the importance of dwPLI as a feature (see e.g. Chennu et al., 2017).

4.7. Limitations

This study was based on data gathered from a limited number of participants in a clinical setting, and the analysis plans were developed only after the data were gathered. Taken together, these limitations reduce the generalizability of the results reported here. In particular, there was a large inter-subject variability in the EEG signals (exemplified in **Fig. 3** and the large error bars in **Figs. S2-S4,** panel B, and the observation that classification at a single patient level was possible with >80% accuracies). This might be expected, as the main purpose of clinical recordings is to allow live monitoring and not to yield high-quality data for research. In spite of their limitations, the results may form the basis for hypothesis generation for future studies on the effects of the Wada test on EEG patterns. In particular, it would be interesting to understand how apparent changes in power and connectivity relate to the apparent changes in complexity measures. Also, this study may pave the way for future attempts to test certain theories of consciousness by exploiting the unique features of the Wada test.

5. Conclusions

Our analysis of EEG data from Wada tests gave four main results: (1) We confirmed that the unilateral injection of anaesthetic in one internal carotid artery/hemisphere caused bilateral changes (e.g. slowing) in the EEG, even though brain function, assessed behaviourally, appeared to be substantially altered only unilaterally, on the injected side. We propose that this may be caused by conduction of slow activity, via the corpus callosum, from the injected hemisphere to the contralateral hemisphere, without substantially affecting the function of the latter hemisphere, thus reflecting a cross-state unreceptiveness (CSUR). (2) Measures of signal power (power spectral density, PSD) increased over both hemispheres, for every canonical EEG frequency band tested, and the functional connectivity inferred using dwPLI changed significantly both within and between hemispheres. (3) Surprisingly, we found no statistically significant difference in signal diversity (LZc) between states in either hemisphere when compared at the group level, but in 4 of 7 individual patients we observed significantly lower values of LZc in the injected than the non-injected hemisphere in the Wada condition. Nevertheless, (4) when attempting to classify both the condition (normal vs. Wada) and injected hemisphere (right vs. left) objectively using the measures, the highest accuracies were achieved when using a combination of LZc and PSD features, indicating that the complexity of the signal carries at least some non-redundant information about the condition and injection site.

Credit authorship contribution statement

Sebastian Halder: Conceptualization, Software, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Bjørn E Juel:** Conceptualization, Software, Writing - review & editing, Funding acquisition. **André S Nilsen:** Conceptualization, Software, Writing - review & editing. **Lashmi Venkat Raghavan:** Investigation, Resources, Data curation, Writing - review & editing. **Johan F Storm:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition, Writing - original draft.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2020.117566.

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