Supplementary Material for

A daily temperature rhythm in the human brain predicts survival after brain injury

Nina M. Rzechorzek, Michael J. Thrippleton, Francesca M. Chappell, Grant Mair, Ari Ercole, Manuel Cabeleira, The CENTER-TBI High Resolution ICU (HR ICU) Sub-Study Participants and Investigators, Jonathan Rhodes, Ian Marshall, John S. O'Neill

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Supplementary Videos 1 and 2 (also located at https://www2.mrc-lmb.cam.ac.uk/groups/oneill/research/heatwave/).

Appendix 1

Investigators for The CENTER-TBI HR ICU Sub-Study

Investigators for The CENTER-TBI HR ICU Sub-Study include: Audny Anke, Department of Physical Medicine and Rehabilitation, University Hospital of North Norway, Tromsø, Norway; Ronny Beer, Department of Neurology, Neurological Intensive Care Unit, Medical University of Innsbruck, Innsbruck, Austria; Bo-Michael Bellander, Department of Neurosurgery & Anesthesia & intensive care medicine, Karolinska University Hospital, Stockholm, Sweden; Erta Beqiri, NeuroIntensive Care, Niguarda Hospital, Milan, Italy; Andras Buki, Department of Neurosurgery, Medical School, University of Pécs, Hungary and Neurotrauma Research Group, János Szentágothai Research Centre, University of Pécs, Hungary; Manuel Cabeleira, Brain Physics Lab, Division of Neurosurgery, Dept of Clinical Neurosciences, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; Marco Carbonara, Neuro ICU, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy; Giorgio Chevallard, NeuroIntensive Care, Niguarda Hospital, Milan, Italy; Arturo Chieregato, NeuroIntensive Care, Niguarda Hospital, Milan, Italy; Giuseppe Citerio, NeuroIntensive Care Unit, Department of Anesthesia & Intensive Care, ASST di Monza, Monza, Italy; and School of Medicine and Surgery, Università Milano Bicocca, Milano, Italy; Endre Czeiter, Department of Neurosurgery, University of Pécs and MTA-PTE Clinical Neuroscience MR Research Group and János Szentágothai Research Centre, University of Pécs, Hungarian Brain Research Program (Grant No. KTIA 13 NAP-A-II/8), Pécs, Hungary; Marek Czosnyka, Brain Physics Lab, Division of Neurosurgery, Dept of Clinical Neurosciences, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; Bart Depreitere, Department of Neurosurgery, University Hospitals Leuven, Leuven, Belgium; Ari Ercole, Division of Anaesthesia, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; Shirin Frisvold, Department of Anesthesiology and Intensive Care, University Hospital of North Norway, Tromsø, Norway; Raimund Helbok, Department of Neurology, Neurological Intensive Care Unit, Medical University of Innsbruck, Innsbruck, Austria; Stefan Jankowski, Neurointensive Care, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK; Daniel Kondziella, Departments of Neurology, Clinical Neurophysiology and Neuroanesthesiology, Region Hovedstaden Rigshospitalet, Copenhagen, Denmark; Lars-Owe Koskinen, Department of Clinical Neuroscience, Neurosurgery, Umeå University, Umeå, Sweden; David K. Menon, Division of Anaesthesia, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; Geert Meyfroidt, Intensive Care Medicine, University Hospitals Leuven, Leuven, Belgium; Kirsten Moeller, Department Neuroanesthesiology, Region Hovedstaden Rigshospitalet, Copenhagen, Denmark; David Nelson, Department of Neurosurgery & Anesthesia & intensive care medicine, Karolinska University Hospital, Stockholm, Sweden; Anna Piippo-Karjalainen, Helsinki University Central Hospital, Helsinki, Finland; Andreea Radoi, Department of Neurosurgery, Vall d'Hebron University Hospital, Barcelona, Spain; Arminas Ragauskas, Department of Neurosurgery, Kaunas University of Technology and Vilnius University, Vilnius, Lithuania; Rahul Raj, Helsinki University Central Hospital, Helsinki, Finland; Jonathan Rhodes, Department of Anaesthesia, Critical Care & Pain Medicine, NHS Lothian & University of Edinburgh, Edinburgh, UK; Saulius Rocka, Department of Neurosurgery, Kaunas University of Technology and Vilnius University, Vilnius, Lithuania; Rolf Rossaint, Department of Anaesthesiology, University Hospital of Aachen, Aachen, Germany; Juan Sahuquillo, Department of Neurosurgery, Vall d'Hebron University Hospital, Barcelona, Spain; Oliver Sakowitz, Klinik für Neurochirurgie, Klinikum Ludwigsburg, Ludwigsburg, Germany; Department of

Neurosurgery, University Hospital Heidelberg, Heidelberg, Germany; Peter Smielewksi, Brain Physics Lab, Division of Neurosurgery, Dept of Clinical Neurosciences, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; Ana Stevanovic, Department of Anaesthesiology, University Hospital of Aachen, Aachen, Germany; Nino Stocchetti, Department of Pathophysiology and Transplantation, Milan University, and Neuroscience ICU, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano, Italy; Nina Sundström, Department of Radiation Sciences, Biomedical Engineering, Umea University Hospital, Umea, Sweden; Riikka Takala, Perioperative Services, Intensive Care Medicine, and Pain Management, Turku University Central Hospital and University of Turku, Turku, Finland; Tomas Tamosuitis, Neuro-intensive Care Unit, Kaunas University of Health Sciences, Kaunas, Lithuania; Olli Tenovuo, Rehabilitation and Brain Trauma, Turku University Central Hospital and University of Turku, Turku, Finland; Peter Vajkoczy, Neurologie, Neurochirurgie und Psychiatrie, Charité–Universitätsmedizin Berlin, Berlin, Germany; Alessia Vargiolu, NeuroIntensive Care Unit, Department of Anesthesia & Intensive Care, ASST di Monza, Monza, Italy; Rimantas Vilcinis, Department of Neurosurgery, Kaunas University of Health Sciences, Kaunas, Lithuania; Stefan Wolf, Interdisciplinary Neuro Intensive Care Unit, Charité–Universitätsmedizin Berlin, Berlin, Germany; Alexander Younsi, Department of Neurosurgery, University Hospital Heidelberg, Heidelberg, Germany; Frederick A. Zeiler, Division of Anaesthesia, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; and Section of Neurosurgery, Department of Surgery, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada.

Supplementary Methods

Actigraphy and chronotyping

Chronotype varies between sexes in an age-dependent manner,[1] as well as between individuals of the same sex. Actigraphy is a validated, non-invasive, and objective tool for studying sleep and circadian patterns.[2-5] We chose the ActTrust2 Wrist Actimeter (Condor Instruments, Sao Paulo, Brazil), sampling the following parameters at a frequency of 60 seconds: external temperature, skin temperature, rest/activity/sleep patterns, and various wavelengths of light (infrared, red, green, blue, UVA and UVB). All scanned participants wore their Actimeter for a minimum of 5 days and nights; they were asked to press an event button on the device once just before trying to sleep each night and again when they first awoke each morning. These reported sleep and wake times were validated against the actigraphy data and confirmed verbally with each participant on their scanning day. Data was transferred using the ActTrust Dock, then processed and analysed using ActTrust Studio software and an in-built Condor Instruments algorithm which uses Proportional Integral Mode (PIM) and Zero Crossing Mode normalized per second (ZCMn) data[6]. The light/dark phase was programmed to reflect the latitude and longitude (time zone with Coordinated Universal Time (UTC) offset of +1h) in Edinburgh, United Kingdom. Devices were retrieved, and the data downloaded and analysed, on the day of scanning; participants were asked to report which (if any) main sleep periods in the preceding week ended naturally ('free') or with an alarm or other disturbance ('scheduled'). Free and scheduled assignments were validated against the data. Sleep scoring parameters were enabled to include one or more main periods of sleep at night. Periods of 'off-wrist' time were excluded from the analysis. Sleep scoring was performed using the Cole-Kripke method (ActStudio software) to extract seven key sleep

parameters for each night of sleep.[6] ActTrust2 data underwent further processing to derive eleven diurnal characteristics, as well as MSF_{sc} and sleep-corrected social jetlag (SJL_{sc}).[7-9]

Scanning protocol

The scanning protocol was adapted from Thrippleton et al.[10] Localizers and structural sequences were acquired prior to MRS to plan voxel placement. Structural acquisition included whole-brain axial T2-weighted (T2w; three-dimensional fast spin-echo; TR/TE = 3200/408 ms; field of view, 240 x 240 mm^2; 18.2% slice oversampling; phase-encoding direction R>>L; 0.9-mm isotropic resolution) and sagittal T1-weighted (T1w; three-dimensional inversion recovery-prepared gradient echo; TR/TI/TE = 2500/1100/4.37 ms; flip angle, 7°; field of view, 256 x 256 mm^2; 0% slice oversampling; phase-encoding direction A>>P; 1-mm isotropic resolution) sequences. Structural imaging was repeated at each scanning session and the last-acquired structural images were used for HRF screening. Magnetic resonance spectroscopic imaging (MRSI; semi-LASER sequence, TR/TE = 1200/144ms; flip angle, 65°)[11] of the cerebrum was acquired from a 10-mm thick axial slice located at the level of the centrum semiovale, generating multiple 10 × 10 × 10 mm voxels (Fig. 3A). Six saturation bands of 40mm thickness were applied to suppress scalp lipid and other signals on all faces of the volume of interest. Automated shimming and partial water suppression (50Hz bandwidth) were applied. For each phase-encoding step, a 512-ms free induction decay (FID) was obtained. Since a single-voxel semi-LASER MRS sequence was not available at the time of the study, single-voxel PRESS MRS acquisition (TR/TE = 1200/144 ms; flip angle, 65°; 50Hz bandwidth water suppression) was used to sample the thalamus (voxel size 15 × 15 × 15 mm) and hypothalamus (voxel size 20 × 10 × 10 mm) (Fig. 3A). Single voxels were positioned to avoid interference from cerebrospinal fluid and/or large blood vessels; spectral quality was visually assessed in real-time to ensure that the water and NAA peaks were of a sufficient signal-to-noise ratio and the acquisition was repeated if necessary. Voxel position and orientation was matched between scanning sessions for each participant by a single radiographer who acquired all data for that individual at each time point. To maximise consistency of voxel positioning between participants, an illustrated placement protocol, specified by a senior neuroradiologist, was used. Foam pads were wedged between the skull and the head coil to eliminate any movement during acquisition. The whole procedure was optimized through pilot scanning, achieving a total scan time of ~30min, including shimming. All structural brain images were reviewed by a consultant neuroradiologist (G.M.). Further details on the scanning protocol are in Supplementary Appendix 3.

MRS data processing

MRS data were processed as described previously.[10] Briefly, free induction decays were apodised (Gaussian function, 4 Hz full width at half height) and the NAA and water chemical shifts determined by time-domain fitting of both resonances. For each voxel, four quality control filters were applied: a cut off for NAA linewidth (13 Hz for multivoxel data; 15.5 Hz for single voxel data), an R-squared measure of the NAA fit quality (0.80), a cut off for H_2O linewidth (13 Hz for multivoxel data; 15.5 Hz for single voxel data), and an R-squared measure of H_2O fit quality (0.93). Whilst the consensus recommendation threshold for H_2O linewidth is 0.1 ppm = 12 Hz,[12] the linewidth cut-offs used here included the effect of the Gaussian apodization applied. Hypothalamus and thalamus are challenging to shim, and the single voxels were larger than those acquired with the multivoxel sequence. For these reasons, the single-voxel data was expected to be of lower quality with a slightly increased linewidth (reflected in the higher linewidth cut-off).

Data from a voxel was discarded if it failed one or more of the set cut-offs and if the resultant data point affected the range of T_{Br} values for that participant at that time point, and/or the range for that voxel at that time point across a given sex group. The means and standard deviations for linewidths for NAA and H_2O are reported in Supplementary Table 3; spectral curve fitting and peak annotation are depicted in Supplementary Fig. 8. Spectral fitting was performed in batch mode without investigator intervention. Processing of MRS data was fully automated.

Rationale for prospective study sampling and linear mixed model

For our primary research question addressing whether there was a time of day variation in human T_{Br} using MRS, we had two main options: (1) sample a few individuals at high temporal resolution, or (2) sample many individuals at low temporal resolution. Given our secondary objectives (to determine T_{Br} variation by brain location and by sex) the second option was clearly preferable, since it offered sufficient power to detect such changes. Three time points were chosen for sampling, equally spaced across the extent of waking hours for an average human, and covering the range of times when patients would most likely be scheduled for advanced diagnostic imaging in the clinic. The null hypothesis was no change in T_{Br} by time of day; given the known, roughly sinusoidal daily variation in human T_{Bo}, a two time-point design carried a small but concerning risk of selecting two points at which T_{Br} returned to baseline. Therefore, three time points was considered the simplest design that would allow T_{Br} variation over time to be detected. Whilst time-dependent analyses exploring only the influence of time on a parameter classically employ nonlinear models, a linear mixed model approach was chosen for the following reasons (i) to accommodate fixed (e.g. time, age, sex, BMI) and random effects (allowing individual participants to have different baseline temperatures and different changes in temperature over time) (ii) to accommodate data nested at multiple levels, not least that T_{Br} measurements would be correlated within individuals, (iii) to accommodate repeated measurements per participant, (iv) to enable the use of data from all participants provided they undergo at least one, but not necessarily all 3 scans, and (v) to enable the incorporation of chronotype-control (with the proviso that 'time' would have to be modelled as a continuous, rather than a categorical variable).

Data management

Source data for the prospective study included electronic screening questionnaire responses (hosted by Jisc online surveys, Bristol, UK), actigraphy data logs (electronic format) and Study Participant Data Forms (Supplementary Appendix 4; hard and electronic format, hosted by the Centre for Clinical Brain Sciences, University of Edinburgh), DICOM files and other raw imaging data (stored at Edinburgh Imaging, hosted by the University of Edinburgh). Clinical imaging reports used for HRF screening were hosted by NHS Lothian; no members of the Study research team had access to these reports apart from the designated Neuroradiologist (G.M.). Data derived from human participants was managed and shared according to terms described in the Consent to Participate Form (Supplementary Appendix 1) and Data Protection Information Sheet, in compliance with the revised GDPR (https://www.eugdpr.org).

Patient temperature data analyses

Temperature data from each patient were subjected to an analysis pipeline to determine the presence/absence of a daily rhythm in T_{Br} and/or T_{Bo}. Since different algorithms frequently produce quite different results from the same data, we aimed to remove any potential for subjective bias in

the choice of analysis method, such that a positive result from any single method should not be sufficient to assign any individual dataset as having a 'daily rhythm'. After confirming that sufficient data were available for rhythmic analysis, the first criterion for assigning a dataset as having a 'daily rhythm' was that a rhythm of period length ~22-26h could be detected by visual analysis of the raw data in GraphPad Prism. This method was prioritised because it simulates what any clinician could practically achieve at the bedside by looking at the raw data without any assumed knowledge of chronobiology or applying algorithms to test for rhythmicity. However, this visual method alone was not considered sufficient, since human error may generate false positives as well as false negatives. Hence the data were then tested using a battery of validated algorithms that are well established in biological rhythms research. The second criterion was that the dataset had to have a 'daily rhythm' according to at least one of three semi-automated methods (cosinor analysis in GraphPad Prism, Harmonic Regression in R, or analysis in Biodare2). For cosinor analysis in Prism, a cosinor fit was tested against the null hypothesis of a straight line; the cosinor fit was modelled as a non-decaying cosine wave oscillating around a baseline that may change in a linear fashion over time:

$$y = baseline + (m * x) + amplitude * cos\left(((2 * \pi * (x - \phi))/\tau)\right)$$

where:
x = time
y = patient temperature (brain or body)
m = linear trend function
$baseline$ = the y (temperature) value around which the wave oscillates
$amplitude$ = height of the top of wave
τ = period (the time it takes for a complete cycle, in units of x)
ϕ = phase (the first time when $y = baseline$, in units of x)

For Harmonic Regression, the oscillation period to estimate and test for (Tau) was set to 24, a polynomial was fitted to each time series and used for normalization, and trend elimination was performed. The scalar used to indicate the polynomial degree (both for normalization and trend elimination) was based on the initial visual analysis in GraphPad Prism. Harmonic Regression results were based on statistical significance as determined by Benjamini-Hochberg-adjusted p-values (qvals). For Biodare2 analyses, amplitude and baseline detrending were performed and a period length constraint of 18 to 34h was applied prior to period analysis using each of the six available period analysis algorithms (FFT NLLS, MFourFit, MESA, LS Periodogram, ER Periodogram, Spectrum Resampling; full details at https://biodare2.ed.ac.uk/documents/period-methods). As recommended by the software developers, a positive result (period length of ~22-26h) had to be returned by at least two of these six algorithms, and the rhythmicity analysis (Classic JTK with no detrending and a preset pattern of a cosine curve with 24h period and phases spread every 1h) had to return a statistically significant 'true' result, in order for the data to be assigned as having a 'daily rhythm'. Results for each patient are presented in Supplementary Extended Data and the entire analysis pipeline for each temperature dataset is summarized in Supplementary Fig.1.

Rationale for generalized linear mixed model

A GLMM requires that (1) the outcome is not normally distributed but the distribution is known

(here, our outcome variable is binomial 'death or survival'), and (2) that there is more than one source of random error. The outcome itself is not linearly related to the predictor(s) but a function of it (logit for logistic regression) is. In our model, death is defined as a success or 'hit', and survival as a 'miss'. Our observations of 'death' or 'survival' are equal to '1' or '0' and the probability of death p lies between these two values. The logit (log of the odds) is thus a way of linking our GLMM to a non-normal distribution of one of two outcomes. In Fig. 5A-C, plots are provided for those readers who prefer to have a graphical presentation of GLMM outputs that enables visualization of all data points. However, since log of the odds is hard to interpret, and for greater accessibility, Fig. 5D presents the regular odds of death for each predictor. The odds of death = probability of death/(1-probability of death) = $p/(1-p)$. We transform from log odds back to regular odds using the exponential function; for this we also have to transform the 95% confidence intervals and in doing so, we are most interested in whether the confidence interval contains 1 (a confidence interval that contains 0 in log odds will always contain 1 when transformed to regular odds).

Our GLMM is designed as a predictive model (which x variables predict the y variable), not an explanatory one (which x variables are truly related to the y variable). For a predictive model, we are not concerned about confounders if the model makes accurate predictions using the variable of interest. To this end, based on our results, we might consider T_{Br} rhythmicity as a proxy variable; in reality, this variable is most probably directly relevant to outcome, but our model is not designed to test this. There are many other factors that may have influenced temperature and/or outcome in our TBI cohort,[13] however for the question we are asking of our retrospective data, we are not interested in how or why the temperature rhythm exists or does not exist—we are simply asking whether the presence/absence of a temperature rhythm correlates with odds of death in intensive care. The limitations of patient T_{Br} data currently available internationally precludes absolute adoption of all recommendations for prognostic modelling.[14] Nevertheless, the effect size for mortality presented herein calls for further validation of T_{Br} variation as a prognostic marker in independent datasets, alongside re-appraisal of existing models for TBI.[13]

Supplementary Text

Internal rhythms and health

The body clock—our internal circadian rhythm—anticipates the day-night cycle, thus optimizing every aspect of our physiology to solar time.[15] Circadian rhythms are fine-tuned by environmental cues, which feed into a multi-oscillator system; a 'master' clock within the suprachiasmatic nuclei (SCN) of the hypothalamus synchronizes cell-autonomous clocks throughout the brain and periphery, coupling systemic processes to light-dark transitions.[16] With age, this timekeeping becomes less robust—a situation compounded by modern living, which dissociates our body clocks from natural cues.[17,18] Shift-work, travel, and 'social jet-jag' all disrupt our sleep and circadian health.[9,19] Globally, neurological conditions are the primary cause of disability and second leading cause of death.[20] Several brain disorders including traumatic and vascular events, neurodevelopmental and mood problems, and neurodegenerative diseases are associated with circadian disruption; others, such as epilepsy, display symptomatic coupling to the body clock.[15,21-29] A mechanistic link between circadian and brain dysfunction is yet to be established, but a poorly

explored factor is T_{Br}.[15] This is despite the fact that elevated temperature and increased temperature variability are considered prognostic indicators after brain injury.[30-36]

Technical limitations of brain thermometry

Direct T_{Br} measurements from human patients are confounded by variable brain pathology, anaesthesia or sedation, TTM protocols, and/or sampling from subdural or intraventricular sites. MRS brain thermometry is attractive because it is non-invasive and can be used to explore multiple brain locations. However, many patients with moderate to severe TBI (as included in this study) are not suitable candidates for MR-based monitoring. In particular, intracranial Licox probes are not MRI-compatible and in most patients with mild TBI, the placement of an intracranial probe is not required nor ethically justified. Thus, it is not currently possible or safe to simultaneously record T_{Br} from a probe whilst a patient is undergoing MRS brain thermometry. Controversy also remains over the ideal calibration method for MRS thermometry. Common approaches use phantoms to mimic brain tissue, or directly-measured T_{Br} curves from other species under anaesthesia (typically neonates), often reporting T_{Br} values that are substantially lower than direct normothermic T_{Br} measurements. Patient MRS studies have focused on relative T_{Br} changes in response to injury or other pathology.[34,37-43] At the time of writing, we are aware of 25 published original research articles that used MRS for brain thermometry in healthy adults (Supplementary Table 1). Sex was reported in 22 of these studies; 21 of them included <36 participants per experiment—a total of 329 participants including 105 women. Only two studies made sex comparisons for T_{Br}, both reporting no statistical difference.[44,45] One retrospective study (n=150 with 90 females) found a 0.1°C higher cerebral T_{Br} in females[46]; smaller than the sex difference found in our cohort. However, no prior studies controlled for menstrual cycle phase, which has enabled us to reveal physiologically-relevant differences between the sexes in all brain regions measured. In addition to menstrual cycle, the large retrospective study of Maudsley et al.[46] did not control for age, time of day, chronotype, or medications, all of which may have contributed to high variance in their dataset. Eight MRS brain thermometry studies reported time period of scanning, but only 6 of these arguably controlled for time of day (scanning window 2–3 hours).

Recent studies have emphasized the need for more accurate calibration and potentially brain region-specific temperature coefficients to account for differences in tissue content, microstructure and orientation.[10,46-48] However, using methods described by Marshall[40] and Thrippleton et al.[10] we have now obtained data that are absolutely consistent with published direct T_{Br} measurements obtained from healthy, conscious, non-human primates, and normothermic human patients.[49-52,53] The limitations of MRS thermometry dictate that 'absolute' temperatures cannot be estimated with the same confidence as relative temperature differences. That said, the mean global temperatures we have observed lie, as expected, between average human core T_{Bo}[54,55] and direct brain temperatures obtained from other species and human patients with brain injury (Supplementary Fig. 3).[49,52,56,57] To avoid concerns arising from potential inaccuracy of the calibration intercept, we have focused our analysis on relative T_{Br} changes, for which MRS thermometry is exquisitely well-suited.[10] Given the propensity for major brain structural changes with age and neurodegeneration, exploring time of day variation at different life stages, rather than single time-point variation over months or years, would circumvent some of the inherent limitations of the technique. Ultimately, physiological and clinical understanding should be informed by experimental observation. MRS thermometry has high spatial resolution and is extremely sensitive to temperature change within a tissue, but the technique can only ever have low temporal

resolution. Moreover, it cannot be used for repeated measurements of brain-injured patients; whereas, direct temperature probes measure absolute temperature with very high temporal resolution, but cannot be used in healthy individuals. We have found these two approaches of T_{Br} measurement to be mutually supportive, with the temperature probe data and repeated measurements of individuals allowing us to be much more confident about the fidelity of the MRS data than in any previous study. Temperature variations were further supported by high temporal resolution actigraphy data in our healthy cohort, and core T_{Bo} data in TBI patients.

Temperature gradients

In our healthy cohort, T_{Br} was always higher than oral temperature. The direction of this brain-body temperature gradient contradicts some studies,[58,59] but agrees with others.[44,58,60] Our findings are corroborated by a case report documenting parallel oesophageal, tympanic, intraventricular, and cortical white matter temperature in an unanaesthetised, normothermic patient.[53] The relationship between sites was fixed, with cortical T_{Br} > ventricular > oesophageal > tympanic.[53] During an overnight recording, cortical T_{Br} declined from ~38.1°C at 9pm to a minimum of ~36.7°C at 3am, as expected during sleep. Considering this 1.4°C decrease over 6 hours, and extrapolating back to the predicted maximum T_{Br} for this male patient (~3pm), maximum T_{Br} in this region would have been ~38.7°C—practically indistinguishable from the mean T_{Br} we found in healthy male parietal white matter in the afternoon (Fig. 4). In the report, T_{Br} exceeded oesophageal temperature by ~0.7°C, and so our measured difference between oral temperature and T_{Br} is realistic. A similar brain-body temperature relationship was found in healthy volunteers with a mean parietal MRS-derived T_{Br} of 38.1°C; 1.3°C higher than rectal temperature.[45] Our retrospective analysis indicates that a positive T_{Br}-T_{Bo} gradient cannot be attributed entirely to local injury (Supplementary Fig. 2) and so, as for non-human primates,[49,50] it is likely a normal physiological phenomenon in humans. Indeed, maintenance of this gradient was associated with a better outcome in severe head injury.[61]

MRS-derived estimates of absolute T_{Br} often conflict with known T_{Br}-T_{Bo} gradients in other species, including non-human primates.[49,50] A recent 7T MRS-based T_{Br} study in healthy sheep found a brain-regional T_{Br} gradient, but global T_{Br} was 0.7°C lower than T_{Bo} (38.5 versus 39.2°C).[56] This inversion of the brain-body temperature gradient might be explained by several factors: (i) that measurements were conducted under general anaesthesia (which inverts temperature gradients in some species), (ii) the species under test has defined neurovascular anatomy to effect selective brain cooling (SBC; a carotid *rete mirabile* is absent in primates and the existence of other SBC mechanisms in humans and non-human primates is highly contentious),[49,52,62,63] and (iii) it remains possible that absolute mean global T_{Br} is the key defended parameter across mammals, whilst T_{Bo} can vary more widely in a species-specific manner (T_{Bo} of most domestic livestock is higher than human T_{Bo}). In seminal work by Fuller et al., diurnal variations in colonic and hypothalamic temperatures were explored simultaneously in unsedated male squirrel monkeys.[52] At various ambient temperatures (T_a of 20, 26, 32, and 36°C), there was no change in the phase or period length of either temperature rhythm, and hypothalamic always exceeded colonic temperature except at T_a 36°C. Mean temperatures at both sites increased with increasing T_a, and there was a clear reduction in amplitude in both temperature rhythms, primarily resulting from an increase in temperature minima during the night. Importantly, hypothalamic temperatures ranged from 37.5–39.1°C between the afternoon and late evening at T_a 26°C, remarkably similar to the hypothalamic temperature range observed by MRS in male subjects here (Fig. 4).[52] Compelling evidence for the

absence of SBC in primates comes from a study in unrestrained baboons, where hypothalamic temperature was consistently higher than carotid arterial or abdominal temperature, ranged between 37.5 and 38.8°C under cycling T_a conditions (15–35°C), and from 37.5–40.5°C when water was restricted.[49] Our results for this brain region are thus entirely consistent with direct temperature measurements from conscious healthy animals of closely related species.

Temperature rhythms and sleep

Temperature rhythms emerge immediately after birth and peak in amplitude during childhood as the brain matures.[15] T_{Bo} rhythms then phase-delay in adulthood, and gradually reduce in amplitude and phase-advance with ageing.[15] Daily variations in heat loss (rather than heat production) are considered to drive these daily changes in temperature[64]; it follows that an increase in minimum T_{Br} (and consequently, a reduced T_{Br} rhythmic amplitude) with ageing must represent a failure of heat loss. It has been estimated that T_{Br} would increase by ~0.28°C/min if the key routes of heat dissipation did not exist,[65] so even modest cerebrovascular compromise is likely to result in net heat gain. This is not necessarily at odds with findings in ischaemic stroke, where T_{Br} is higher in the lesion penumbra than the lesion core.[35,40] At the core, total loss of perfusion would lead to a T_{Br} drop as observed during brain death.[66] A vascular-driven disruption of T_{Br} rhythm reconciles various risk factors for non-convulsive seizures during sleep in dementia patients with some of the temperature-sensitive molecular aberrations in animal models of these disorders,[67,68] and potentially even disease-specific distribution of protein pathology. Rather than an inherent vulnerability of specific, synaptically-connected neurons, or an age-related decline in glymphatic clearance,[19] the predictable 'spread' of protein aggregates through the brain in neurodegenerative disease may simply reflect age-related progression of cooling failure through vascular compromise. This is conceptually straightforward for chronic traumatic encephalopathy, where tau pathology is concentrated at cerebral sulci surrounding repetitive microvascular trauma.[69] The propensity of mutant protein forms to aggregate in familial disease may signify a heightened sensitivity to temperature-driven effects on biochemical modification in regions of greatest diurnal T_{Br} variability.[70,71] Notably, the hypothalamus (and in particular the SCN) is one of the most densely vascularized regions of the mammalian brain.[72] This is the first study to measure T_{Br} in the human hypothalamus; future studies should consider whether the position and vascularity of this structure optimizes its circadian and thermoregulatory roles, whilst shielding it from high absolute temperatures. Epilepsy is bimodally distributed but the highest incidence is in adults >75 years, where cerebrovascular disease causes more than a third of cases.[67,73] Alzheimer's disease and late-onset epilepsy may be pathologically linked through vascular changes, tau pathology, or both, and sleep deprivation can unmask a life-long tendency to seizures.[67] Given the temperature-sensitivity of tau protein modifications, the role of brain vasculature in effecting T_{Br} changes, and the importance of temperature in sleep, impaired T_{Br} rhythms might contribute to this pathological link.[70,74] In everyday clinical terms however, cerebrovascular impairment is not the only route to a T_{Br} increase; nasal airflow is clearly inhibited during prolonged wearing of facemasks,[75,76] or indeed bypassed all together in intubated patients. That daily T_{Br} variation is such as strong predictor of mortality (Fig. 5) underpins an urgent need for further chronotype-controlled research in this area.[30,53]

Superimposed on daily temperature rhythms is a daily oscillation in thermoregulatory capacity which is relatively impaired during sleep.[64] This may have contributed to the increased variability in T_{Br} in participants that reportedly slept during their scans (Fig. 3B). Whilst the preoptic area and

anterior hypothalamus are the main loci for T_{Bo} regulation, their thermoregulatory function appears to suppress, rather than coordinate, the circadian organization of T_{Bo}—a tenet of the SCN.[15] Studies in several mammalian species including humans have suggested that circadian T_{Br} and sleep-wake cycles are not interdependent, but that under free-running conditions, the period length of the sleep-wake cycle is negatively correlated with average core T_{Bo}.[77-81] In rats, ultradian (<24-hour) T_{Br} rhythms are superimposed on circadian T_{Br} rhythms.[82] These high-frequency rhythms are closely associated with sleep-wake states, and persist in the absence of SCN input which abolishes the circadian rhythm in T_{Br}.[82] Rodents however are not small humans; there are critical differences in thermoregulation strategy and rate of heat gain/loss across mammals which make it difficult to translate temperature-related phenomena between species of vastly different sizes.[83] Such caveats aside, it is clear that changes in T_{Br} can affect synaptic structure, function, and plasticity in mammals,[84,85] and may even influence the diurnal variation in glymphatic clearance of 'brain waste'.[19,86] This clearance in awake rats seems to be greatest during the rest phase, at a time when T_{Br} would be lowest and CBF increased.[86-88] On the one hand, increased CBF might simultaneously enhance the removal of brain heat whilst favouring pulsatile glymphatic clearance; on the other it could reduce the perivascular space available for glymphatic flow through hydrostatic means, particularly in the presence of hypertensive cardiovascular disease.[19] We found no significant correlation between T_{Br} and subject-reported sleep during scanning (Supplementary Appendix 5). However, we did not employ a gold standard method for sleep measurement (polysomnography) and since the scan time was around only 30 minutes (and the MRS acquisition much less than this), we would not expect to observe a significant effect. Irrespectively, our late evening T_{Br} data confirms that sleep onset coincides with a reduction in both core T_{Bo} and T_{Br}, assisted by a selective redistribution of blood flow (Fig. 2B-C), and presumably modulated by melatonin.[64,89,90-92] Moving forward, it will be important to consider the role of this hormone, and whether its rhythmic disruption in intensive care patients affects its rhythmic differential modulation of regional vasculature.[92,93]

Limitations of retrospective TBI analysis

There are many factors (internal and external to the patient) that might impact upon T_{Bo}, T_{Br}, or mortality risk in TBI patients.[13] We could only test for relationships among the limited number of parameters for which data were available in our patient cohort, and that could be accommodated by our sample size without overfitting the outcome model. Our model thus posed a very simple question of whether the presence or absence of a daily T_{Br} rhythm correlates with the odds of death, without implying causality in either direction. Despite our inability to adjust for several more conventional parameters such as injury severity, our analysis suggests that loss of a daily T_{Br} rhythm is a very powerful predictor of mortality compared to prognostic factors reported historically, either alone, or in combination.[13] One interpretation is that a daily T_{Br} rhythm together with age might be a very simple way to accurately predict ICU outcome after brain injury—independent validation and a rigorous prospective clinical study is of course needed test this hypothesis.[14] The fact that presence of a daily T_{Br} rhythm did not correlate with either age or mean T_{Br} indicates that this parameter has additional predictive value in its own right.

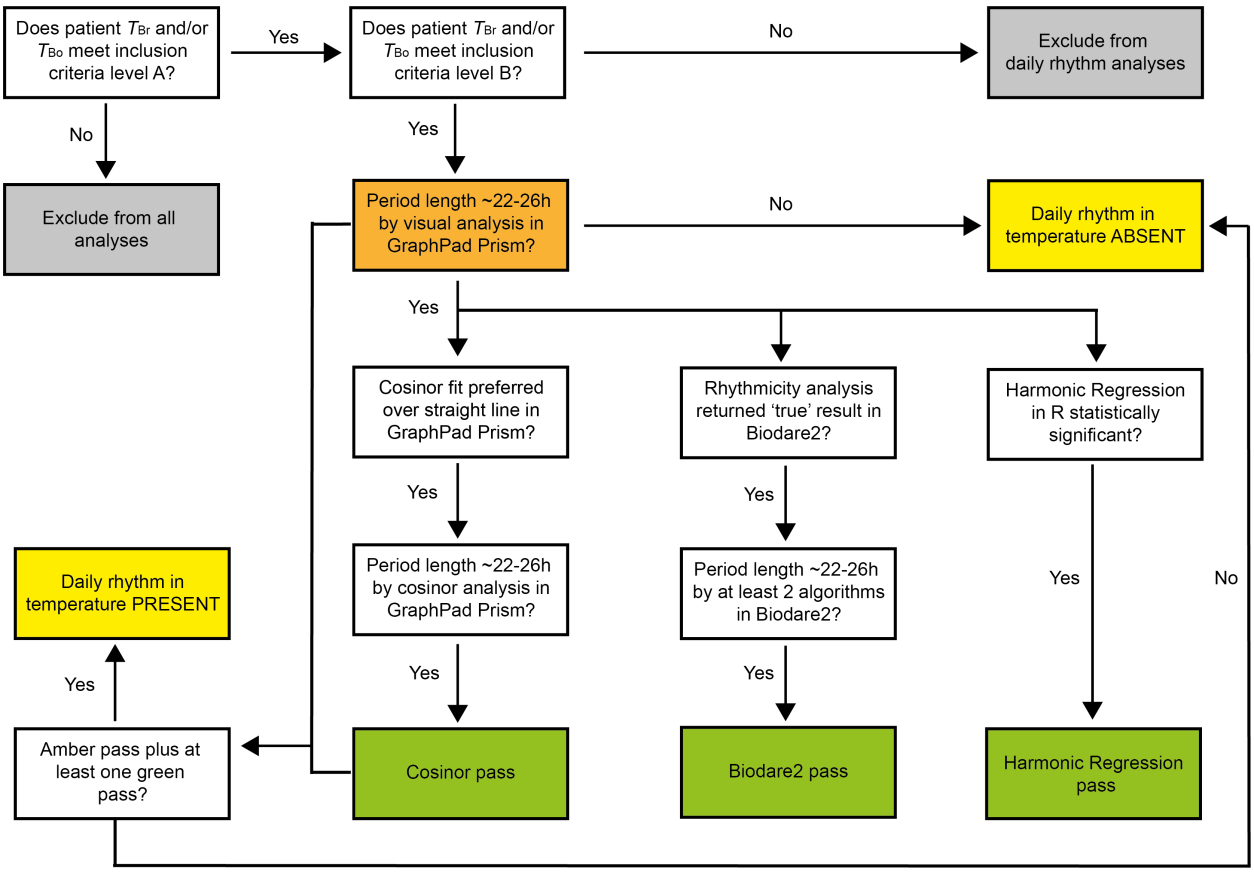
Clinical applications

Impaired daily core T_{Bo} rhythmicity correlates with injury severity and worsened behavioural measures for patients in vegetative or minimally conscious states.[21,22] One study reported that only 29% of trauma patients had a T_{Bo} rhythm period of 22–26h, and circadian disruption has been

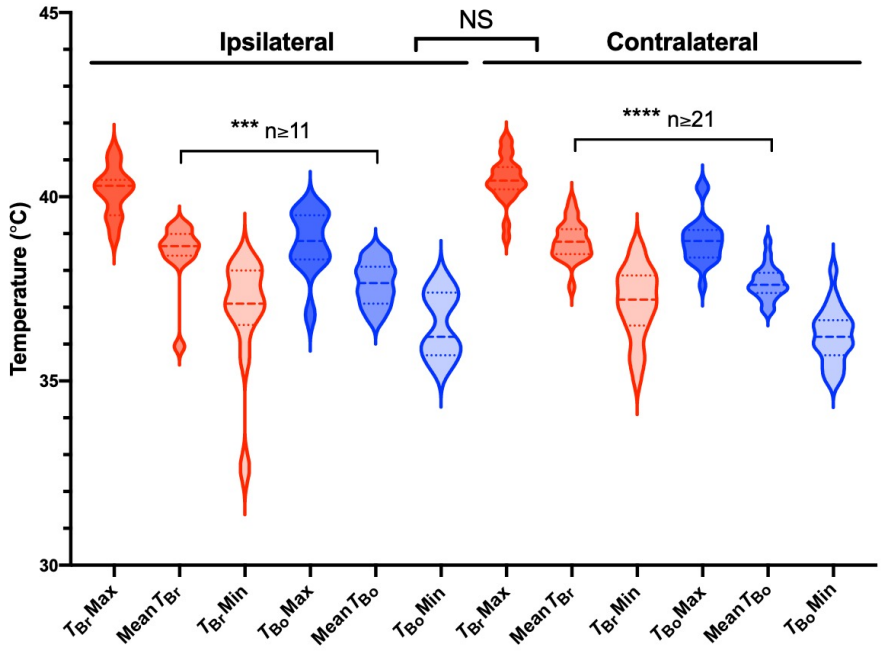
associated with poor outcome after both head trauma and subarachnoid haemorrhage.[94,95] T_{Br} was not measured in these patients, but our findings (Fig. 5) suggest that relationships between temperature rhythm and outcome would be clearer if T_{Br} was included for monitoring and prognostication. Indeed, functional scoring of comatose patients varies by time of day, with better scores around maximum T_{Bo}.[94] Based on our new data, we recommend review of existing guidelines for management of TBI patients in intensive care. Specifically, we propose that direct T_{Br} (in addition to core T_{Bo}) becomes the clinical standard for temperature monitoring in these patients, and that, alongside other clinical parameters, daily *temperature variation* should be considered a core element of treatment plans and prognostication. At the very least, time of day should be considered when scheduling assessments, and ideally, patient-specific temperature rhythm and chronotype should be accounted for when interpreting results that influence life-changing clinical decisions. Future studies should standardize the method of T_{Bo} measurement, and the location of intracranial probes with respect to focal brain pathology. By contrast, HEATWAVE would find greatest practical utility for chronic brain disease, since our voxel maps give an approximation of the T_{Br} expected in each brain location at three clinically-relevant times of day, and how much T_{Br} should vary between voxels at a given time point. Since each data point in each map is averaged from multiple healthy individuals, it incorporates the range of ages, BMIs, and chronotypes for each sex in the demographic tested.

Beyond brain injury and disease, our results further question the value of single-point temperature measurements using peripheral thermometers.[96] We have shown that more sophisticated analyses can better exploit temperature as a clinical tool. Wearable devices now permit easy and convenient recording of daily rhythms in many physiological parameters. Algorithm-based temperature profiling will help accomplish the goals of precision medicine, not just for individuals,[54] but at scale. For example, in an infectious disease outbreak, real-time screening for fever development could rapidly identify high-risk individuals by deviation from their own temperature rhythm, rather than a population ‘mean’ or by random testing. Personalized, digital, round-the-clock temperature monitoring would thus advance remote health tracking and evidence-based enforcement of global health policy in the context of emerging disease.

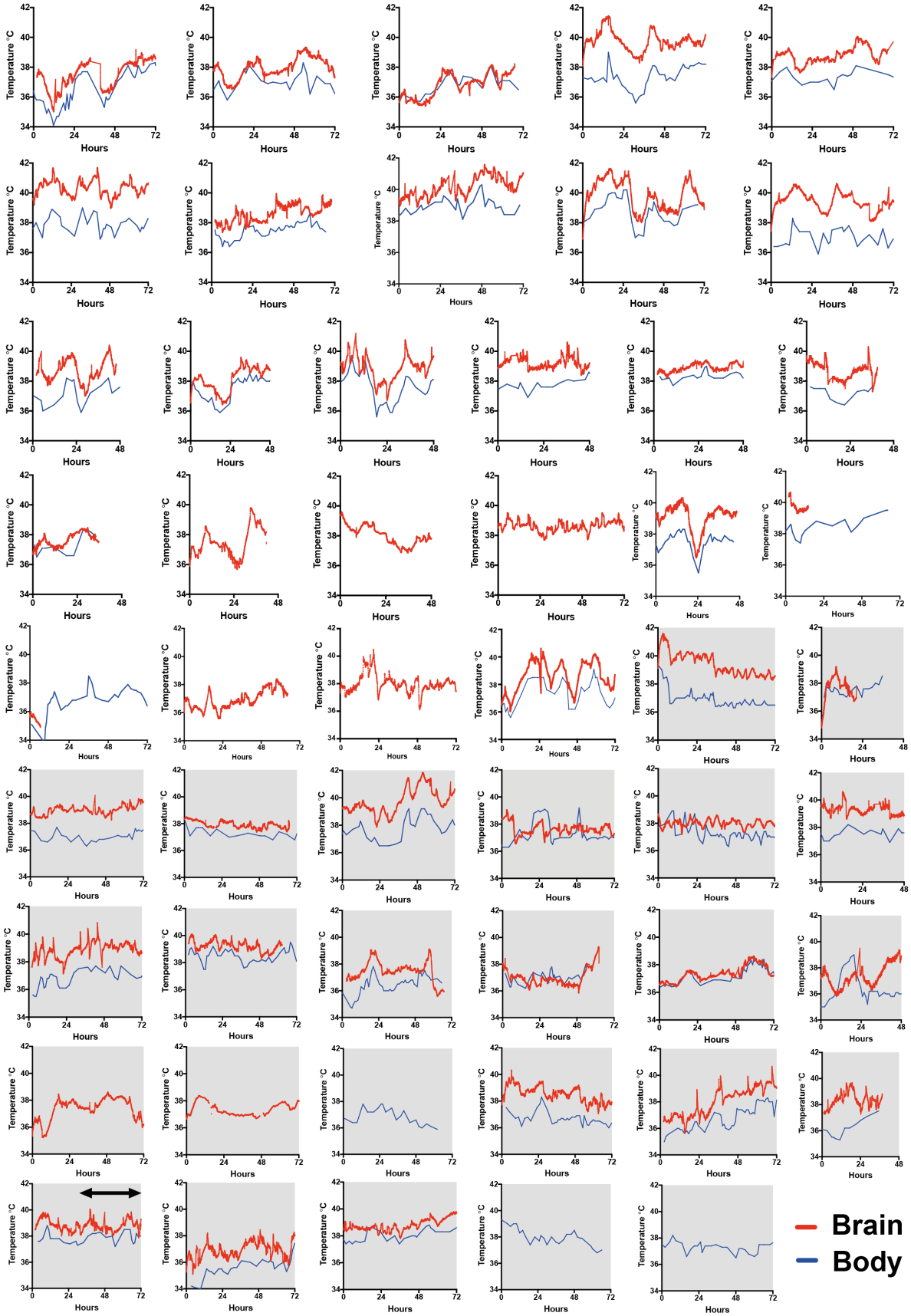
Supplementary Figures and Tables



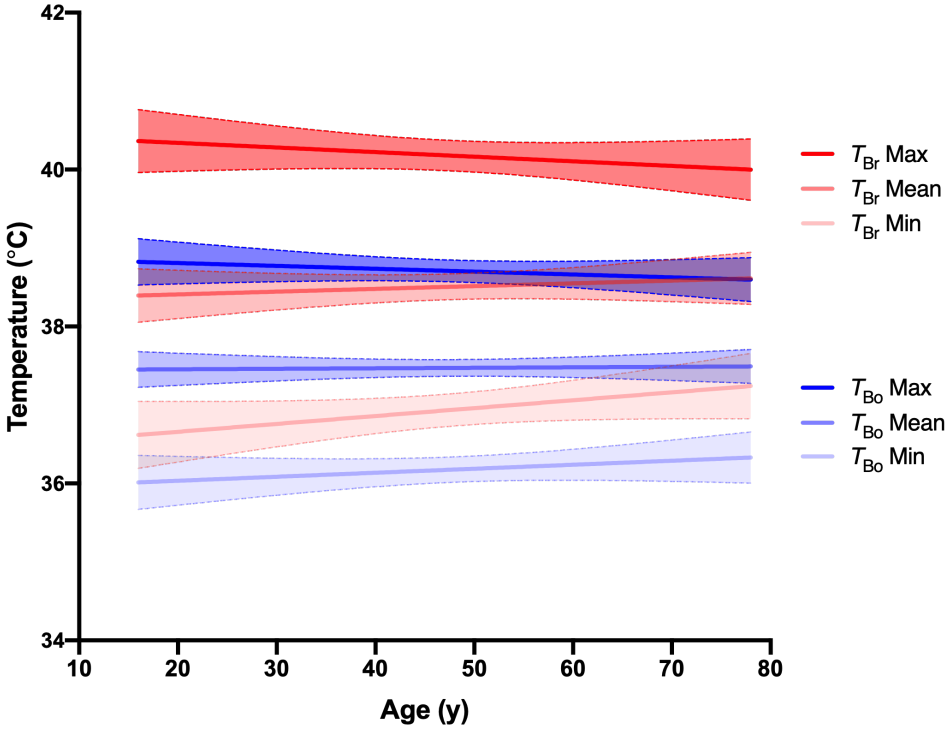
Supplementary Figure 1 Patient temperature analysis pipeline.



Supplementary Figure 2 Patient temperatures according to probe site. Violin plot of T_{Br} and T_{Bo} in TBI patients with focal brain injury. The nature of brain injury was available for 46 patients (34 focal, 12 diffuse); of the focal injury cases, the brain probe was placed ipsilateral and contralateral to the site of injury in 12 and 22 cases, respectively. Mean T_{Br} was greater than mean T_{Bo}, regardless of site of probe placement (mixed effects analysis with Tukey's for multiple comparisons ***P=0.0007, ****P<0.0001, n refers to number of individual patients; one patient in each probe site group lacked T_{Bo} data). There were no significant differences in temperature according to site of probe placement.

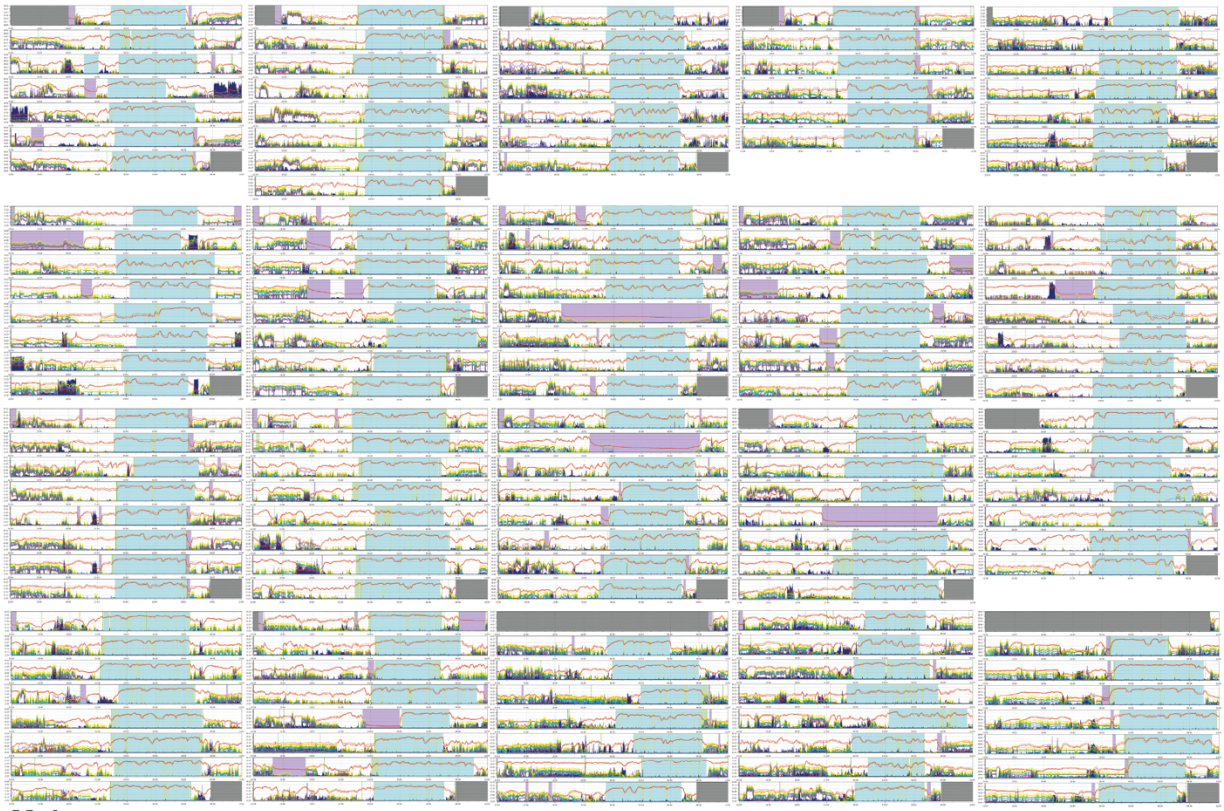

Temperature °C
Hours
Brain
Body

Supplementary Figure 3 Raw temperatures in non-survivors and patients with daily rhythms. Upper panel (white plots) show raw T_{Br} and/or T_{Bo} data from 26 patients that survived intensive care and were deemed to have evidence of a daily rhythm in one or both temperature parameters at some point during recording. Lower panel (grey plots) show the 25 patients that did not survive in intensive care after TBI. All but one of these patients (lower left) showed a complete lack of a daily temperature rhythm during recording. Rhythmicity was apparent in some of these patients but it did not meet our assigned daily cut-off for period length of 22–26h. Note that in the patients who died, transitory inversion of the T_{Br}–T_{Bo} gradient was more commonly observed than in those that survived (in which T_{Br} was almost always higher than T_{Bo}). The patient in the lower left corner was classified as having a daily rhythm based on the time period highlighted by the horizontal black arrow, although it is clear that the period length was >26h earlier on in the recording.

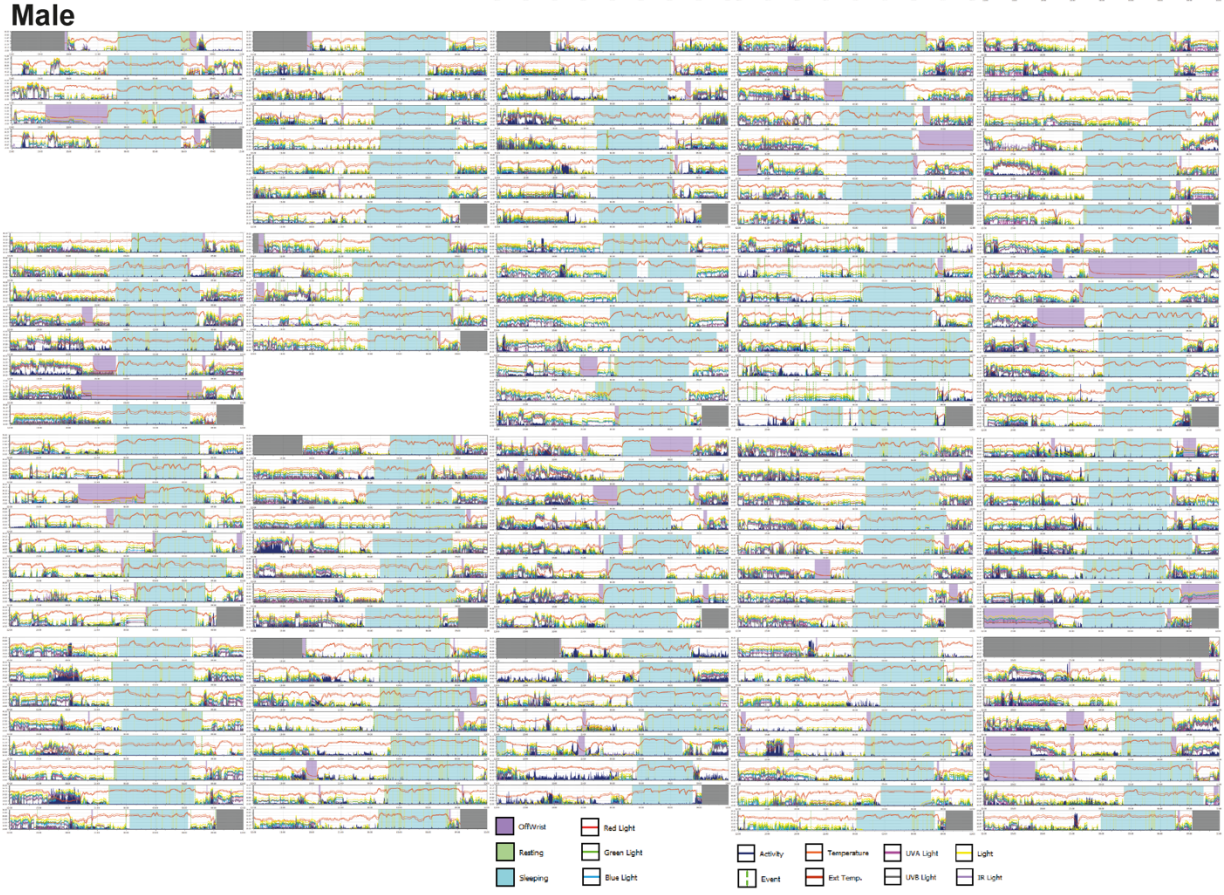


Supplementary Figure 4 Minimum brain temperature trends upwards with age in TBI patients. Linear regression of patient temperatures with age (n=105 for T_{Br}Mean, n=104 for T_{Br}Max and T_{Br}Min, n=101 for T_{Bo}Max and T_{Bo}Min, n=94 for T_{Bo}Mean); the apparent upward trend for minimum T_{Br} was not significantly different from zero (slope 0.010, 95% confidence interval -0.002 to 0.022, P=0.096). Shaded areas represent 95% confidence intervals.

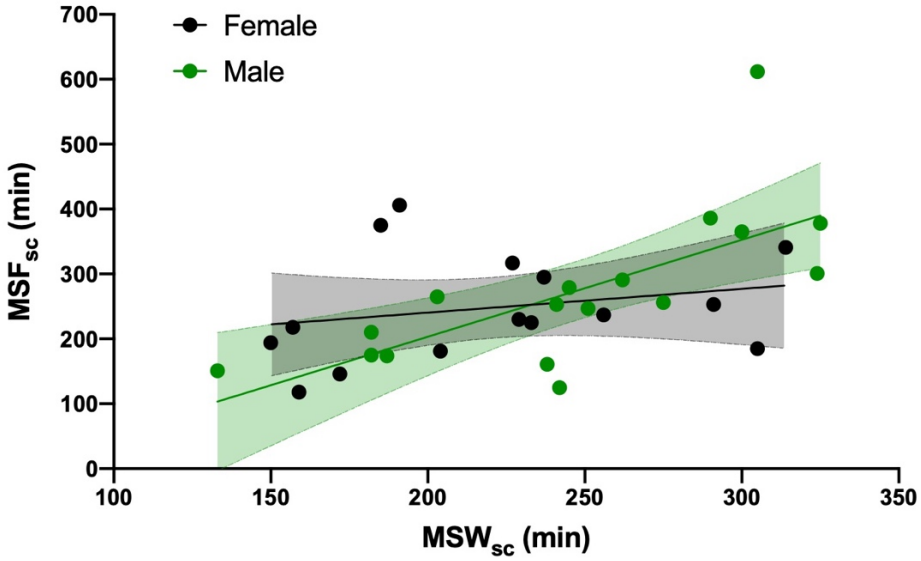
Female



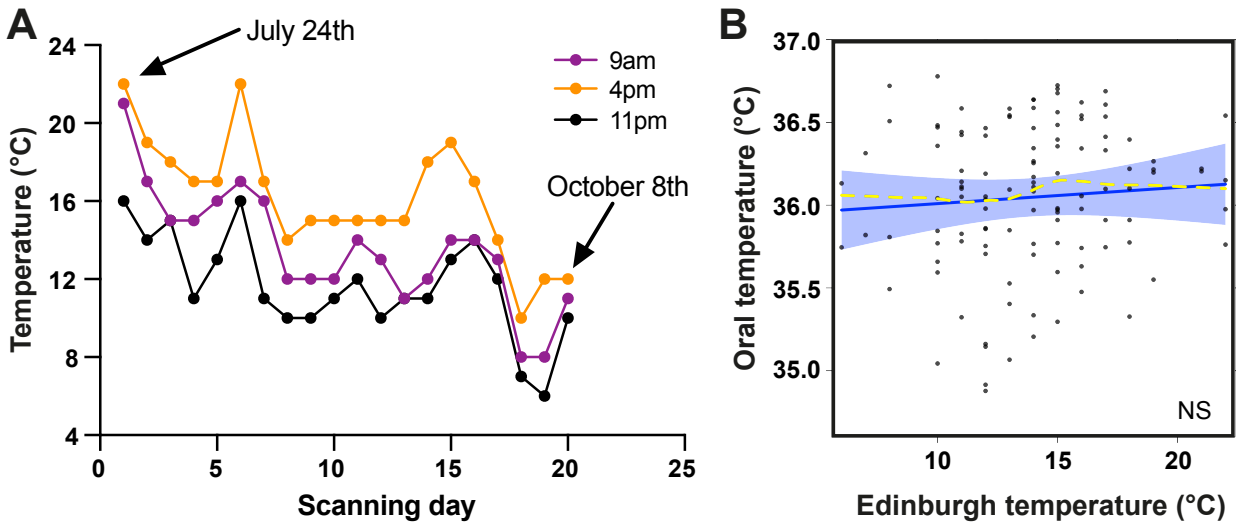
Male


Off/Wrist
Resting
Sleeping
Red Light
Green Light
Blue Light
Activity
Event
Temperature
Ext Temp.
UVA Light
UVB Light
Light
IR Light

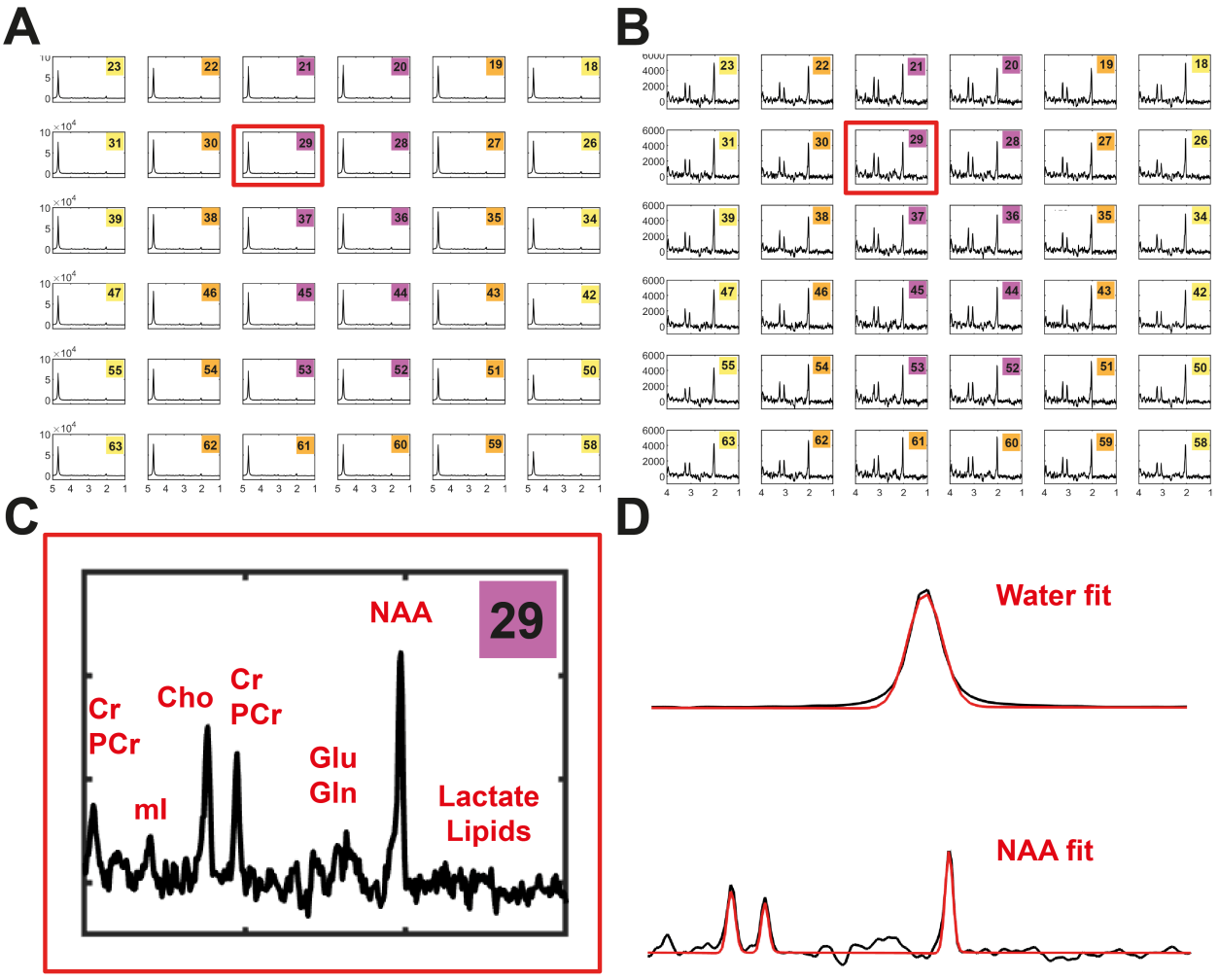
Supplementary Figure 5 Actograms in healthy volunteers. Montage of actograms in 40 healthy volunteers. Most participants wore an ActTrust2 device for eight days. Actograms are presented in order of acrophase (earliest top left, latest bottom right) in females and males separately. Note increase in participant wrist skin temperature during sleep phase, reflecting peripheral vasodilation (core body temperature is expected to drop in anti-phase to a skin temperature increase during sleep).[97]



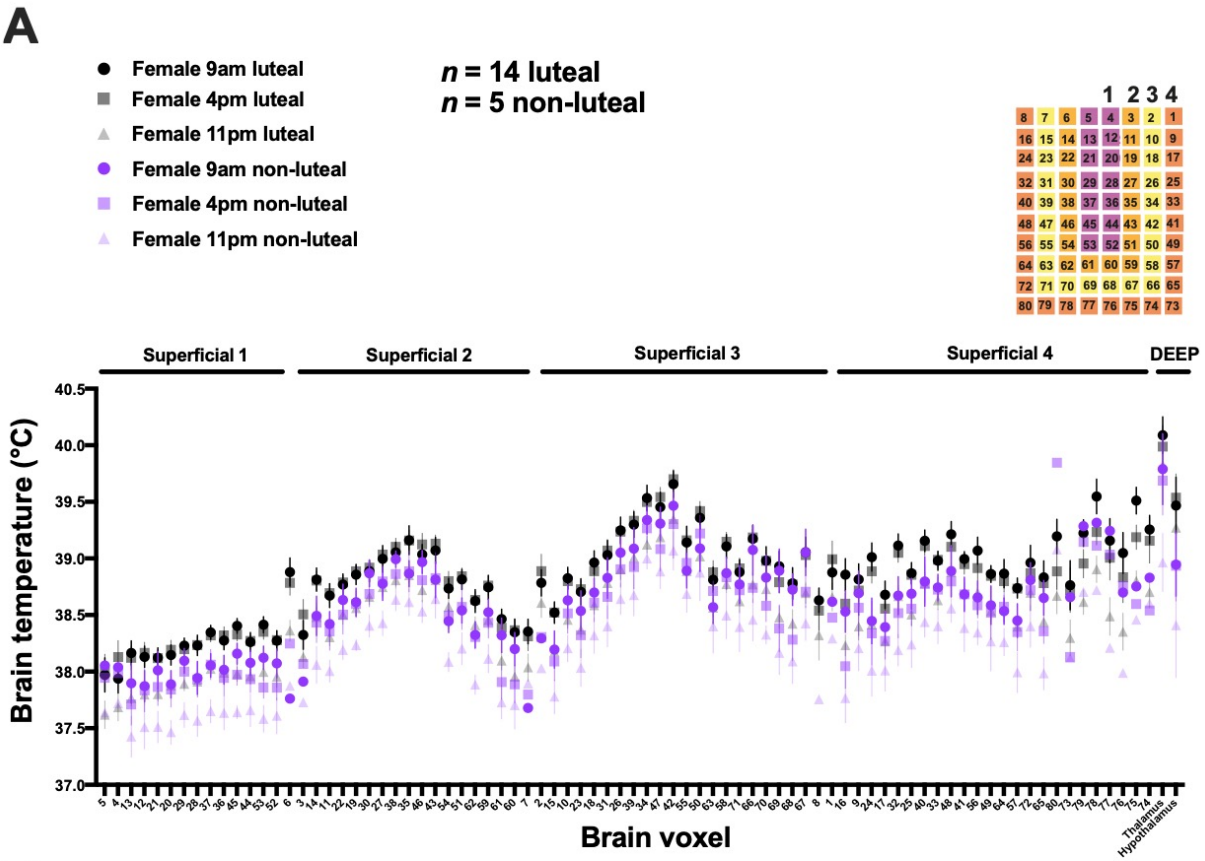
Supplementary Figure 6 Interpolation of MSF_{sc}. Five females and three males reported no free days during actigraphy sampling; their sleep-corrected midpoint of sleep on free days (MSF_{sc}) was interpolated by linear regression with the sleep-corrected midpoint of sleep on work days (MSW_{sc}). Regression was performed for males and females separately.

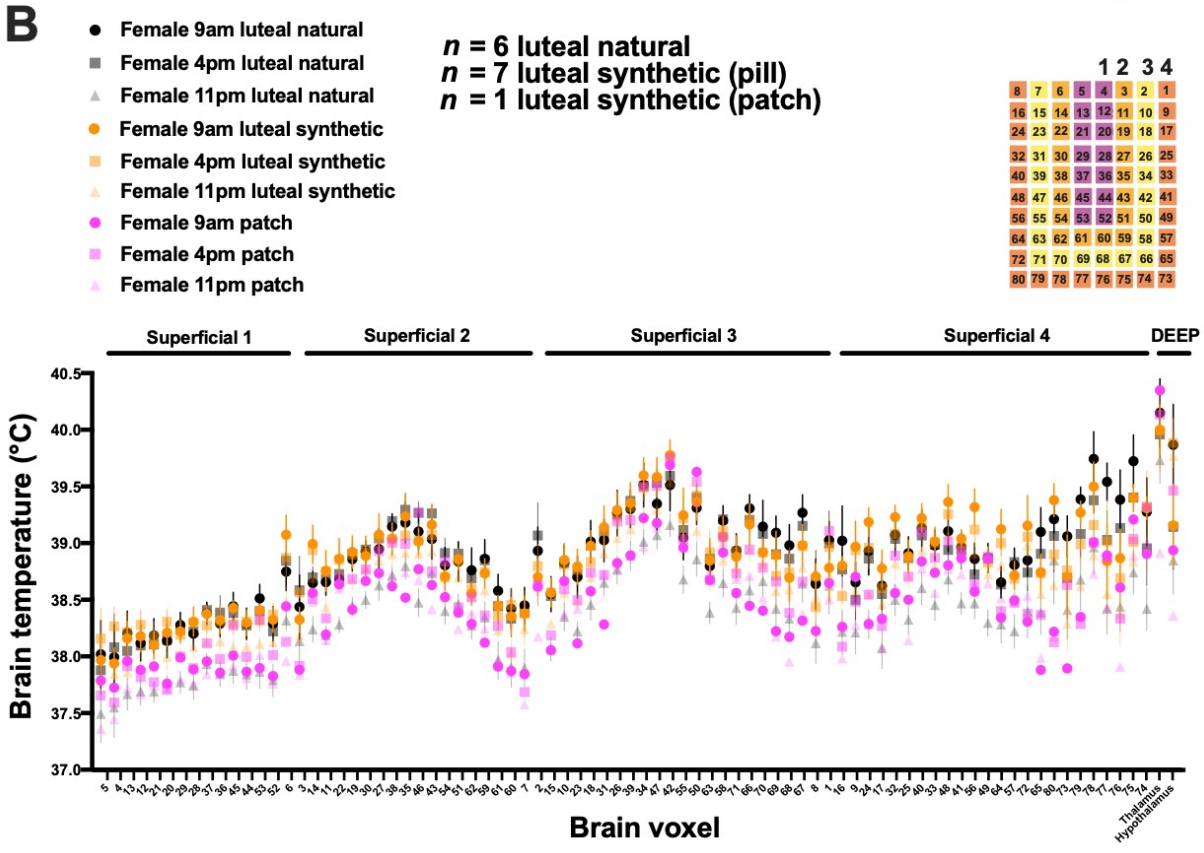


Supplementary Figure 7 Environmental temperature variations. (**A**) Recorded outdoor air temperatures at 1.5m above ground level in Edinburgh at times of scanning sessions, on each day of scanning during data collection period. Collection period limited to 14 weeks to minimize any effect of seasonal variation in environmental temperature and light. For actigraphy data extraction from each participant, daily changes in sunrise and sunset times were adjusted according to date of device retrieval using date and location function in ActStudio. (**B**) Linear mixed model results for oral temperature by environmental temperature. Solid blue line represents model fit, shaded areas represent 95% confidence intervals, dark grey circles display residuals (single temperature data points), and smoothed dashed yellow line represents partial residuals.

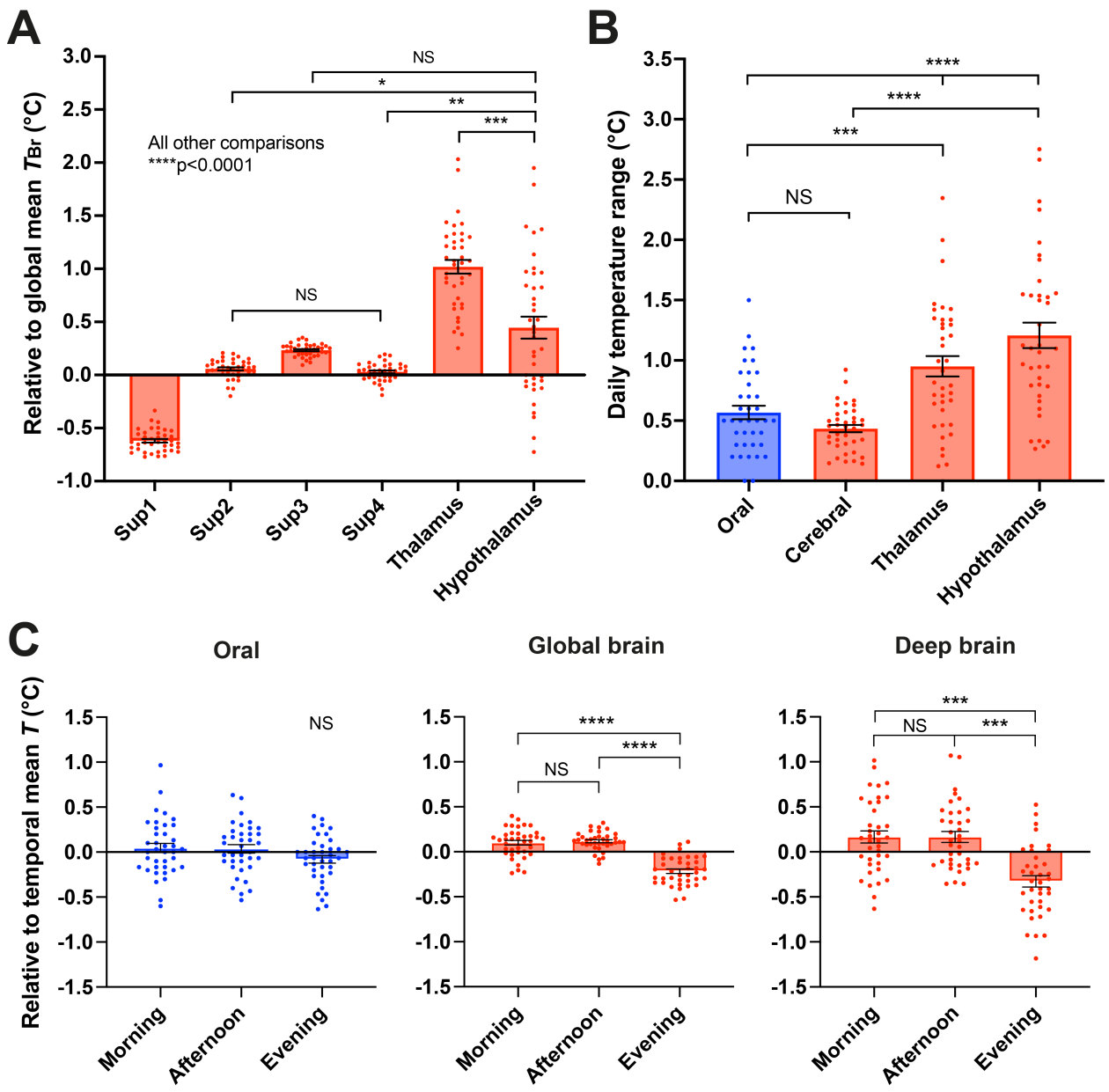


Supplementary Figure 8 MRS data processing. (**A**) Example spectra from central 36 voxels from cerebral region; voxel numbering as in Fig. 3. At this scale, only the large water peak to the left of each spectrum is clearly visible; smaller peaks to the right of this are barely visible. Selected voxel (29) shown in red box. (**B**) Zoomed-in representation of voxel 29, focusing on smaller peaks; the water peak is off-chart (and off-scale) to the left. The largest peak visible in the spectrum is now NAA. (**C**) Enlargement of voxel from (**B**) to show peak annotation; Cr, creatine; PCr, phosphocreatine; mI, myo-inositol; Cho, choline; Glu, glutamate; Gln, glutamine; NAA, N-acetylaspartate. (**D**) Representative examples of spectral fitting of water and NAA peaks for voxel 29. All but one of the 24 rejected T_{Br} data points were obtained from the most rostro-lateral voxel of the right frontal lobe.


A
Female 9am luteal
Female 4pm luteal
Female 11pm luteal
Female 9am non-luteal
Female 4pm non-luteal
Female 11pm non-luteal
n = 14 luteal
n = 5 non-luteal
1 2 3 4
Superficial 1
Superficial 2
Superficial 3
Superficial 4
DEEP
Brain temperature (°C)
Brain voxel
Thalamus
Hypothalamus


B
Female 9am luteal natural
Female 4pm luteal natural
Female 11pm luteal natural
Female 9am luteal synthetic
Female 4pm luteal synthetic
Female 11pm luteal synthetic
Female 9am patch
Female 4pm patch
Female 11pm patch
n = 6 luteal natural
n = 7 luteal synthetic (pill)
n = 1 luteal synthetic (patch)
1 2 3 4
Superficial 1
Superficial 2
Superficial 3
Superficial 4
DEEP
Brain temperature (°C)
Brain voxel
Thalamus
Hypothalamus

Supplementary Figure 9 Female brain temperature by menstrual cycle phase. (A) Female T_{Br} data split into luteal and non-luteal groups. Menstrual cycle phase explained 15.2% (P=0.0006) and time of day explained 21.2% (P=0.0004) of the variation in T_{Br}, respectively (two-way ANOVA). The interaction between these two factors was not significant. (**B**) Luteal-phase female T_{Br} plotted according to contraception type. T_{Br} did not differ between luteal females with natural menstrual cycles and those taking the combined contraceptive pill (mixed-effects analysis with Tukey's multiple comparisons test). Synthetic steroid hormones influence T_{Bo} differently from endogenous forms; women taking oral contraceptives have persistently raised T_{Bo}, similar to naturally cycling women in the luteal phase.[53] We suggest this is also true for T_{Br}. Only one participant was using the combined patch; note that T_{Br} for this participant was generally lower (more consistent with non-luteal females and males). For each voxel in (**A**) and (**B**), data are plotted as mean ±SEM. Voxel numbering on x-axis refers to numbers shown in overlay (top right); voxels were grouped into 4 concentric U-shaped regions labelled 1–4 based on T_{Br} variation. Note relatively high T_{Br} in cerebral white matter ('Superficial 3') and thalamus, and low T_{Br} in grey matter surrounding the sagittal sulcus ('Superficial 1').



Supplementary Figure 10 Daily temperature ranges by location. (**A**) Time-averaged spatial variation in T_{Br} by brain region; temperature deviations relative to global temporal mean T_{Br} were calculated for each individual. Sup1-4, superficial cerebral layers 1-4. Comparisons between regions were significant unless specified as NS (n=39, repeated measures one-way ANOVA with Tukey's multiple comparisons test, *P=0.01, **P=0.004, ***P=0.0002, ****P<0.0001). (**B**) Daily temporal temperature ranges (maximum versus minimum across the three tested time points) are plotted by measurement site (n=39). Note that temperature varied more by time of day in the thalamus and hypothalamus than in the cerebrum or orally (repeated measures one-way ANOVA with Sidak's multiple comparisons test ***P=0.0004, ****P<0.0001). 'Cerebral' refers to mean T_{Br} from all cerebral voxels combined. (**C**) Temporal variations in temperature; temperature deviations relative to temporal mean were calculated for each participant for three sites (body, global brain, deep brain) at each time point. Note drop in T_{Br} in late evening (n=38, mixed-effects

analysis (REML) with Tukey's multiple comparisons test, ****P<0.0001, ***P=0.0004 for morning versus evening and 0.0001 for afternoon versus evening). NS, non-significant. Note that analyses in (**A**) to (**C**) were performed without controlling for sex, age, BMI, menstrual cycle stage, or chronotype. Irrespectively, the effects of brain region and time of day on T_{Br} remain significant.

Supplementary Box 1 Definitions for key terms and phrases with context-dependent meaning

Term	Definition
Circadian	'About a day'; here also used as an adjective to describe physiological phenomena that vary in a repeating, approximately 24-hour pattern as a result of the internal timekeeping mechanism of a human (the 'body clock'), but which cannot be formally confirmed to be 'circadian' in nature unless observed/measured under constant environmental conditions. None of the data collected from healthy volunteers or TBI patients in this study can be described as circadian, but a subject/patient's circadian rhythm ('body clock') will have impacted on the key variables observed/measured.[a]
Circadian rhythm	A self-sustained cyclic biological phenomenon that (i) persists under constant conditions with a period length of around 24 hours, (ii) can be entrained by or synchronises to environmental cues, and (iii) proceeds at approximately the same rate (same period length) across a physiological range of temperatures for a given organism.[b]
Daily	Something that happens once every day or varies in an approximately 24-hour pattern.
Daily rhythm	A cyclic phenomenon that repeats every day.[c]
Diurnal	Something that changes by time of day in the manner expected for a daytime active mammal (e.g. a human) under natural environmental conditions.
Diurnal rhythm	A cyclic phenomenon that repeats approximately every 24 hours in the manner expected for a daytime active mammal (e.g. a human) under natural environmental conditions.[d]
Diurnal variation	A parameter that varies with time of day in the manner expected for a daytime active mammal (e.g. a human) under natural environmental conditions.
Rhythmic variation	A parameter that varies in a repetitive and predictable manner.
Spatiotemporal variation	A parameter that varies with space and time; in the context of this work, a change that happens according to different brain locations, at different times of the day, and/or according to age of the subject/patient.
Time of day variation	A parameter that varies according to the (external) clock time at which it is observed/measured which may also be influenced by the phase of the (internal) circadian rhythm ('body clock') at which it is observed/measured.

[a]'Circadian' is used also in reference to cited works that have used this specific term.
[b]This means that the rhythm is temperature compensated.
[c]A daily rhythm may also prove to be a circadian rhythm if observed/measured under constant environmental conditions; such conditions do not apply to the data described in this manuscript.
[d]A diurnal rhythm may also prove to be a circadian rhythm if observed/measured under constant environmental conditions; such conditions do not apply to the data described in this manuscript.

Supplementary Table 1 Published studies using MRS brain thermometry in healthy adults

Study	Subject age	Females/total	TOD control	T_{Br}	Calibration method
Childs et al. (2007)[58]	23–52y	3/8	No	36.5	Anaesthetised piglets[59]
Corbett et al. (1997)[59]	NR	5/10	No	37.2/37.7[a]	Anaesthetised piglets[59]
Corbett & Laptook (1998)[98]	22–47y	NR/10	No	37.0/36.6[b]	Anaesthetised piglets[59]
Curran et al. (2017)[99]	Mean 33y	NR/6	No	NA	Phantom[d99]
Covaciu et al. (2010)[45]	18–57y	5/18	No (10:30–17:30)	38.1	Phantom[e45]
Covaciu et al. (2011)[100]	21–62y	2/10	No	NA	Anaesthetised adult pigs[101]
Fujiwara et al. (2016)[102]	21–50y	3/15	No	38.3[c]	Anaesthetised piglets[103]
Harris et al. (2008)[104]	31–48y	2/5	No	NA	Phantom[f40]
Inoue et al. (2013)[105]	NR	NR/5	No	37.1	NR
Kaupinnen et al. (2008)[106]	21–51y	5/14	No	37.4	Anaesthetised piglets[103]
Kickhefel et al. (2010)[107]	25–43y	5/9	No	NA	Ex vivo swine muscle
Marshall et al. (2006)[40]	23–38y	0/4	No	36.5	Phantom referenced[f40]
Maudsley et al. (2017)[46]	18–84y	90/150	No	37.5	Combination[103,48,108,109]
Onitsuka et al. (2018)[110]	Mean 26.9	0/8	No	~37.2	Anaesthetised piglets[103]
Posporelis et al. (2018)[44]	Mean 23.1y	7/20	Yes (12:00–14:00)	NA	Anaesthetised piglets[103]
Rango et al. (2012)[41]	49–78y	5/10	Yes (14:00–16:00)	36.8	Anaesthetised piglets[59]
Rango et al. (2014)[65]	Mean 43y	6/14	Yes (14:00–16:00)	37.6	Anaesthetised piglets[59]
Rango et al. (2015)[111]	23–53y	10/20	Yes (14:00–16:00)	37.38	Anaesthetised piglets/rats[59,111]
Sharma et al. (2020)[112]	23-46y	7/18	No	37.2	Combination[103,48,108,109]
Shiloh et al. (2008)[113]	Mean 30.6y	0/10	No	37.7	Anaesthetised piglets[103]
Sumida et al. (2016)[114]	25–78y	18/35	Yes (17:00–19:00)	36.04	Anaesthetised piglets[103]
Thrippleton et al. (2014)[10]	22–40y	0/51	Yes (afternoon)	37.4	Referenced phantom[f40]
Verius et al. (2019)[37]	22–37y	15/30	No (08:00 to 15:50)	37.78	Phantom[g37]
Weis et al. (2012)[115]	20–61y	2/10	No	NA	Anaesthetised adult pigs[115]
Zhang (2020)[116]	19-49y	5/10	No	36.9	Combination[103,48,108,109]

Subject age is reported as available in publication; either mean or range. Females/total states number of female subjects included relative to total number of subjects, where this information was available. NR, not reported; NA, not applicable; TOD, time of control. 'No' means there was no reported control for TOD when scanning subjects; time period during which scans were performed are given in brackets if reported. T_{Br} = mean/median absolute T_{Br} (if reported) in °C.

[a]frontal lobe/thalamus

[b]superficial cortex/thalamus

[c]defined as upper threshold in healthy control subjects

[d]Water and N-acteylaspartate (NAA) solution

[e]Aqueous solutions of glycero-phosphocoline (GPC), creatine (Cr), and NAA

[f]Homogenous solution of metabolites

[g]pH-buffered aqueous solution of NAA, Cr, methyl protons of Cr (Cr2), dimethyl silapentane sulfonic acid (DSS), and sodium formate (NaFor)

Supplementary Table 2 Inclusion and exclusion criteria for prospective study

Criteria for inclusion	Criteria for exclusion
• Healthy men • Healthy women with regular natural menstrual cycles for a minimum of 6 months, or women that have taken monophasic hormonal contraception (fixed dose oestradiol and synthetic progestin) for a minimum of 3 months prior to scanning and will continue to do so during the month of scanning. • Age 20–40 years • Body mass index 18.5–29.9 • Live within 5 mile radius of Edinburgh Imaging (Royal Infirmary of Edinburgh) Facility • Capacity to understand written and verbal information provided in English, and able to provide valid written informed consent to participate • Able to wear an actigraphy wristband for a week prior to scanning • Able to commit to 3 x 45min scanning protocol within selected 24h period • Able to commit to fixed times of food and caffeine consumption on the day of scanning • Able to avoid alcohol and excessive physical activity on the day of scanning	• Pregnancy • Early menopause, irregular menstrual cycles, premenstrual syndrome • Lack of luteinizing hormone surge prior to two scheduled scanning days (naturally cycling women only) • MRI contraindications (e.g. cardiac pacemaker, cochlear implant, claustrophobia, any prior accident in which metal penetrated one or both eyes) • Oral temperature outside of normal range (33.2–38.1°C) prior to any scan • Medical history that might limit activity or alter cerebral blood flow (hypothyroidism, stroke, severe arthritis, Parkinson's disease, dementia, history of brain trauma, brain tumour or epilepsy, significant mental illness besides clinical depression, spinal cord injuries, recent serious burn, diabetes, dehydration) • Taking medications (except seasonal allergy medication, over-the-counter NSAIDs, or contraceptives), paracetamol, drug abuse • Known neurodevelopmental, neuropsychiatric or neurodegenerative disorder • Known sleep or chronotype disorder (delayed sleep phase syndrome, familial advanced sleep phase syndrome) • Known family history of cardiovascular disease at < 40 years of age • Failure to attend, or late attendance at, two independently scheduled morning scans • Participant-reported ill-health on the day before or the morning of scanning

Supplementary Table 3 MRS linewidths by brain region, time of day, and sex

	NAA			**H_2O**		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening
Females						
Cerebrum	7.44 (0.19)	7.31 (0.16)	7.31 (0.18)	9.70 (0.22)	9.45 (0.19)	9.40 (0.20)
Thalamus	8.64 (0.64)	8.46 (0.78)	8.46 (0.75)	11.5 (0.98)	11.3 (0.84)	11.3 (0.73)
Hypothalamus	10.8 (1.63)	10.0 (0.67)	9.86 (1.19)	14.1 (1.42)	14.0 (1.38)	13.9 (1.39)
Males						
Cerebrum	7.42 (0.29)	7.36 (0.27)	7.35 (0.28)	9.68 (0.34)	9.57 (0.35)	9.52 (0.31)
Thalamus	8.65 (0.58)	8.61 (0.62)	8.44 (0.71)	11.5 (0.92)	11.7 (0.85)	11.3 (0.69)
Hypothalamus	10.4 (1.44)	10.3 (1.57)	9.83 (0.88)	13.6 (1.48)	13.7 (1.27)	13.5 (1.48)

Spectral linewidth data presented for NAA and H_2O as mean (SD) for n=19 females and n=20 males. All data for the female participant with an HRF was excluded.

Supplementary Extended Data

A supplementary Excel spreadsheet is provided containing brain and body temperature rhythmicity analysis results for each TBI patient.

Captions for Supplementary Videos

Supplementary Video 1 Introduction to HEATWAVE. Dynamic 3D brain temperature map showing aggregate data from luteal females at one time point and highlighting brain regions from which data was extracted in healthy volunteers. Inferno colour scale is used to assign a temperature to each tissue voxel, to 0.1°C resolution. The colour of each voxel represents the mean raw temperature of that voxel calculated from the data obtained from all luteal females (n=14). For enhanced accessibility, the mapping reverses the radiological convention used in Fig. 3A such that from all perspectives, the data presented on the right side of the brain in HEATWAVE are from the right side of the human brain. Note that the thalamus is illuminated as a relatively hot, core brain structure.

Supplementary Video 2 HEATWAVE by sex. Comparison of diurnal brain temperature variation in luteal females and males (aggregate data from each group, without controlling for age, BMI, or chronotype), enabling both time-of-day and sex differences to be visualized in accelerated time, side-by-side. Two days' worth of data are presented in an accelerated looped fashion at 1 hour resolution. At the data collection points (9am, 4pm, 11pm), the colour of each voxel represents the mean raw temperature of that voxel calculated from the data obtained from either all luteal females (n=14), or all males (n=20). Between data collection points, data were extrapolated via non-linear regression of the aggregate data in GraphPad Prism version 8.2, fitting to a sine function (see Fig. 4D).

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Supplementary Appendices

Supplementary Appendix 1 - Participant Information Sheet and Consent to Participate Form
Supplementary Appendix 2 - Prospective Study Protocol
Supplementary Appendix 3 - MRI Protocol
Supplementary Appendix 4 - Study Participant Data Form
Supplementary Appendix 5 - Analytic code for statistical models

Participant Information Sheet

Diurnal brain temperature mapping by Magnetic Resonance Spectroscopy (MRS) in healthy volunteers

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Contact us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Background and rationale

Circadian (daily) rhythms co-ordinate many biological functions. These rhythms are driven by molecular 'clocks' that exist in every cell of your body. Good timekeeping in brain cell clocks is essential for brain health and for maintaining body temperature. Research suggests that brain cell clocks fail to keep time in neurodegenerative diseases such as dementia. Risk of these diseases increases with age, and elderly people struggle to regulate their own body temperature.

In healthy people, body temperature varies with a daily rhythm, but we do not know whether brain temperature also varies in this way. Previous studies suggest that brain temperature is higher than body temperature, but we do not know if this difference persists throughout the day. Body temperature is higher during some parts of the menstrual cycle in women, relative to men, but we do not know if this is the case for brain temperature. There is some evidence that hormones in contraceptive pills are associated with an increased body temperature throughout the menstrual cycle, but whether this affects brain temperature is unknown.

Brain temperature can be measured using a tiny thermometer inserted into the surface of the brain. This invasive method can only be justified in critically ill patients, and it measures only a tiny part of the brain. MRS uses a magnetic resonance imaging (MRI) scanner and is currently the only way to measure human brain temperature non-invasively. A recent study at Edinburgh Imaging showed that MRS can measure temperature in many brain regions in healthy male volunteers.

What is the purpose of this study?

Our main objective is to determine how human brain temperature varies according to time of day. To achieve this we will measure brain temperature in healthy volunteers at three different time points in the 24-hour cycle using MRS. **This study will provide critical information about daily variation in human brain temperature in healthy adults.**

Circadian Brain Temperature Study

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Our study may also determine:

- How much brain temperature varies in the course of a day relative to oral temperature
- Whether there is a difference in temperature between different parts of the brain
- Whether there is a difference in brain temperature between healthy men and women

How many healthy volunteers will be involved?

36 volunteers (18 men and 18 women) are needed to answer our main research question. Four 'extra' volunteers will be recruited for scanning. This will account for potential losses due to participant-elected withdrawal from the study, unexpected technical failure of the scanner, and exclusion of participants after scanning (see below).

What areas are being studied?

We will use a non-invasive method called Magnetic Resonance Spectroscopy (MRS) to scan your brain. MRS can be used to estimate temperature in different parts of your brain. Because MRS is based on MRI, we will also obtain limited information about the structure of your brain.

Where will the study be conducted?

Scanning will take place at the Edinburgh Imaging (Royal Infirmary of Edinburgh) Facility, Little France. Scans will be conducted using an MRI scanner in a temperature-controlled room. Most image processing and analysis will occur on-site. Some secondary analysis of anonymised brain temperature data will take place at the MRC Laboratory of Molecular Biology in Cambridge. Anonymised data cannot be linked back to you as an individual.

When will the study start and end?

We started recruiting volunteers in Spring 2019. Scanning will take place between June and September 2019. We expect to submit the results of the study for publication in Spring 2020.

What will it mean to take part?

Taking part in this study requires that you meet the criteria for inclusion, that you understand the information provided, and that you have the capacity to provide valid, written, informed consent to participate.

Overall, your involvement will likely span 14-17 days. This includes today's consenting interview, a 7-10 day period during which you will wear a special wristband, one day of scanning, and up to 7 days after scanning. During the week after scanning, you must report any unexpected abnormal events to the Chief Investigator by email (even if these are completely unrelated to the scans). The total duration of your involvement could be longer (up to a maximum of 2 months) if we need to reschedule your scanning day.

Why have I been invited to take part?

You are a healthy volunteer for a study investigating the normal daily variation in human brain temperature.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and you will be asked to sign a 'Consent to Participate' Form. If you decide to take part you are still free to withdraw at any time and without giving a reason. Deciding not to take part or withdrawing from the study will not affect the healthcare that you receive, or your legal rights. Before participating you should consider if this will affect any insurance you have and seek advice if necessary

What will happen if I take part?

Today the Chief Investigator will go through this Information Sheet and the Consent to Participate Form with you to check you have understood the information provided, and that you remain willing to take part. You will be invited to initial each statement on the Form and then sign it to give your consent to take part. You will then be given a special wristband and asked to wear this until your scanning date. The wristband will monitor your rest, sleep, and activity patterns, as well as your skin temperature and light exposure. If you decide to take part in the scanning, the data collected by the wristband will be downloaded via USB connector to a secure University of Edinburgh computer for processing. If you decide not to take part in the scanning, your wristband data will be safely destroyed without further processing.

In 7-10 days' time, you will be scheduled to attend the Edinburgh Imaging (Royal Infirmary of Edinburgh) Facility in the morning. The Chief Investigator will remove your wristband and then measure your height, weight, and oral temperature (under your tongue). A Radiographer (scanning specialist) will then **check that it is safe for you to be scanned**. A changing cubicle will be provided. You will be asked to place any metal objects (keys, phones, credit cards) in a locker. **Please do not wear any make-up or talcum powder and be prepared to remove contact lenses if you use them.** You will be asked to change into clean hospital clothing and/or a hospital gown (provided) before entering the scanning room.

You will be positioned in the scanner for your first scan in the morning, and asked to return 15 minutes in advance of your second and third scans in the afternoon and late evening, respectively on the same day. **It is important that you are not late for your scans, which will each last around 45 minutes.** The scanner makes quite loud noises whilst it operates. You will be provided with headphones during scanning. If at any stage during a scan you become worried, or wish to ask a question, you will be able to speak to the Radiographer via an intercom.

During each scan, your whole brain will be imaged using standard MRI to collect information about the structure of your brain. An MRS scan will then be run across a section of your brain at its surface, and again in a deeper region, to collect information

on brain temperature.

It is not essential to remain at the hospital between scans, but it may be convenient for you to do so.

No adverse effects are expected as a result of scanning, however you must report to us any abnormalities experienced during the week before, and the week after, scanning. **We cannot provide any information to you about the results of your scans on the day of scanning.** Data from the wristband will be used to determine your normal rest/activity pattern so that we can effectively interpret your brain temperature data. **To safeguard your anonymity when publishing our findings, we cannot provide you with your individual wristband data** (although commercially available products can provide you with very similar data). No adverse effects are expected as a result of wearing the wristband. Although extremely unlikely, as with any new wearable item, an allergic reaction is possible; **you should contact your GP and the Chief Investigator immediately if you notice any itching or irritation in the contact area of the wristband with the skin.**

Screening and exclusion

Your eligibility to take part will be confirmed on the day of scanning. Any of the following would mean that you cannot take part:

- The wristband was not worn for two or more days preceding the scanning day
- Your body mass index (BMI) sits above or below the range stated in the inclusion criteria
- Your oral temperature sits above or below the normal range before the first scan (we will invite you to reschedule your scanning day).
- You report feeling unwell on the day before, or the morning of, scanning
- You are a woman with natural menstrual cycles, and you cannot confirm ovulation in the preceding 7-10 days (we will invite you to reschedule your scanning day for the following month).

The following circumstances would result in exclusion of your data from our study:

- Your oral temperature sits above or below the normal range before your second or third scans
- Detection of a significant health-related finding (HRF) in your brain scans
- Consumption of food or caffeine during prohibited time periods on the day of scanning
- Consumption of alcohol on the day of scanning
- Participation in excessive physical activity on the day of scanning
- Failure to attend, or late attendance at two independently scheduled morning scans

If you or your data are excluded, you will still be reimbursed for any expenses incurred as a result of taking part in our study.

Health-related findings (HRFs)

When apparently healthy volunteers take part in an imaging study, there is always a

small chance that an abnormality (unrelated to the purpose of the study) may be found. For standard MRI scans of the brain, **this is occurs in roughly 1 in 20 people**. Some abnormalities have no known health implications; such findings are considered harmless in medical terms, but knowing about them may harm an individual by causing unnecessary anxiety. **Potentially serious HRFs are those that indicate the possibility of a health condition which, if confirmed, would likely impact upon lifespan, major body functions, or life quality.** For standard MRI scans of the brain, potentially serious HRFs are **noted in around 1 in 70 people**. However, based on current literature, serious diagnoses are confirmed only in 1 in 350 people.

Your scans will be reviewed by an expert in brain imaging. **If any significant HRFs are noted, these will be reported back to you and your GP in strictest confidence. A significant HRF is defined as one that could have health implications for you, and where the potential benefits of knowing about this HRF outweigh any potential harm caused to you.** Please note that diagnostic MRI studies include the injection of a chemical into the bloodstream to highlight some types of brain abnormality. **No injections will be administered during this study, and so some types of brain abnormality will not be detected.** Only data obtained from structurally normal brains can be included in our brain temperature analysis. The Chief Investigator will be notified if your data must be excluded on the basis of an HRF, but they will not be given any specific information about the reasons for exclusion.

Will my GP be involved?

By providing the details of your registered GP, you agreed to us contacting them to notify them of your interest in taking part in our study. If you report any adverse events to us during the study, we will report these back to your GP (in confidence). **If any significant HRFs are noted on your scans, these will be discussed with you and your GP. If recommended, your GP will arrange any further tests or specialist referrals for you.**

Expenses and payments

You will not be substantially out of pocket as a result of taking part in our study. We will reimburse travel expenses, and meal vouchers will be provided for Royal Infirmary of Edinburgh catering facilities on the day of scanning. Reimbursement for expenses incurred for childcare or loss of earnings will be considered on a case-by-case basis upon discussion with the Chief Investigator.

Is there anything I need to do or avoid?

Please wear the wristband provided until your scanning date and proceed with your normal daily routine. **The band is splash proof so it can be worn during hand washing, however please remove it when taking a bath or shower, or if swimming, and reapply it immediately afterwards. On the day of scanning, you must adhere to strict times for consumption of food** (6am-8am, 12 noon to 2pm and 6pm-8pm), **and caffeine** (6am-8am, and 12 noon to 2pm). You must **not consume alcohol or undergo any excessive physical activity on the day of scanning** (e.g. gym workout, running, cycling).

Additional requirements for women with natural menstrual cycles:

If you are a woman and not taking oral contraceptives, **your scans must be scheduled during the post-ovulation phase of your menstrual cycle**. We will confirm this with urine testing kits (ClearBlue). A kit with several test strips is provided today (on or just before your expected ovulation date). You can test your urine on up to three consecutive days (if needed) to confirm ovulation. **You must record the date and time of a positive result and notify the Chief Investigator.** Alternatively you can take a digital photograph of the result (i.e. on a mobile phone) and bring this with you on the morning of scanning (the photograph will be viewed by the Chief Investigator but not transferred or stored). If you are unable to confirm ovulation, further test strips will be provided, and you will be invited to reschedule your scanning day for the following month.

What are the possible benefits of taking part?

There are no direct financial or health benefits associated with taking part in this study. We do not know what the outcome will be, which is why we are conducting the research. Some volunteers may experience indirect benefits as follows:

- Better awareness of BMI and menstrual pattern (where applicable)
- Potential identification of an HRF that might be treated at an earlier stage **(please note that not all HRFs have health implications, and those that do, may not have treatment options)**
- Better engagement with research, researchers, and health professionals
- Contributing data from an under-researched group (women)
- Helping to address a fundamental knowledge gap that may help patients in the future

What are the possible disadvantages of taking part?

MRS scans are completely safe as long as routine MRI safety procedures are followed. MRI uses a magnet and radio waves to generate brain images. MRI and MRS scans do not involve any radiation, so there is no additional risk to having multiple scans. In the event of an unexpected medical emergency, the Imaging Facility has easy access to emergency medical care on-site. It is not possible to eliminate all risks of taking part in this study. You should be aware that stress or anxiety may result from the following:

- Reviewing the Participant Information Sheet or completing the Consent to Participate Form
- Attending in person for the consenting procedure and scanning day
- Wearing the wristband for 7-10 days
- BMI and oral temperature checks
- Urine testing (for some women)
- Attending 3 scans on time including a late evening scan
- Being positioned within the MRI scanner
- Restricted food and caffeine consumption on the day of scanning
- Prohibition of alcohol consumption on the day of scanning
- Restricted activity on the day of scanning

- Requirement to report any adverse events
- Identification of a HRF (especially for lesions that have no effective treatment options)
- Exclusion from the study after consenting to participate

Please be aware that any HRFs identified in your brain scans might affect insurance policies you may have, or plan to take out.

What if there are any problems?

If you have a concern about any aspect of this study, please contact Dr Nina Rzechorzek (Chief Investigator) who will do her best to answer your questions. You may also speak to a Consultant Radiologist (imaging specialist) who is not involved in the study. Contact details are provided at the end of this Information Sheet.

If you have a medical problem that occurs during the study and you cannot consent to take part any longer, or if you become unable to make decisions for yourself, we will exclude you from the study but we will use the information we have collected up to that point.

In the unlikely event that something goes wrong, and you are harmed during the study due to someone's negligence, then you may have grounds for legal action for compensation against the NHS or the University of Edinburgh. You may however have to pay your own legal costs. The normal NHS complaints mechanisms will still be available to you (if appropriate). The University of Edinburgh is liable for its employees' actions (undertaken as part of their job) and is insured against the risk of claims relating to research studies that their staff design and undertake. This insurance covers both negligence and no-fault compensation.

What will happen if I don't want to carry on with the study?

You can withdraw from our study at any stage, without giving a reason. Your decision to withdraw will be notified to your GP, but will not have any impact on your future care. There will be no penalty or loss of benefits (i.e. you will still be reimbursed for any expenses incurred as a result of being part of the study).

If you wear the wristband but then decide not to proceed with the scans, the data already collected from you will be safely destroyed. If you withdraw after your scans have been completed, but before analysis is complete, your data will no longer be included in the study. **However, any significant HRFs identified on your brain scans must still be reported to you and your GP (in confidence)**. You must also understand that once anonymised, it will not be possible to exclude your brain temperature data from the analysis and publication of the results, or from future research studies that use this anonymised data. The reason for this is that once the data is anonymised, we cannot identify which data belongs to you.

What happens when the study is finished?

The use of your data in further research

The data from your wristband and the brain temperature data generated from your scans will be anonymised prior to further analysis. Anonymised data will be shared between members of the Study Team (including Dr O'Neill, an expert in circadian biology at the MRC Laboratory of Molecular Biology Cambridge). Anonymised data will also be made available for use by other researchers internationally via deposit in a secure online database and will be retained indefinitely. The data collected during this study will be used as a standard against which to compare similar data from individuals of other age groups, and also patients with neurological disorders.

Some of the data collected for this study will be linked to you as an individual and will be retained securely for a minimum of 20 years. Such data will be used only for communicating with you and your GP as outlined above. With your consent, your email address will be used to notify you when the study results are published, and to inform you of future imaging studies that might interest you. **You can decline to be contacted for these purposes using the Consent to Participate Form.**

Will my taking part be kept confidential?

All the information we collect during the research will be kept confidential and there are strict laws that safeguard your privacy at every stage. Wristband data, brain images, BMI results, oral temperatures, and urine test results (where applicable) will only be identifiable by your participant number for the study and will not be labelled with any person-identifiable information.

With your consent, we have notified your GP that you are interested in taking part in this study. We will ask your permission to use your Community Health Index (CHI) number to communicate with your GP. The CHI is a population register, which is used in Scotland for healthcare purposes. The CHI number uniquely identifies a person on the index. Should the need arise, we will notify your GP of any significant HRFs. To ensure that the study is being run correctly, responsible representatives from the University of Edinburgh and/or NHS Lothian may need to access your medical records and data collected during the study, where it is relevant to you taking part. These institutions are Co-Sponsors for this study and are together responsible for its management and for providing insurance and indemnity.

What will happen to the results of the study?

You will not be provided with any specific rest/activity or brain temperature results relating to you as an individual. We will analyse the results from all participants as a group, and publish our findings in publically-accessible scientific journals and at scientific conferences. We will also make the results available on University of Edinburgh (Edinburgh Imaging) and MRC Laboratory of Molecular Biology websites. All data are anonymised so it will not be possible to identify you when we make our results public.

The results of this study will be used to design studies in the lab. These studies will use human stem cell-derived brain cells to understand how brain temperature

interacts with the molecular clock inside brain cells. Your data may also be used to design future studies looking at how brain temperature changes with healthy ageing and shift work, and in neurological diseases such as dementia, brain cancer, mental illness, and sleep disorders.

Who is organising and funding the research?

This study has been organised by an Edinburgh Imaging Study Team, led by Dr Nina Rzechorzek (Chief Investigator) and supervised by Professor Ian Marshall at the University of Edinburgh. The study is being co-sponsored by the University of Edinburgh and NHS Lothian. The work is being funded by the Medical Research Council (MRC).

Who has reviewed the study?

The study proposal underwent independent external review as part of a MRC Clinician Scientist Fellowship application, led by Dr Rzechorzek. All research conducted using NHS facilities is reviewed by an independent group of people, called a Research Ethics Committee (REC), to protect your interests. This study has been reviewed and given a favourable opinion by the Academic and Clinical Central Office for Research & Development (ACCORD) medical research ethics committee (AMREC). NHS management approval has also been given.

Researcher Contact Details

If you would like further information about the study, or if you need to change your scheduled scan date, please contact the **Chief Investigator:** Dr Nina Rzechorzek, MRC Clinician Scientist Fellow, email: nina.rzechorzek@ed.ac.uk, or ninar@mrc-lmb.cam.ac.uk, telephone: 07900 815450 (mobile).

Independent Contact Details

Alongside the Edinburgh Imaging Radiologist team, Dr Dilip Patel or Dr Graham McKillop, Consultant Radiologists at the Royal Infirmary of Edinburgh (Tel 0131 536 1000) are happy to answer any further questions you may have. These persons are not directly involved in this study, and so will be able to give you independent advice.

Complaints

If you wish to make a complaint about the study please contact:

Patient Experience Team
2 – 4 Waterloo Place, Edinburgh, EH1 3EG
feedback@nhslothian.scot.nhs.uk
0131 536 3370

Participant ID:		Centre ID (if applicable)	

IRAS ID: 244533 Centre Number: Study Number: AC 18038

Participant Identification Number:

CONSENT TO PARTICIPATE FORM

Study title: Can we measure a diurnal shift in brain temperature in healthy human volunteers using Magnetic Resonance Spectroscopy (MRS)?

Chief Investigator: DR NINA RZECHORZEK

Please initial each box

1. I have read the Participant Information Sheet and Consent to Participate Form (version 1.9, 14th May 2019), and Data Protection Information Sheet (version 1.1, 6th April 2019) in relation to this study. I have been given ample opportunity to consider this information, ask questions about both documents, and have had these answered to my satisfaction. I have all the information I need to **provide informed consent to take part in this study.** ☐

2. I understand that my participation is voluntary; **I am free to withdraw at any time** without giving any reason, and without my medical care or legal rights being affected. As a volunteer, I am aware that I am not being scanned at the request of a doctor for any specific medical condition. ☐

3. I am aware that my scans will be viewed by a doctor qualified in medical imaging. I understand that images of my brain are being collected for research purposes only. These images are **not for diagnostic use** and would not be sufficient to rule out certain brain disorders. ☐

4. I understand that relevant sections of data collected during the study may be looked at by individuals from the Sponsors (NHS Lothian and the University of Edinburgh) where it is relevant to my taking part in this research. I give permission for those individuals to have access to my records and my CHI number. ☐

5. I understand that images obtained during my scan will be stored and processed using computers. After the study has ended, I am aware that these images may be copied onto a **permanent record** which might be studied again at a later date. ☐

6. I am aware that **incidental unexpected findings can occur when scanning healthy volunteers**. I understand that if any significant health-related findings are revealed through imaging my brain, these will be reported back to my GP who will then contact me to discuss their implications, and whether or not further tests are required. ☐

7. I am aware that unexpected findings **may affect insurance policies** ☐

Participant ID:		Centre ID (if applicable)	

8. I understand that the information collected about me might be used to support other research in future, and might be shared anonymously with other medical and scientific researchers. This will be subject to strict laws and University of Edinburgh policies intended to safeguard my privacy. ☐

9. **I know of no reason why I should not undergo Magnetic Resonance Imaging** or take part in this study. If I lose capacity during the study, I understand that the Study Team will **retain and use** the data collected from me prior to this. ☐

	YES	NO
I give consent for my email address to be used to inform me of the study results, and details of new studies that I might be interested in taking part in.	☐	☐

10. **By signing below, I give consent to take part in this study.**

Signature of volunteer

Name of volunteer (please print in block capitals)

Person taking consent (signature)

Name of person taking consent (please print in block capitals)

DR NINA RZECHORZEK

Date

CHI Number

Name and address of Volunteer's GP

Circadian Brain Temperature Study

Academic and Clinical Central Office for Research and Development

Non-CTIMP Study Protocol

Can we measure a diurnal shift in brain temperature in healthy human volunteers using Magnetic Resonance Spectroscopy (MRS)?

	The University of Edinburgh and Lothian Health Board ACCORD The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ
Protocol authors	Dr Nina Rzechorzek Professor Ian Marshall Dr Francesca Chappell Dr Michael Thrippleton Dr Duncan Martin Dr John O'Neill
Funder	Medical Research Council
Funding Reference Number	MR/S022023/1
Chief Investigator	Dr Nina Rzechorzek (MRC Clinician Scientist Fellow)
Sponsor number	AC 18038
REC Number	18-HV-045
Project registration	UKCRN (NIHR CPMS) Portfolio (registration pending)
Version Number and Date	Version 1.7 May 14th 2019

Amendment classification and number:	**Summary of change(s)**
Version 1.4	Prospective Funder updated to MRC Abbreviations list updated to include MRC, GDPR and UKRI 9.0 Addition of local SOP title and number 11.2.5 Data Protection section extended in line with MRC Data Management Plan 13.1 Authorship Policy extended in line with MRC Data Management Plan
Version 1.5	Study logo and funder logo added Title adjusted from 'MRSI' to 'MRS' Funder confirmed as MRC 1.1 Actigraphy wristband added as potential burden and requirement of entry into the study to establish participant-specific chronotype. Pilot scan added to optimize MRS acquisition in deep brain region. 2. Secondary objective/endpoint relating to cooling effect of positioning/scan length removed 3.0 Study design adjusted to 'prospective, single-site, cohort', data collection period extended to 4 months (June to September 2019). T_{Br} data to be normalized to individual chronotype. Study procedure adjusted to include actigraphy and scanning times/procedure simplified. BRIC SOP cited for HRF feedback pathway. CI employment details updated. Study schedule modified to remove public open seminar. 4.0 Inclusion criteria to include wearing of actigrpahy wristband 5.0 Identifying participants – actigraphy data to be destroyed if no consent is obtained to proceed with scans 7.0 Data collection to include actigraphy. Scanning protocol simplified/adjusted.

Circadian Brain Temperature Study

Academic and Clinical Central Office for Research and Development

	8.0 Data analysis to include chronotype References – additional reference added relating to chronotype differences between sexes
Version 1.7	Further clarification on collection, transfer and processing of actigraphy wristband data including potential risks. Obtaining written informed consent to participate rescheduled (signatures will now be obtained prior to issue of actigraphy wristbands or urine test kits)

CONTENTS

LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development - Joint office for The University of Edinburgh and Lothian Health Board
AE	Adverse Event
AR	Adverse Reaction
BMI	Body mass index
CI	Chief Investigator
CiBraT	Circadian brain temperature
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HRF	Health-related finding
ICH	International Conference on Harmonisation
MRC	Medical Research Council
MRS	Magnetic Resonance Spectroscopy
QA	Quality Assurance
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SPDF	Study Participant Data Form
SUSAR	Suspected Unexpected Serious Adverse Reaction
T_{Bo}	Body temperature
T_{Br}	Brain temperature
UKRI	UK Research and Innovation

1 INTRODUCTION

1.1 BACKGROUND

Circadian rhythms are fundamental to brain health
Circadian clocks drive near-24-hour rhythmic changes in biological processes, adapting organisms to Earth's periodic rotation. These imprecise clocks are reset by environmental cues, which in mammals feed into a hierarchical multi-oscillator system; a 'master' clock deep in the brain synchronizes cell-autonomous clocks in the rest of the brain and periphery, coupling systemic adaptations to light-dark cycles. Age-related deterioration of the clock is compounded by modern living, which dissociates endogenous clocks from natural cues. Neurodegeneration — as occurs in dementia — is increasingly linked to clock disruption and presents a burgeoning socioeconomic threat to our healthcare system. To maintain brain health throughout life, we must understand how to maintain neural cellular timekeeping.

Thermal resilience of the clock is mechanistically elusive
The phase of mammalian circadian oscillations is highly sensitive to temperature shift, whilst circadian period (clock speed) remains constant across physiological temperatures. This 'temperature compensation' of periodicity comprises a hallmark feature of *bona fide* circadian rhythms, and is poorly understood at the molecular level. Failure of this mechanism may dictate the loss of neural cellular timekeeping in neurodegenerative disease. Thermoregulatory capacity declines with age; suboptimal thermal control could contribute to — and arise from — neural clock dysfunction. Brain temperature (T_{Br}) generally exceeds core body temperature (T_{Bo}), however, despite the importance of T_{Br} to neural health, a basic understanding of human T_{Br} fluctuation is missing. T_{Br} is difficult to measure and varies by brain region, with neural activity, state of consciousness, and pathology. Although circadian T_{Bo} cycles are well recognized in mammals, and have reduced amplitudes in aged humans, no studies have examined how human T_{Br} varies across circadian time. To extract the relevance of thermal-clock interactions to brain health, the molecular clockwork must be studied at physiological human brain temperatures.

CICs interact with the clock but causal molecular mechanisms are unknown
CICs are 'accessory oscillators'; they are themselves circadian, serving both as inputs to, and outputs from, the core clock. Links between CICs and the circadian clock are expanding, but the influence of CICs on timekeeping in response to temperature change remains unexplored. If temperature compensation — and thus clock integrity — depends on CIC-clock interactions, these might be exploited to re-establish clock fidelity in vulnerable neural cells. In order to explore this possibility in the lab, we first need to know how temperature varies around the clock in the human brain. The proposed imaging study forms part of a Fellowship proposal to address the following question:

How does T_{Br} vary across the circadian cycle?

Although we know that T_{Bo} in healthy individuals varies in a circadian manner, it is currently not known whether T_{Br} also varies in this way. In the clinic, human T_{Br} can be

measured directly using probes inserted into the surface of the brain. However, this invasive recording method can only be justified in critically ill patients with brain injury, and it only captures data from one focal brain region. MRS is currently the only method available to measure T_{Br} in a non-invasive way. A previous study conducted by Edinburgh Imaging researchers demonstrated that this technique can generate T_{Br} data from multiple brain regions in healthy human volunteers. The proposed study will extend our understanding of how human T_{Br} varies around the clock by recording MRS temperature data in each volunteer at three different time points in a single 24-hour cycle. This study will therefore provide critical baseline information about diurnal variation in human T_{Br} in healthy adults. The data produced will be used to inform mechanistic studies in the lab using human stem cell-derived brain cells to understand how T_{Br} interacts with the molecular clockwork. The data will also be used to design future studies looking at how T_{Br} changes in healthy elderly individuals as well as those with neurological diseases, such as Alzheimer's disease.

Risk, burdens and benefits

Potential risks to participants

- Inadequate informed consent - considered to cause possible harm to an individual, with potentially moderate long-term consequences of harm to that individual.
- Hazards of the interventions - the long-term effects of MRS scanning on the brain are considered negligible. The routine safety risks of MRI are considered negligible as long as the appropriate health and safety procedures are followed. Any participant could potentially experience a local allergic reaction to any new wearable device (e.g. actigraphy wristband), with possible minor harm. This hazard is considered extremely unlikely and would be easily managed without any long-term effects.
- Hazards of assessment methods – identification of a health-related finding (HRF) is considered to cause possible harm to an individual with potentially moderate consequences of harm to that individual.
- Failure to act appropriately upon health information discovered as a consequence of participating in the study – considered to cause possible harm to an individual, with potentially moderate consequences of harm to that individual (depending on the nature of the HRF).
- Failure to protect the privacy of participants – considered to cause possible harm to an individual, with potentially moderate consequences of harm to that individual.

Potential burdens to participants

- Requirement to attend in person for the consenting procedure and scanning day
- Requirement to wear a single wristband-based monitor (actigraphy wristband) that measures rest/activity patterns, skin temperature, and light exposure for one week prior to scanning
- Requirement to attend 3 MRI scans on time including a late evening scan
- Requirement to fix times of food and caffeine consumption, and avoid alcohol and excessive physical activity, on the day of scanning

Potential benefits to participants

- Better awareness of own health status including body mass index (BMI), sleep hygiene, and menstrual pattern (where applicable)
- Potential identification of an HRF that might be successfully treated at an earlier stage
- Engagement with research, researchers and health professionals

- Contributing data from an under-researched group (women)
- Learning about circadian biology, brain imaging, MRI safety, and non-invasive T_{Br} measurement
- Helping to address a fundamental knowledge gap (contribution to knowledge and understanding of diurnal brain temperature variation in healthy individuals)
- Understanding that the results of the study will provide critical information to inform mechanistic studies *in vitro*
- Understanding that the results of this study may inform the development of novel treatment approaches for neurodegenerative diseases
- Understanding that the results of this study may inform new guidelines for T_{Br} measurement and interpretation in humans

Overall the potential benefits of this study to the wider public outweigh the potential risks and burdens to individual participants.

1.2 RATIONALE FOR STUDY

Circadian ('around 24 hour') rhythms orchestrate many biological functions and are driven by molecular clocks that exist in every cell - including cells of the brain. These biological clocks are essential for optimal brain health and the regulation of T_{Bo}. Emerging evidence suggests that brain cell clocks are disrupted in neurodegenerative diseases such as Alzheimer's disease. The risk of these diseases is highest in elderly individuals, who are notoriously poor at regulating their own temperature. In healthy brain cells, molecular clocks maintain circadian rhythms at constant speed, despite temperature fluctuation. Cold-inducible chaperone (CIC) proteins interact with cellular clocks and are highly sensitive to temperature change. We propose that CICs are critical to clock fidelity under T_{Br} fluctuation, and that failure of this mechanism advances neurodegeneration. First, we must determine how human T_{Br} varies with time; this represents a fundamental knowledge gap.

T_{Br} fluctuation is reported in rodents, but circadian effects may be confounded by the sleep-wake cycle. T_{Br} also depends on cerebral haemodynamics, and given species-specific variation in cerebral circulation, extrapolating animal data to humans is problematic. The current gold standard technique for human T_{Br} measurement requires an intracranial probe, retrieving data from a focal point in the brain parenchyma. This is reserved for patients undergoing neurosurgery, or those with traumatic brain injury. With MRS, high-resolution T_{Br} data can now be obtained non-invasively in healthy subjects. Pre-existing round-the-clock T_{Br} data from brain-injured patients has informed the design of an MRS study in human volunteers, to measure healthy T_{Br} at different times of the day. Derived maximum and minimum T_{Br} will be used to explore how CICs are involved in the resilience of clocks to physiological temperature shifts using human stem cell-derived brain cells in the lab, potentially leading to novel therapeutic targets for chronic brain disorders.

Hypotheses:
(i) A diurnal shift in mean human T_{Br} can be measured in healthy volunteers.
(ii) Mean human T_{Br} exceeds oral temperature, irrespective of time.
(iii) Mean brain and oral temperatures are higher in luteal-phase women than in men.
Method: alongside brain injury effects, several confounders limit extrapolation of

patient data to the general population. Using MRS, we will map diurnal changes in T_{Br} in healthy volunteers to determine normal human maximum and minimum T_{Br} at unparalleled spatial resolution. Participants will be recruited locally and screened for suitability via electronic questionnaire. If eligible and willing to participate, they will be provided with the Participant Information Sheet and Consent to Participate Form. One visit 7 days prior to scanning will be used as the consenting procedure, and to issue actigraphy wristbands, and also urine test kits (where needed).. 40 subjects (20 men, 20 women) will each be scanned at 3 specific times (morning, afternoon, and late evening) within 24h, using a 3 Tesla MRI scanner. The scanning protocol will include whole brain, T1- and T2-weighted structural acquisition followed by MRS in a superficial and deeper brain location . All scans will be conducted within four months (minimizing seasonal variation) in a temperature-controlled room. Meal times and caffeine consumption will be fixed on the day of scanning and participants will be requested to avoid alcohol and excessive physical activity. Oral temperature will be measured prior to each scan, and hormonal influences controlled through urine testing (naturally cycling women only). Protocols for data extraction and temperature estimation by MRS are already validated and published. Anonymised MRS data will be processed blind (to time of day), and then analysed using a linear mixed model that accounts for time of day, subject-specific chronotype (based on activity data), and repeated measures for each participant.

2 STUDY OBJECTIVES

2.1 OBJECTIVES

2.1.1 Primary Objective

To determine whether human T_{Br} varies according to time of day using a non-invasive brain imaging technique (MRS).

2.1.2 Secondary Objectives

- Quantify the difference in variability of brain and oral temperatures across a 24-hour period in healthy human volunteers
- Quantify the difference between healthy male and luteal-phase female T_{Br} at each time of day
- Quantify the difference between temperatures at the brain surface and the brain core at each time of day in healthy human volunteers

2.2 ENDPOINTS

2.2.1 Primary Endpoint

A statistically significant change in mean human T_{Br} (across all measured voxels per subject) between time points.

2.2.2 Secondary Endpoints

- The difference in variation of brain and oral temperatures across a 24-hour period

- The difference in brain and oral temperatures between men and luteal-phase women at each time point
- The difference between deep and superficial T_{Br} at each time point

3 STUDY DESIGN

This is a prospective, single-site, cohort study in healthy human volunteers based at the Edinburgh Imaging (Royal Infirmary of Edinburgh) Facility. The study duration is 12 months from the start of recruitment to the completion of data analysis and submission for publication. Data collection will be scheduled over a 4-month period from June to September 2019. The anticipated duration of involvement for each participant is up to 17 days (from the date of obtaining informed consent to participate 7-10 days prior to scanning, to 7 days post scanning in case any adverse events need to be reported to the Study Team). The study length will be longer if scans need to be rescheduled for any participant. All participants will have the option to be notified by email when the results of the study are published (with Open Access).

The first key decision during the study design process was to determine the number of scans to be conducted for each participant. The logistical and financial burden of attending for scans was considered, and even though all participants will be local to the scanning facility, our approach is to minimize the impact of scanning on daily life, work, and family commitments. We decided that each participant should be requested to commit only to one scanning day, and that we would capture data at three time points during that single day. Although this removes the effects of day-today variation, it clearly cannot account for it, and this is an accepted risk of our method. The 3 time points were chosen based on available literature relating to T_{Br} fluctuation in rodents, known T_{Bo} cycles in healthy humans, and also pre-existing data collected from brain-injured patients as part of routine critical care. A 2-time point approach was considered but carried the risk of sampling twice at the midpoint between the temperature peak and trough. Overall our 3-time point approach, together with normalization of T_{Br} data to individual participant chronotype, will maximize the opportunity to fulfill our recruitment target and meet primary outcome objectives.

The second key decision was for the inclusion of female participants. Women were excluded from previous studies using MRS to measure T_{Br} in order to remove the potential confounding effect of the menstrual cycle on body (and possibly brain) temperature. We believe it is important, where possible, to include both sexes in studies of human brain physiology, such that the results of our study are as widely applicable as possible. Whilst it is well established that T_{Bo} can fluctuate during different stages of the menstrual cycle, and that there is a clear difference in chronotype between sexes at certain life stages, the primary outcome for this study is whether there is an intra-individual shift in T_{Br} between different times of the day. As such, each individual acts as their own control, and a linear mixed model approach can be used to address the effects of nesting at multiple levels when considering data within and between participants. It is also reasonably straightforward (and inexpensive) to control for chronotype differences (using activity monitors) and the effects of the menstrual cycle (by scanning all naturally cycling female participants at the same respective phase of their cycle i.e. post-ovulatory/luteal phase). Whilst this adds an additional non-invasive measurement (actigraphy) for all participants and an

additional procedure (urine testing) for some female participants, we do not believe this would have more than a negligible impact on subject recruitment. Indeed, it means that unexpected chronotype extremes can be accommodated, and both naturally cycling women and women taking monophasic contraceptive medication can be included in the study. It will impact on logistics, reducing the number of dates on which some female participants are eligible for scanning, but this is a manageable risk within the 4-month duration of data collection. Commercially available ovulation urine testing kits were deemed to be a minimally invasive, inexpensive, and effective means of confirming the luteal phase of the cycle in female participants, in a way that could be readily confirmed and recorded by the CI, without compromising the privacy or health of the participant. Commercially-available actigraphy wristbands are widely validated and increasingly used by researchers as well as the health-conscious general public. The data collected from the wristband will be identifiable only by a unique participant identification number and will be downloaded via USB port to a secure University of Edinburgh computer prior to further processing. The data will be safely destroyed without use by the Study Team if a participant decides not to proceed with scanning.

The final key decision was the choice of method for T_{Bo} measurement in order to exclude participants with T_{Bo} beyond the normal range. After discussion of the relative risks and merits of more invasive methods (e.g. rectal temperature) we decided that oral temperature measurement would be widely acceptable to potential participants, would be more reliable than aural temperature measurement, and would be least likely to impact negatively on recruitment.

Null hypothesis (1): There is no statistically significant change in human T_{Br} measured at different times of the day within a single 24-hour period
Alternative hypothesis (1): There is a statistically significant diurnal shift in human T_{Br} within a single 24-hour period

This alternative hypothesis was chosen on the basis of known circadian fluctuation in human T_{Bo} in healthy humans, recently observed circadian fluctuation in T_{Br} in brain-injured patients that mimics T_{Bo} cycling (unpublished), diurnal T_{Br} fluctuation in rodents, and the ability of 3T MRS to detect small changes in human T_{Br}.

Null hypothesis (2): The variability in temperature within a single 24-jour period will not differ significantly between brain and oral regions of healthy human volunteers.

Alternative hypothesis (2): Human T_{Br} variation will exceed oral temperature variation within a single 24-hour period in healthy human volunteers.

There is conflicting data comparing brain and core body temperatures in humans and other animals, but generally, T_{Br} is considered to exceed core T_{Bo}, and we have recently observed a greater daily variability in T_{Br} relative to T_{Bo} in patients with traumatic brain injury (see References).

Null hypothesis (3): There is no statistically significant difference between brain temperatures measured in healthy men and women
Alternative hypothesis (3): T_{Br} will be higher in luteal-phase women and in women taking monophasic contraceptive medication compared to men, after accounting for chronotype differences.

It is established that an increase in progesterone during the post-ovulatory (luteal) phase of the menstrual cycle increases T_{Bo}, and there is published evidence that women in the luteal phase of their cycle have higher body temperatures throughout the circadian cycle relative to men. There is also evidence to suggest that the synthetic steroids (progesterone and oestrogen) provided in the monophasic contraceptive pill are associated with a persistently increased T_{Bo} throughout the cycle compared to the T_{Bo} observed during the natural follicular phase in women not taking oral contraceptives. We predict that this may also be reflected in T_{Br}, and therefore we would expect luteal-phase women and women taking monophasic contraception to have higher brain temperatures that men, irrespective of the time of the day.

Null hypothesis (4): There is no statistically significant difference between mean temperatures at the brain core and the brain surface in healthy human volunteers
Alternative hypothesis (4): Mean temperature at the brain core will be consistently higher than that at the brain surface, irrespective of time.

Intuitively, we would expect mean temperature deeper within the brain to exceed that at the brain surface, based on the laws of physics.

Study procedure

Each participant will be scheduled to attend for three brain scans (approximate duration 45 minutes) at specific times on a single day (morning, afternoon, and late evening) using a 3 Tesla MRI scanner located in a temperature-controlled room at the Edinburgh Imaging (Royal Infirmary of Edinburgh) Facility. After consent is obtained, all participants will wear an actigraphy wristband for one week prior to scanning to establish their normal rest/activity, skin temperature, and light exposure patterns (and therefore their individual chronotype). On arrival at the imaging facility on their scanning day, each participant will have their height and weight recorded, and their actigraphy wristband will be removed and the data downloaded. Prior to each scan, the participant will be asked to change into hospital clothing and to complete an MRI safety and quality checklist (i.e. no metallic/cochlear implants, no metallic jewellery, no makeup that might create imaging artefacts). The participant's temperature will then be measured with an oral thermometer and if the temperature lies above or below the normal oral temperature range (33.2–38.1 °C for women; 35.7–37.7 °C for men), the participant will be rescheduled for an alternative day and any scans already performed on that participant will be excluded from the analysis. For female participants with natural menstrual cycles, a commercially-available urine testing kit (Clearblue) will be provided to confirm ovulation 7-10 days prior to their scheduled scanning date (several test strips supplied during the consenting interview so that urine can be tested for a luteinizing hormone surge on 3 consecutive days if necessary). Should ovulation not be confirmed, further test strips will be provided and the scans rescheduled for the following month. Kits will not be required for women taking monophasic contraception. No urine samples will be collected or stored from any participant; women using the kits must confirm ovulation by notification to the CI ahead of the planned scanning date, and will have the option of bringing a digital photo of the test result with them on the morning of scanning. The result will be viewed by the CI but no photos will be transferred or stored.

For each scan, the protocol will start with structural imaging of the whole brain using standard structural MRI sequences. The MRS protocol will immediately follow the

structural scan and will consist of acquiring a 1cm thick section of MRS data near the brain surface, and MRS data from another region situated more deeply (including the hypothalamus). The participant will be given written guidance on which activities and consumable products are permissible in between scans and at what time. Meal times will be fixed on the day of scanning to 6am to 8am, 12noon to 2pm, and 6pm to 8pm. Caffeine consumption will be limited to 6am to 8am and 12 noon to 2pm. Participants will be permitted to leave the site between scans but must return 15 minutes prior to their next scheduled scan. If a participant fails to attend their first morning scan (or attends late), they will be invited to reschedule their scanning day once. Participants will be advised that if they attend late to their afternoon or evening scans, these cannot proceed, since the data would be invalid. Participants will be asked to inform the CI via email of any adverse events in the 7 days post-scanning.

All structural scans will be reviewed by a neuroradiologist to check for any abnormalities. All relevant HRFs will be confidentially reported to the participant and their respective GP, adhering to Edinburgh Imaging CRFSOP 19.02 BRIC v03 Radiological Reporting of Research Scans. The neuroradiologist will inform the CI whether or not a participant's data is suitable for inclusion in the analysis (but the CI will not be given any specific information about reasons for exclusion). Only MRS data from structurally-normal brains will be included for analysis.

The study will be led by Dr Nina Rzechorzek (the CI; MRC Clinician Scientist Fellow at the MRC Laboratory of Molecular Biology, Cambridge, and Honorary Staff Member at the Centre for Clinical Brain Sciences, University of Edinburgh), supervised by Prof Ian Marshall (Centre for Clinical Brain Sciences and Edinburgh Imaging). Dr Rzechorzek is a European Board-eligible Specialist in Veterinary Neurology and Neurosurgery and has previous research experience in both advanced neuroimaging (using MRI in canine patients), and also in conducting human clinical trials. Dr Rzechorzek has also completed GCP training. Prof Marshall holds a Personal Chair in Magnetic Resonance Physics and supervised a previous study testing MRS to measure brain temperature in healthy human volunteers. The Edinburgh Imaging Study Manager (Dr Duncan Martin) will oversee logistics and has extensive experience in managing MRI-based research studies with human volunteers. Dr Francesca Chappell (a Medical Statistician based at the Centre for Clinical Brain Sciences) has performed calculations to estimate the number of volunteers required to answer our research question, and she will oversee the statistical analysis of the data generated. Dr Michael Thrippleton (a Medical Physicist in MRI based at the Centre for Clinical Brain Sciences) led a previous study on brain temperature mapping using MRS and will provide intellectual support for this work. This study is part of a wider Fellowship programme designed by Dr Rzechorzek to understand the interaction between T_{Br} fluctuation and the molecular clock in human brain cells. The Core Sponsor for this programme is Dr John O'Neill (MRC Laboratory of Molecular Biology, Cambridge), who is an internationally-recognised expert in circadian biology and has assisted with the design of the imaging arm of the proposal and the selected time points for imaging.

Reasoning for study design and methodology:
MRS is currently the only method available to accurately measure human T_{Br} non-invasively. MRI-based brain imaging provides exceptional resolution of brain structure and is the gold-standard means by which brain lesions are diagnosed routinely in the clinic. MRS is a special form of MRI that can be used to estimate T_{Br} very precisely and carries no additional risk to participants compared to MRI alone. MRI is a very

safe method of brain imaging with no known lasting effects on the brain after repeated imaging. Although intravenous contrast methods are sometimes used for MRI diagnostics, contrast is not required for MRS and will not be used in this study. Therefore no invasive procedures will be performed, but the lack of post-contrast imaging means that some HRFs may require follow-up imaging to verify them. The feedback and follow-up pathway is explained in the Participant Information Sheet.

A 3 Tesla (3T) MRI scanner is proposed for this work, because a recent study published by members of the Study Team showed that 3T MRS was more reliable than 1.5T MRS in estimating T_{Br} in healthy men. In that study, all scans were carried out at the same time of day to control for any effects of diurnal temperature fluctuation. In this study, the diurnal variation in human T_{Br} is the key parameter of interest and so each volunteer will be scanned at specific times in the morning, afternoon, and late evening on their scheduled scanning date. MRI-based brain imaging remains a fairly time-consuming process requiring high technical skill for acquisition and processing. Repeated brain imaging of each volunteer on a single day places a level of burden on that individual and on staff running the scanner. A three time point design was chosen to maximize the opportunity to meet our primary research objective whilst minimizing the burden on individual volunteers and radiographers. The selected time points are based on preliminary analysis of pre-existing data from human brain injury patients showing when T_{Br} was recorded at its maximum and minimum level via intracranial probe. Whilst we cannot conclude that T_{Br} peaks and troughs will occur at these same times in healthy human volunteers, the spacing between the time points should ensure that we do not inadvertently capture two times at which T_{Br} will be the same within a 24 hour cycle. The selected time points are also sympathetic to volunteer and staff needs, and aim to prevent any disruption to sleep patterns that may affect the results. More frequent sampling would be neither practical, nor cost-effective and would likely present a major barrier to recruitment.

The inclusion and exclusion criteria have been specifically developed to maximize participant safety, compliance, and wellbeing, whilst controlling for confounding effects that might impact upon T_{Br} and T_{Bo}, and/or thermoregulation generally. In particular, T_{Br} is highly dependent on blood flow to the brain, and on the sleep/wake cycle. We have therefore excluded people with known sleep disorders or with medical conditions that would be expected to affect general circulation, cerebral blood flow, or thermoregulation. Thermoregulation is also known to decline with age and is subject to variation in body mass, and so the age range has been limited to 20-40 years, and the BMI to 18.5-29.9. The proposed age range is consistent with the ages of healthy adult males recruited into the previously published study, and from which T_{Br} data was successfully acquired through MRS. The inclusion of women is a step forward from the previous study, which excluded women to avoid potential confounding effects of the menstrual cycle on T_{Br}. For the results of the prospective study to be widely applicable, it is important to include women whilst controlling for these effects. We believe that our approach is scientifically justified, easy to achieve, inexpensive, and logistically feasible within the duration of the study. We accept that for naturally cycling women there will be additional burden (urine testing in a location of their choosing, and a reduced selection of scanning dates) that may impact on their recruitment. However, we believe that this small impact is outweighed by the negative impact on recruitment if these women were to be excluded. Finally, the actigraphy data will allow us to control for differences in chronotype which are known to vary between the sexes, as well as between individuals of the same sex.

Proposed timetable

- September 2018 IRAS submission for R&D and ethics approval
- March 2019 Funding outcome announced
- March 2019 Study Team meeting to review/update Study Protocol and finalise recruitment strategy
- April/May 2019 revised and finalised Study Documents submitted for updated approvals ahead of recruitment
- May 2019 Commence recruitment
- May/June 2019 GP letters sent for 50 prospective participants
- June/July 2019 Complete initial recruitment (with 40 subjects called for interview and 10 subjects on 'stand-by' list)
- June to September 2019 Scanning, data collection, preliminary MRS data processing
- October to November 2019 Data analysis, statistical analysis
- December 2019 Manuscript preparation (followed by submission when finalized)
- From December 2019 Presentation of results at conferences/symposia, aggregate feedback to participants
- Deposition of averaged anonymised MRS data in a secure electronic Open Access repository upon publication

Procedures to detect and compensate for potential researcher bias

- The first 20 men and 20 women that satisfy inclusion/exclusion criteria will be invited for a consenting interview; an additional 5 men and 5 women will be invited onto a 'stand-by' list and may be invited to attend for scanning if there are interim withdrawals or exclusions.
- Recruitment will close after 25 eligible men and 25 eligible women are identified, and will re-open only if necessary.
- Volunteers will be randomised to a single scanning weekday (according to their availability) within the 7-10 days following consent
- No subjects will be scanned on a weekend
- All MRS data processing will be performed blind to sex and time of day
- After processing is complete, the CI will be unblinded in order to perform data interpretation and statistical analysis
- Statistical analyses will be supervised by a medical statistician (Dr Chappell)

4 STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

The required sample to achieve our primary endpoint is 36 healthy human volunteers, aged 20-40 years, and living within a 5 mile radius of the Imaging Facility. All scans will be conducted at one site. The study cohort will include equal numbers of men and women. 40 subjects will be recruited for scanning to account for participant withdrawal, exclusion due to HRFs, and scan failure (for technical reasons). Recruitment will take place over a 5-month period (May 2019 to September 2019).

4.2 INCLUSION CRITERIA

- Healthy adult human volunteers (men or women)
- Age 20-40 years
- Body mass index 18.5-29.9
- Live within 5-mile radius of Edinburgh Imaging (Royal Infirmary of Edinburgh) Facility
- No MRI contraindications
- Women with regular natural menstrual cycles for a minimum of 6 months, or women that have taken monophasic hormonal contraception (fixed dose oestrodiol and synthetic progestin) for a minimum of 3 months prior to scanning and will continue to do so during the month of scanning.
- Capacity to understand written and verbal information provided in English, and able to provide valid written informed consent to participate
- Able to commit to 3 x 45-minute scanning protocol within selected 24-hour period (in the post-ovulation phase of their cycle for women with natural menstrual cycles), and within the specified study period.
- Able to wear an actigraphy wristband for at least 7 days prior to scanning
- Able to commit to fixed times of food and caffeine consumption on the day of scanning
- Able to avoid alcohol and excessive physical activity on the day of scanning

4.3 EXCLUSION CRITERIA

- Pregnant women
- Early menopause, irregular menstrual cycles, premenstrual syndrome, lack of luteinizing hormone surge (according to urine test) prior to two scheduled scanning days (women with natural menstrual cycles only)
- Body mass index < 18.5 or > 29.9
- Age < 20 years or > 40 years
- MRI contraindications (e.g. cardiac pacemaker, cochlear implant, claustrophobia, any prior accident in which metal penetrated one or both eyes)
- Oral temperature outside of normal range (33.2–38.1°C for women; 35.7–37.7°C for men) prior to any scan
- Medical history that might limit activity or alter cerebral blood flow (hypothyroidism, stroke, severe arthritis, Parkinson's disease, dementia, history of brain trauma, brain tumour or epilepsy, significant mental illness besides clinical depression, spinal cord injuries, recent serious burn, diabetes, dehydration)
- Taking medications (except seasonal allergy medication, over-the-counter NSAIDs, or contraceptives), acetaminophen (paracetamol), drug abuse
- Known neurodevelopmental, neuropsychiatric or neurodegenerative disorder
- Known sleep or chronotype disorder (delayed sleep phase syndrome (CRY1), familial advanced sleep phase syndrome (PER2))
- Known family history of cardiovascular disease at < 40 years of age
- Consumption of food or caffeine out with the specified time periods on the day of scanning
- Consumption of alcohol or participation in excessive physical activity on the day of scanning
- Failure to attend, or late attendance at, two independently scheduled morning scans

- Participant-reported ill-health on the day before or the morning of scanning

5 PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

Recruitment will be conducted locally by the CI using standard approaches employed by Edinburgh Imaging. This will include a recruitment drive using mailshots to University of Edinburgh and NHS staff (including Edinburgh Imaging and Edinburgh Neuroscience subscribers), social media posts, and posters displayed at University of Edinburgh campuses and NHS Lothian hospitals. Only names and email addresses will be collected at the initial recruitment stage. Prospective participants will then be invited by email to complete an electronic questionnaire during which they can view the full list of inclusion and exclusion criteria for the study, the Participant Information Sheet, and the Consent to Participate Form. They will then be asked whether they meet the study criteria and whether or not they wish to participate. Non-willing participants will be invited to comment on why they have declined to participate. Willing participants will be asked to indicate their availability for consenting interview and scanning, and to provide written consent for the CI to contact their GP, by providing the name and address of their GP, and their own post code.

Review of medical records may only be conducted by the direct care team (the participant's GP) and/or regulatory authorities of the co-sponsors (where it is relevant to an individual's participation in the study). Letters will be sent by the CI to each prospective participant's GP, to notify them of the participant's willingness to take part in the study and listing the inclusion/exclusion criteria. GPs will have the opportunity to contact the CI with any concerns, but will not be obliged to review medical records or confirm eligibility to participate. GPs will be advised that they will be contacted by the Study Team if HRFs need to be discussed or acted upon.

The first 20 eligible men and 20 eligible women will be invited by the CI via email to attend a consenting interview at the Imaging Facility. During this interview, each participant will be provided with hard copies of the Participant Information Sheet and Consent to Participate Form to review. The participant will be invited to ask any questions and then initial and sign the Form (witnessed and co-signed by the CI). They will then be issued with an actigraphy wristband (and urine test kit if needed). Recruitment will be finalised by the CI on the morning of scanning, during which willingness to participate will be rechecked and documented. Only the minimum required person-identifiable data (name, email address, postcode, and GP details) will be collected during recruitment. Actigraphy data will be downloaded via USB port to a secure University of Edinburgh computer and will be used only if volunteers choose to proceed with scanning, otherwise it will be safely destroyed. For collection and transfer, the actigraphy data will be identifiable only by a unique participant identification number.

Participants will not be financially advantaged or disadvantaged as a result of participating in the study, nor will any forms of coercion be used to encourage recruitment. No incentives (promises of therapeutic benefit, diagnostic information, or

access to personal imaging data) will be offered during recruitment. There will be no financial incentives for participation, but travel and meal expenses to take part in the study will be provided. Participation in the study will be entirely voluntary. No undue influence will be exerted when approaching potential participants and no sanctions will follow if the participant decides to leave the research at any time. Vulnerable persons and adults lacking capacity will not be knowingly approached for recruitment.

5.2 CONSENTING PARTICIPANTS

The CI is a practising referral-level veterinary clinician who has completed specialist training in Neurology and Neurosurgery. As such, the CI has extensive experience of neuroimaging (particularly MRI techniques) and has undergone both GCP and Consent and Transparency training. The CI was also involved with the recruitment of healthy human participants for two successful double-blinded randomized controlled trials. The CI therefore understands the ethical principles underpinning informed consent and is familiar with the legal frameworks governing the consenting procedure. This procedure will ensure that each prospective participant has been provided with sufficient information and has the capacity to:

- Understand the purpose and nature of the research
- Understand what the research involves, its benefits (or lack of benefits), risks and burdens
- Understand the alternatives to taking part
- Retain the information long enough to make an effective decision
- Make a free choice
- Make this particular decision at the time it needs to be made

Consent will be obtained electronically to notify prospective participants' GPs of the participant's willingness to take part. The first 40 eligible participants (20 men and 20 women) will be invited (by emailed letter) for interview 7-10 days in advance of their selected scanning date to conduct the consenting procedure. Remaining eligible participants will be informed (by emailed letter) that they are on a 'stand-by' list and may be invited to participate at a later date in the event of subject withdrawal/exclusion.

All prospective participants will be given a Participant Information Sheet to explain the purpose and procedures of the study and valid, written informed consent to participate will be obtained once remotely (to notify respective GPs), and again during face-to-face interview with the CI at the Royal Infirmary of Edinburgh, 7-10 days prior to scanning. During this interview all participants will be provided with an actigraphy wristband to wear until their scan date and female participants with natural menstrual cycles, will be provided with a commercially-available urine testing kit (Clearblue) to confirm ovulation prior to their scheduled scanning date.

Following local guidelines, all participants must agree during the consenting procedure to be informed of any relevant HRF information arising from their brain scan. They must also understand that this information will be reported confidentially to their GP. Written consent to participate will be obtained once the participant has had time to consider all of the information provided, and after willingness to participate has been rechecked.

In addition, the consenting procedure will cover the following:

- Current and potential future risks of data use
- The risks of uncovering an HRF and how this will be handled
- The right and ability to withdraw; the circumstances under which it will not be possible to withdraw pre-existing unlinked anonymised participant data will be made clear

It is a condition of this study that all participants have the capacity to understand written and verbal information provided in advance of obtaining written informed consent to participate. Whilst the risks of MRI are very few and can be effectively controlled through appropriate safety screening, the harm to an individual if they do not understand these risks is potentially very serious. The CI will be responsible for ensuring that each participant is provided with sufficient information to understand the nature of the research and the risks involved. A number of techniques (written, visual, and interactive verbal) will be used to repeat the most salient points and confirm understanding of each participant.

The consent material and the consent process is appropriate for the study population, and meets ethical requirements. Potential participants will have sufficient time to discuss the study or consider whether to take part. The proposed study and consenting procedure will be subject to appropriate ethics review. A record of consent and willingness to participate will be kept for each interaction of the participant with Study Team members. The CI fulfills the inclusion criteria for participation and is well placed to understand the needs and perspectives of the study population.

5.2.1 Withdrawal of Study Participants

Participants are free to withdraw from the study at any point or a participant can be withdrawn by the Investigator. If withdrawal occurs, the primary reason for withdrawal will be documented in the Study Participant Data Form (SPDF), if possible. The participant will have the option of withdrawal from all aspects of the study but it is made clear in the Participant Information Sheet that anonymised data collected from that individual up to the point of withdrawal may still be used by the Study Team. If a participant loses capacity to consent during the study, they would be withdrawn from the study. However, identifiable and anonymised data already collected with consent would be retained and used in the study. No further data would be collected or any other research procedures carried out on or in relation to the participant.

The right and ability to withdraw is clearly explained in the Participant Information Sheet and Consent to Participate Form; the circumstances in which withdrawal of pre-existing unlinked anonymised participant data will not be possible is also articulated in these documents. Notification of withdrawal can be indicated verbally but should also be confirmed in writing (email or letter) to the Study Manager who will also inform the CI using linked anonymisation. The decision to withdraw will be documented by the Study Manager in the source data on the date of receipt of notification. The respective participant's GP will also be notified in writing of a withdrawal, which they must then record in the participant's clinical notes. There are no anticipated safety issues post-withdrawal. All members of the Study Team know the process to follow in the event of a withdrawal (i.e. who to inform and how it should be flagged that a participant has withdrawn).

The CI reserves the right to withdraw any participant at any time for one or more of

the following reasons:

- Identification of an HRF
- A participant becomes unable to meet the inclusion criteria during the course of the study
- A participant satisfies a criterion for exclusion during the conduct of the study
- The participant loses capacity to give valid consent to participate during the study

6 STUDY ASSESSMENTS

6.1 STUDY ASSESSMENTS

Each participant will undergo the same assessments as part of the research protocol. Women with natural menstrual cycles will undergo one additional assessment; this will comprise a urine test to confirm post-ovulation status during the scanning sessions. A urine testing kit will be provided during the consenting procedure (7-10 days prior to scanning) and urine testing will be performed by the participant at home; optionally they may record the result by digital photograph (e.g. on a personal mobile phone). The participant will be asked to confirm ovulation by notification to the CI prior to attending the scanning day. On the morning of scanning, the participant will have the option of showing the CI photographic evidence of the urine test result. The test result will be viewed and recorded by the CI but no photographic images will be transferred or stored.

Each participant will undergo three (approximately 45-minute) brain scanning sessions in a 3T MRI scanner at the Edinburgh Imaging (Royal Infirmary of Edinburgh) Facility on their scheduled scan date (in the morning, afternoon, and late evening). Just prior to their first scheduled scan, each participant will have their actigraphy wristband removed, and their height and weight measured to calculate their body mass index (BMI). They will then be asked to complete an MRI safety checklist before their oral temperature is measured by digital thermometer. The MRI safety checklist and oral temperature measurement will be repeated prior to the second and third scans of the day. During each scan, the whole brain will be imaged to check for any structural abnormalities and to provide structural information to aide localisation and interpretation of T_{Br} data. MRS scanning will then be performed in superficial and deep regions of the brain in order to obtain data that can be used to estimate brain temperature.

7 DATA COLLECTION

Details of observational components of the research methodology and how these will be carried out:

- Time points for data collection will be specified for each participant in the morning, afternoon, and late evening on their designated day of scanning. BMI will only be measured once immediately prior to the first scan.
- Body weight, height, and oral temperature measurements will be collected by the CI; structural and MRS brain scanning data will be collected by the attending radiographer

- Standardised tools will include wristband-based actigraphy in the 7 days preceding scanning, BMI calculation from body weight and height measurements, oral temperature measurements via digital thermometer, urine testing for ovulation checks (where applicable), structural brain analysis via MRI, T_{Br} estimation via MRS.
- Methods to maximise completeness of data collection will involve the CI sending an email reminder to each participant 24 hours before scanning. Participants who fail to attend for their first morning scan will be emailed to reschedule their scanning day (there will be one opportunity to reschedule for failed attendance)

Participant rest/activity, skin temperature, and light exposure patterns will be monitored non-invasively over a minimum of 5 days prior to scanning using an actigraphy wristband.

Oral temperature will be measured by the CI immediately prior to subject positioning on the scanner using a precalibrated digital thermometer covered in a disposable one-use thermometer sleeve. The thermometer will be positioned under the tongue with the mouth closed and held in place for 40 seconds. Date, time and temperature will be recorded. Scanning will proceed only if the temperature is within the normal range.

Urine testing will only be necessary for naturally cycling women recruited into the study. The CI will have prior knowledge of which women require urine testing and a urine testing kit will be provided during the consenting procedure. Participants that require urine testing will be asked to perform the test as directed in the manufacturer's instructions and then take a note of the result together with the date and time it was obtained (optionally they can take a digital photograph of the result using a mobile phone or alternative). They must notify the CI of the result prior to the scanning day so that confirmation of a luteal surge can be recorded by the CI in the Source Data. Participants have the option of bringing a photographic image of the result on the morning of scanning but no images will be transferred or stored.

At each scanning time point the scanning protocol will consist of whole-brain T1- and T2-weighted structural acquisition followed by MRS acquisition in a superficial brain region then a deep brain region. Protocols for data extraction and temperature estimation are established (see Thrippleton *et al. NMR Biomed* (2014)). Spectroscopic acquisition from the deep brain region will include the hypothalamus (which contains the suprachiasmatic nucleus where the 'master clock' is situated), and the procedure will be optimized through a pilot scan at Edinburgh Imaging. Scanning will be performed using a PRISMA 3-T clinical MRI scanner (Siemens AG, Healthcare Sector, Erlangen, Germany). MRS T_{Br} data will be obtained using sequences optimized for each brain region. All images and MRS data will be analysed offline using validated methods (see References).

7.1 Source Data Documentation

Source documents will include the following:

Electronic questionnaire — emailed to prospective participants who have given consent to be contacted in this way by providing their email address. The questionnaire will document whether or not a participants believes they meet the criteria for inclusion; whether or not they are willing to participate; and their availability for the consenting interview and scanning. It will also provide an option to provide

reasons for non-willingness to participate. By providing the name and address of their GP as well as their own postcode, prospective participants will be giving consent for their GP to be contacted.

Consent to Participate Form — to be completed by the participant with CI immediately prior to issue of the actigraphy wristband (7-10 days prior to scanning). Three copies must be signed by the participant and co-signed by the CI; one copy for the participant to keep, one copy to be added to the medical notes (sent to GP), and one copy for the Source Data File.

Source Data notes in standardized hard copy File Note and Appointment Checklist formats — on the day of scanning the CI will record participant ID, document willingness to participate, document any adverse events occurring since the consenting interview (and during the 7 days post-scanning), record confirmation of actigraphy wristband use (minimum 5 days) and ovulation (where applicable), record date, time, body weight, height, oral temperature, any deviations from or non-compliance with the Study Protocol or GCP, and any Serious Breaches.

Image files — MRI and MRS data will be stored on secure servers at Edinburgh Imaging prior to further processing.

Actigraphy data files – data will be identifiable only by unique participant number during collection and transfer via USB port to a secure University of Edinburgh computer. Data will be processed and used only if the participant proceeds with scanning, otherwise it will be safely destroyed.

7.2 Study Participant Data Forms

Source Data (participant ID, date, time, sex, DOB, height, weight, BMI, oral temperature, confirmed actigraphy recording and ovulation where applicable) will be carefully copied to a standardized paper-format SPDF template by the CI on the day of scanning. Completed SPDFs will be added to the Site File.

8 STATISTICS AND DATA ANALYSIS

8.1 SAMPLE SIZE CALCULATION

The sample size was estimated by a medical statistician (Dr Francesca Chappell) based on achieving the primary outcome using a linear mixed model for the analysis, and using previously published data exploring the reliability of MRS to measure T_{Br} in healthy human subjects. With a sample size of 36 subjects, and conservative true mean T_{Br} difference of 0.5°C for the primary outcome measure, there is 80% power to detect a statistically significant difference between time points at the 5% significance level. In total we aim to fully consent and scan 40 eligible participants to account for losses due to withdrawal, pertinent incidental HRFs that may require subject exclusion, and failed scans (e.g. technical failure of the scanner). A completion of a feedback pathway for 2 volunteers is expected (based on 5% prevalence of incidental HRFs using high-resolution MRI (see References). A 5-month recruitment period is considered entirely feasible to meet the required sample size.

8.2 PROPOSED ANALYSES

Dependent variables = T_{Br}; oral temperature. Means and standard deviations will be reported
Independent variables = time of day; sex; BMI; chronotype; phase of menstrual cycle (for naturally cycling women); brain region (deep/superficial); duration of scan

The data collected during the course of this study is nested at several levels (participant, sex, chronotype, time of day, duration of scan etc), and as such, complex statistical modelling is required. The expertise of a medical statistician are critical to the analysis of the data; Dr Chappell will oversee and supervise all statistical analyses required for this study. A linear mixed model approach will be used to accommodate data incorporating repeated measurements per participant. A linear mixed model will account for the fact that T_{Br} measurements will be correlated within individuals in the analysis; it will also allow us to use data from all participants provided they undergo at least one, but not necessarily all 3 scans. This will ensure best use of participant and staff time.

HRF-based exclusion, and non-compliance with the Study Protocol will render the entire data set retrieved from a given individual unusable. The consequences of non-compliance are clearly explained in the Participant Information Sheet. Participants who are late for, or fail to attend, their first scan will be invited to reschedule their entire scanning day. Naturally cycling female participants who cannot demonstrate ovulation via urine test prior to their scheduled scanning day will be invited to reschedule urine testing and scanning for the following month.

The core analysis focuses on comparing mean T_{Br} between different time points across the cohort. Pre-defined subgroup analyses will include mean T_{Br} comparisons between men and women at each time point, and mean T_{Br} between deep and superficial brain regions at each time point.

Given the small scale of this study, interim analyses and reports are not appropriate. Additional volunteers will be recruited if sufficient eligible participants are not identified from the initial approach

9 ADVERSE EVENTS

Adverse events will be recorded in the Source Data; for HRFs reporting will adhere to Edinburgh Imaging CRFSOP 19.02 BRIC v03 Radiological Reporting of Research Scans.

10 OVERSIGHT ARRANGEMENTS

10.1 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit the review of all study documentation by the sponsors and the REC. In the event of audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation.

11 GOOD CLINICAL PRACTICE

11.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).

Before the study can commence, all required approvals will be obtained and any conditions of approvals will be met.

11.2 INVESTIGATOR RESPONSIBILITIES

The Chief Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

11.2.1 Informed Consent

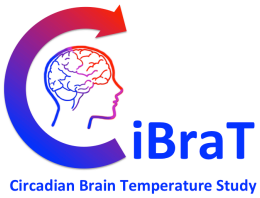
The Chief Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants will receive adequate oral and written information – appropriate Participant Information Sheets and informed Consent to Participate Forms will be provided. The oral explanation to the participant will be performed by the Chief Investigator, and must cover all the elements specified in the Participant Information Sheet and Consent to Participate Form.

The participant will be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant will be given sufficient time to consider the information provided. It will be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsors.

The Chief Investigator and the participant will sign and date the informed Consent to Participate Form to confirm that consent has been obtained. The participant will receive a copy of this document and a copy will be filed in the Investigator Site File (ISF) and participant's medical notes (complete form sent to GP).



11.2.2 Study Site Staff

The Chief Investigator must be familiar with the protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the protocol and their study-related duties.

11.2.3 GCP Training

For non-CTIMP studies all researchers are encouraged to undertake GCP training in order to understand the principles of GCP. However, this is not a mandatory requirement unless deemed so by the Sponsor. GCP training status for the Chief Investigator is indicated in their respective CV.

11.2.4 Confidentiality

All evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

11.2.5 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of relevant UK Data Protection legislation with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to individuals from the research team interacting with the participants and representatives of the sponsors.

Computers used to collate the data will have limited access measures via user names and passwords. Published results will not contain any personal data that could allow identification of individual participants.

All data collected will be compliant with the UK Data Service Data Security standards (https://www.ukdataservice.ac.uk/manage-data/store/security) and the revised GDPR (https://www.eugdpr.org). MRC guidance on confidentiality and data security (https://mrc.ukri.org/publications/browse/mrc-policy-and-guidance-on-sharing-of-research-datafrom-population-and-patient-studies/) and UK Data Service guidance on anonymisation and controlling access to shared research data (http://www.ukdataservice.ac.uk/manage-data/legalethical/ access-control) will be followed.

12 STUDY CONDUCT RESPONSIBILITIES

12.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Amendments will be submitted to a sponsor representative for review and authorisation before being submitted in writing to the appropriate REC, and local R&D for approval prior to participants being enrolled into an amended protocol.

12.2 MANAGEMENT OF PROTOCOL NON COMPLIANCE

Prospective protocol deviations, i.e. protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, and local R&D for review and approval if appropriate.

Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to the sponsor within 3 days of becoming aware of the violation. All protocol deviation logs and violation forms should be emailed to QA@accord.scot

Deviations and violations are non-compliance events discovered after the event has occurred. Deviation logs will be maintained for each site in multi-centre studies. An alternative frequency of deviation log submission to the sponsors may be agreed in writing with the sponsors.

12.3 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the participants of the study; or

(b) the scientific value of the study.

If a potential serious breach is identified by the Chief Investigator or delegates, the co-sponsors (seriousbreach@accord.scot) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the study, to determine whether the incident constitutes a serious breach and report to research ethics committees as necessary.

12.4 STUDY RECORD RETENTION

All study documentation will be kept for a minimum of 3 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

12.5 END OF STUDY

The end of study is defined as the last participant's last visit (anticipated September 2019).

The Investigators or the co-sponsors have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC, and R&D Office and co-sponsors within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved. End of study notification will be reported to the co-sponsors via email to resgov@accord.scot.

A summary report of the study will be provided to the REC within 1 year of the end of the study.

12.6 INSURANCE AND INDEMNITY

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been designed by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's National Health Service will have the benefit of NHS Indemnity.

13 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

13.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team. Aggregated results of the study will be fed back to participants and published with Open Access. The UKRI Open Access Policy and MRC Data Sharing Policy will be

followed to maximize opportunities for data linkage and interoperability (https://mrc.ukri.org/documents/pdf/mrc-data-sharing-policy/). Data preservation will comply with the UK Data Service Preservation Policy (https://data-archive.ac.uk/media/514523/cd062-preservationpolicy.pdf). Metadata will be provided according to DataCite Metadata Schema (https://schema.datacite.org/) allowing datasets to be discovered, interpreted, and used by others. Human datasets will be de-identified via the Safe Harbor Method (https://www.hhs.gov/hipaa/forprofessionals/privacy/special-topics/deidentification/index.html#standard) prior to deposit. Positive and negative research findings will be published with Open Access and according to FORCE11 Data Citation Principles (https://www.force11.org/group/joint-declaration-data-citation-principles-final). Datasets of value to the research community will be shared immediately upon publication. For data derived from human participants, intention to publish and re-use this data for research purposes will be explained during the consenting procedure. Datasets will be uploaded to recognised repositories such that the data can be preserved and curated beyond the lifetime of the funded study. Anonymised and averaged MRS-derived T_{Br} maps will be deposited securely in NeuroVault (http://neurovault.org) under a Creative Commons Public Domain Dedication License (CC0), or will be made available via the University of Edinburgh Datashare (https://datashare.is.ed.ac.uk). Open Access manuscripts will detail DOIs and repositories of deposited datasets, including details of software (version/accessibility) required to view/re-use data, or to replicate analyses. Data derived from human participants will be managed and shared according to terms described in the Participant Consent Form, in compliance with the revised GDPR (https://www.eugdpr.org). Terms will include provision for sharing anonymised and aggregate data that maximizes its value for wider research use, whilst retaining participant confidentiality. Current and potential future risks of data use will be explained during the consenting procedure. UKRI Knowledge Exchange Principles will be followed.

14 REFERENCES

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\\Study Protocols\BRAIN\Other\CiBraT_E192051\t2_space_v4

TA: 3:42 PM: REF Voxel size: 0.9×0.9×0.9 mmPAT: 4 Rel. SNR: 1.00 : spcR

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Position	Isocenter
Orientation	Transversal
Phase enc. dir.	R >> L
AutoAlign	Head > Brain
Phase oversampling	0 %
Slice oversampling	18.2 %
Slices per slab	176
FoV read	240 mm
FoV phase	100.0 %
Slice thickness	0.90 mm
TR	3200 ms
TE	408 ms
Averages	1.4
Concatenations	1
Filter	Raw filter, Distortion Corr.(2D), Prescan Normalize
Coil elements	HEA;HEP

Contrast - Common

TR	3200 ms
TE	408 ms
MTC	Off
Magn. preparation	None
Fat suppr.	None
Blood suppr.	Off
Restore magn.	On

Contrast - Dynamic

Averages	1.4
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution - Common

FoV read	240 mm
FoV phase	100.0 %
Slice thickness	0.90 mm
Base resolution	256
Phase resolution	100 %
Slice resolution	100 %
Phase partial Fourier	Allowed
Slice partial Fourier	Off
Interpolation	Off

Resolution - iPAT

PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	2
Ref. lines 3D	24
Reference scan mode	Integrated

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	On
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	On
Elliptical filter	Off

Geometry - Common

Slab group	1
Slabs	1
Position	Isocenter
Orientation	Transversal
Phase enc. dir.	R >> L
Slice oversampling	18.2 %
Slices per slab	176
FoV read	240 mm
FoV phase	100.0 %
Slice thickness	0.90 mm
TR	3200 ms
Series	Interleaved
Concatenations	1

Geometry - AutoAlign

Slab group	1
Position	Isocenter
Orientation	Transversal
Phase enc. dir.	R >> L
AutoAlign	Head > Brain
Initial Position	Isocenter
L	0.0 mm
P	0.0 mm
H	0.0 mm
Initial Rotation	90.00 deg
Initial Orientation	Transversal

Geometry - Saturation

Fat suppr.	None
Restore magn.	On
Special sat.	None

Geometry - Navigator

Geometry - Tim Planning Suite

Set-n-Go Protocol	Off
Table position	H
Table position	0 mm

Geometry - Tim Planning Suite

Inline Composing	Off

System - Miscellaneous

Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Performance
AutoAlign	Head > Brain
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Tune up
B1 Shim mode	TrueForm
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
A >> P	263 mm
R >> L	350 mm
F >> H	350 mm
Reset	Off

System - pTx Volumes

B1 Shim mode	TrueForm
Excitation	Slab-sel.

System - Tx/Rx

Frequency 1H	123.244480 MHz
Correction factor	1
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
Trigger delay	0 ms
TR	3200 ms
Concatenations	1

Physio - Cardiac

Magn. preparation	None
Fat suppr.	None
Dark blood	Off
FoV read	240 mm
FoV phase	100.0 %
Phase resolution	100 %

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

Subtract	Off
Measurements	1
StdDev	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Composing

Inline Composing	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	On

Sequence - Part 1

Introduction	On
Dimension	3D
Elliptical scanning	Off
Reordering	Linear
Flow comp.	No
Echo spacing	3.61 ms
Adiabatic-mode	Off
Bandwidth	723 Hz/Px

Sequence - Part 2

Echo train duration	910 ms
RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
Flip angle mode	T2 var
Turbo factor	282

Sequence - Assistant

Allowed delay	30 s

\\Study Protocols\BRAIN\Other\CiBraT_E192051\t1_mprage_sag_p3_iso_Munich

TA: 3:45 PM: REF Voxel size: 1.0×1.0×1.0 mmPAT: 3 Rel. SNR: 1.00 : tfl

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	On
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Slab group	1
Slabs	1
Dist. factor	50 %
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
AutoAlign	Head > Basis
Phase oversampling	0 %
Slice oversampling	0.0 %
Slices per slab	192
FoV read	256 mm
FoV phase	100.0 %
Slice thickness	1.00 mm
TR	2500.0 ms
TE	4.37 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	HEA;HEP

Contrast - Common

TR	2500.0 ms
TE	4.37 ms
Magn. preparation	Non-sel. IR
TI	1100 ms
Flip angle	7 deg
Fat suppr.	Water excit. fast
Water suppr.	None

Contrast - Dynamic

Averages	1
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution - Common

FoV read	256 mm
FoV phase	100.0 %
Slice thickness	1.00 mm
Base resolution	256
Phase resolution	100 %
Slice resolution	100 %
Phase partial Fourier	7/8
Slice partial Fourier	Off

Resolution - Common

Interpolation	Off

Resolution - iPAT

PAT mode	GRAPPA
Accel. factor PE	3
Ref. lines PE	24
Accel. factor 3D	1
Reference scan mode	Integrated

Resolution - Filter Image

Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	On
Prescan Normalize	On
Unfiltered images	Off
Normalize	Off
B1 filter	Off

Resolution - Filter Rawdata

Raw filter	Off
Elliptical filter	Off

Geometry - Common

Slab group	1
Slabs	1
Dist. factor	50 %
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Slice oversampling	0.0 %
Slices per slab	192
FoV read	256 mm
FoV phase	100.0 %
Slice thickness	1.00 mm
TR	2500.0 ms
Multi-slice mode	Single shot
Series	Interleaved
Concatenations	1

Geometry - AutoAlign

Slab group	1
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
AutoAlign	Head > Basis
Initial Position	Isocenter
L	0.0 mm
P	0.0 mm
H	0.0 mm
Initial Rotation	0.00 deg
Initial Orientation	Sagittal

Geometry - Navigator

Geometry - Tim Planning Suite

Set-n-Go Protocol	Off
Table position	H
Table position	0 mm
Inline Composing	Off

System - Miscellaneous

Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Coil Combine Mode	Adaptive Combine
Save uncombined	Off
Matrix Optimization	Off
AutoAlign	Head > Basis
Coil Select Mode	On - AutoCoilSelect

System - Adjustments

B0 Shim mode	Standard
B1 Shim mode	TrueForm
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	Isocenter
Orientation	Sagittal
Rotation	0.00 deg
A >> P	256 mm
F >> H	256 mm
R >> L	192 mm
Reset	Off

System - pTx Volumes

B1 Shim mode	TrueForm
Excitation	Non-sel.

System - Tx/Rx

Frequency 1H	123.244480 MHz
Correction factor	1
Gain	Low
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	2500.0 ms
Concatenations	1

Physio - Cardiac

Magn. preparation	Non-sel. IR
TI	1100 ms
Fat suppr.	Water excit. fast
Dark blood	Off
FoV read	256 mm
FoV phase	100.0 %
Phase resolution	100 %

Physio - PACE

Resp. control	Off
Concatenations	1

Inline - Common

Subtract	Off

Inline - Common

Measurements	1
StdDev	Off
Save original images	On

Inline - MIP

MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Inline - Composing

Inline Composing	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	On

Inline - MapIt

Save original images	On
MapIt	None
Flip angle	7 deg
Measurements	1
TR	2500.0 ms
TE	4.37 ms

Sequence - Part 1

Introduction	Off
Dimension	3D
Elliptical scanning	Off
Reordering	Linear
Asymmetric echo	Off
Flow comp.	No
Multi-slice mode	Single shot
Echo spacing	11.1 ms
Bandwidth	140 Hz/Px

Sequence - Part 2

RF pulse type	Fast
Gradient mode	Fast
Excitation	Non-sel.
RF spoiling	On
Incr. Gradient spoiling	Off
Turbo factor	192

Sequence - Assistant

Mode	Off

\\Study Protocols\BRAIN\Other\CiBraT_E192051\csi_centrum_semiovale_TBr_144

TA: 4:52 PM: REF Voxel size: 10.0×10.0×10.0 mmRel. SNR: 1.00 : csislsr

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	L0.3 A12.2 H15.4 mm
Orientation	T > C-2.8 > S-2.4
Rotation	1 deg
Slices	1
Vol A >> P	100 mm
Vol R >> L	90 mm
FoV A >> P	160 mm
FoV R >> L	160 mm
Thickness F >> H	10 mm
TR	1200 ms
TE	144 ms
Averages	3
Filter	Prescan Normalize, Hamming
Coil elements	HEA;HEP

Contrast

TR	1200 ms
TE	144 ms
Averages	3
Averaging mode	Long term
Flip angle	65 deg
Water suppr.	Weak water suppr.
Water suppr. BW	50 Hz
Measurements	1

Resolution - Common

FoV R >> L	160 mm
FoV A >> P	160 mm
Thickness F >> H	10 mm
Scan res. R >> L	16
Scan res. A >> P	16
Interpol. res. R >> L	16
Interpol. res. A >> P	16
Hamming	On
Width	50
Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	L0.3 A12.2 H15.4 mm
Orientation	T > C-2.8 > S-2.4
Rotation	1 deg
FoV R >> L	160 mm
FoV A >> P	160 mm
Thickness F >> H	10 mm

Geometry - Common

Vol R >> L	90 mm
Vol A >> P	100 mm
Sat. region	1
Thickness	40 mm
Position	R67.7 P0.2 H6.1 mm
Orientation	S > T5.2 > C0.2
Sat. delta frequ.	-3.40 ppm
Sat. region	2
Thickness	40 mm
Position	L67.1 A1.0 F2.4 mm
Orientation	S > T2.0 > C0.9
Sat. delta frequ.	-3.40 ppm
Sat. region	3
Thickness	40 mm
Position	L5.5 A7.2 F13.3 mm
Orientation	T > C-2.7 > S-2.5
Sat. delta frequ.	-3.40 ppm
Sat. region	4
Thickness	40 mm
Position	R1.6 A84.8 H4.3 mm
Orientation	C > T2.9 > S-1.0
Sat. delta frequ.	-3.40 ppm
Sat. region	5
Thickness	40 mm
Position	L0.9 P58.8 F3.2 mm
Orientation	C > T3.1 > S-0.9
Sat. delta frequ.	-3.40 ppm
Sat. region	6
Thickness	40 mm
Position	L7.9 A4.3 H42.4 mm
Orientation	T > C-3.0 > S-2.5
Sat. delta frequ.	-3.40 ppm

Geometry - AutoAlign

Slice group	1
Position	L0.3 A12.2 H15.4 mm
Orientation	T > C-2.8 > S-2.4
Phase enc. dir.	A >> P
AutoAlign	Head > Brain
Initial Position	L0.7 A0.5 H33.7
L	0.7 mm
A	0.5 mm
H	33.7 mm
Initial Rotation	91.75 deg
Initial Orientation	Transversal

System - Miscellaneous

Positioning mode	REF
Table position	F
Table position	23 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
AutoAlign	Head > Brain
Coil Select Mode	Default

System - Adjustments

B0 Shim mode	Brain

System - Adjustments

B1 Shim mode	TrueForm
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	L0.3 A12.2 H15.4 mm
Orientation	T > C-2.8 > S-2.4
Rotation	91.00 deg
R >> L	90 mm
A >> P	100 mm
F >> H	10 mm
Reset	Off

System - pTx Volumes

B1 Shim mode	TrueForm

System - Tx/Rx

Frequency 1H	123.244480 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Sequence - Common

Preparation scans	4
Dimension	2D
Delta frequency	-2.70 ppm
Phase encoding	Weighted
Bandwidth	2000 Hz
Acquisition duration	512 ms
Remove oversampling	Off

\\Study Protocols\BRAIN\Other\CiBraT_E192051\svs_hypothalamus_TBr_144

TA: 5:13 PM: REF Vol: 10 ×20 ×10 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	Isocenter
Orientation	Transversal
Rotation	0 deg
Vol R >> L	20 mm
Vol R >> L	20 mm
Vol F >> H	10 mm
TR	1200 ms
TE	144 ms
Averages	256
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	1200 ms
TE	144 ms
Averages	256
Flip angle	65 deg
Water suppr.	Weak water suppr.
Water suppr. BW	50 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	Isocenter
Orientation	Transversal
Rotation	0 deg
Vol R >> L	20 mm
Vol A >> P	10 mm
Vol F >> H	10 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	Isocenter
L	0 mm
P	0 mm
H	0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	Default

System - Adjustments

B0 Shim mode	Brain
B1 Shim mode	TrueForm
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
A >> P	10 mm
R >> L	20 mm
F >> H	10 mm
Reset	Off

System - pTx Volumes

B1 Shim mode	TrueForm

System - Tx/Rx

Frequency 1H	123.244480 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	1200 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	4
Delta frequency	-2.3 ppm
Ref. scan mode	Save all
No. of ref. scans	1
Phase cycling	Auto
Bandwidth	2000 Hz
Acquisition duration	512 ms
Remove oversampling	Off

\\Study Protocols\BRAIN\Other\CiBraT_E192051\svs_thalamus_TBr_144

TA: 2:40 PM: REF Vol: 15 ×15 ×15 mmRel. SNR: 1.00 : svs_se

Properties

Prio recon	Off
Load images to viewer	On
Inline movie	Off
Auto store images	On
Load images to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Auto close inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	Single measurement

Routine

Position	Isocenter
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol R >> L	15 mm
Vol F >> H	15 mm
TR	1200 ms
TE	144 ms
Averages	128
Filter	Prescan Normalize
Coil elements	HE1-4

Contrast

TR	1200 ms
TE	144 ms
Averages	128
Flip angle	65 deg
Water suppr.	Weak water suppr.
Water suppr. BW	50 Hz
Spectral suppr.	None
Measurements	1

Resolution - Common

Prescan Normalize	On
Vector size	1024

Geometry - Common

Position	Isocenter
Orientation	Transversal
Rotation	0 deg
Vol R >> L	15 mm
Vol A >> P	15 mm
Vol F >> H	15 mm

Geometry - AutoAlign

AutoAlign	---
Initial Position	Isocenter
L	0 mm
P	0 mm
H	0 mm
Initial Rotation	0.00 deg
Initial Orientation	Transversal

Geometry - Navigator

System - Miscellaneous

Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	F >> H
Save uncombined	Off
Save single averages	Off
AutoAlign	---
Coil Select Mode	Default

System - Adjustments

B0 Shim mode	Brain
B1 Shim mode	TrueForm
Adj. water suppr.	On
Adjust with body coil	Off
Confirm freq. adjustment	On
Only after freq. change	On
Assume Dominant Fat	Off
Assume Silicone	Off
Adjustment Tolerance	Auto

System - Adjust Volume

Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
A >> P	15 mm
R >> L	15 mm
F >> H	15 mm
Reset	Off

System - pTx Volumes

B1 Shim mode	TrueForm

System - Tx/Rx

Frequency 1H	123.244480 MHz
Gain	High
Img. Scale Cor.	1.000
Reset	Off
? Ref. amplitude 1H	0.000 V

Physio - Signal1

1st Signal/Mode	None
TR	1200 ms

Physio - PACE

Resp. control	Off

Sequence - Common

Preparation scans	4
Delta frequency	-2.3 ppm
Ref. scan mode	Save all
No. of ref. scans	1
Phase cycling	Auto
Bandwidth	2000 Hz
Acquisition duration	512 ms
Remove oversampling	Off

Supplementary Appendix 4

16th July 2019 Version 1.0

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Academic and Clinical Central Office for Research and Development
NHS Lothian

IRAS Number:	244533	REC Number:	18-HV-045	R&D Number:	2019/0133
Sponsor Number:	AC 18038	Site ID:	E192051	Study Acronym:	CiBraT
NIHR CPMS ID:	42644	Participant ID:	CiBraT_	Participant Initials:	

Study Participant Data Form (Case Report Form)

Abbreviations: Y = yes, N = no, NA = not applicable, NR = not recruited, NK = not known, ND = not done, WD = withdrawn, RS = rescheduled, AE = adverse event, DC = declined

Study Details

Study title	Can we measure a diurnal shift in brain temperature in healthy human volunteers using Magnetic Resonance Spectroscopy (MRS)?		
Short study title	Circadian Brain Temperature (CiBraT) Study		
Chief Investigator	Dr Nina Rzechorzek (also Principal Investigator)		
Medical Statistician	Dr Francesca Chappell	Study Manager	Dr Duncan Martin
Local Collaborator	Prof Ian Marshall	Medical Physicist	Dr Michael J Thrippleton
Neuroradiologist	Dr Grant Mair	Location	Edinburgh Imaging (RIE) Facility
Study design	Prospective, single site, cohort study in healthy volunteers		
Jisc URL	https://mrc.onlinesurveys.ac.uk/cibrat		

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD: ; MMM: ; YYYY: 2019

Participant Details

Initials		Sex	Male / Female
Age	YY: ; MM:	Study ID	CiBraT_
Postcode		Within 5 miles?	Y / N
Jisc response date	DD: ; MMM: ; YYYY:	Urine kit needed?	Y / N / NA

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD: ; MMM: ; YYYY: 2019

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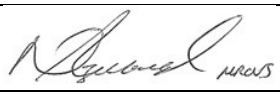
IRAS Number:	244533	REC Number:	18-HV-045	R&D Number:	2019/0133
Sponsor Number:	AC 18038	Site ID:	E192051	Study Acronym:	CiBraT
NIHR CPMS ID:	42644	Participant ID:	CiBraT_	Participant Initials:	

NHS Details

GP name					
GP address					
GP notified?	Y / N		GP letter date	DD / MMM / 2019	
GP concerns?	Y / N / NK	Cycle control	Pill / Patch / NA	Admin time	HH / NA
CHI number			Brand/NA		

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD / MMM / 2019

Consenting Interview (Pre-Scan Visit)

Date	DD / MMM / 2019	Time	HH / MM
Location			
Data entry	Dr Nina Rzechorzek	Willingness to participate rechecked?	Y / N / NR
Has participant read and understood PIS, CTPF, DPIS?			Y / N
Three copies of CTPF completed and signed?			Y / N / NR
Hard copies of PIS, signed CTPF, and DPIS given to participant?			Y / N / NR
Participant elected to be emailed about (a) study results and/or (b) future studies?			Y / N / Y / N
MRI checklist completed?	Y / N	Checklist queries reported to radiography team?	Y / N / NA
Urine Test Kit supplied?	Y / N / NA / NR	Number of days to scan date	
ActTrust2 device issued		ActTrust2 participant ID	CiBraT_
Scans booked?	Y / N / NA	Travel expenses received?	Y / N / NA

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD / MMM / 2019

accord Academic and Clinical Central Office for Research and Development

IRAS Number:	244533	**REC Number:**	18-HV-045	**R&D Number:**	2019/0133
Sponsor Number:	AC 18038	**Site ID:**	E192051	**Study Acronym:**	CiBraT
NIHR CPMS ID:	42644	**Participant ID:**	CiBraT_	**Participant Initials:**	

Scan Visit 1 (Morning)

Date	DD / MMM / 2019		Time	HH / MM	
Participant arrived by 8.45am (female) or 9.15am (male)?	Y / N / NA		Room temp.	.	
Location	Edinburgh Imaging (RIE) Facility (BRIC2)				
Data entry	Dr Nina Rzechorzek		Radiographer		
Participant well?	Y / N	Any AEs?	Y / N	AEs followed up?	Y / N / NA
Willingness checked?	Y / N / WD / RS		Actigraph removed?	Y / N	
Number of days Actigraph worn	Free / Scheduled		Patch removed?	Y / N / NA	
MRI checklist signed?	Y / N		Ovulation confirmed?	Y / N / NA	
Oral temperature (°C)	.		Within range?	Y / N	33.2–38.1 °C women 35.7–37.7 °C men
Height (m)	.		Weight (kg)	.	
BMI (kg/m^2)	.	BMI within range (18.5-29.9)?		Y / N	
Prohibited medications or alcohol?	Y / N		Food or caffeine after 8am?	Y / N	
Vigorous exercise this morning?	Y / N		Eligibility confirmed?	Y / N	
Participant changed into hospital clothing?	Y / N		All MR sequences completed?	Y / N	
Reason for reschedule (if applicable, otherwise NA)					
Reason for withdrawal (if given, otherwise NA)					
Participant reported falling asleep during scan?	Y / N / NK		Scan duration (minutes)		
Meal vouchers (lunch) issued?	Y / N / DC		Visit duration (minutes)		
Actigraphy data transferred?	Y / N		ActTrust2 data erased?	Y / N	

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD / MMM / 2019

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NHS Lothian

IRAS Number:	244533	**REC Number:**	18-HV-045	**R&D Number:**	2019/0133
Sponsor Number:	AC 18038	**Site ID:**	E192051	**Study Acronym:**	CiBraT
NIHR CPMS ID:	42644	**Participant ID:**	CiBraT_	**Participant Initials:**	

Scan Visit 2 (Afternoon)

Date	DD: ; MMM: ; YYYY: 2019	Time	HH: ; MM:
Participant arrived by 3.45pm (female) or 4.15am (male)?	Y: ; N: ; NA:	Room temp.	.
Location	Edinburgh Imaging (RIE) Facility (BRIC2)		
Data entry	Dr Nina Rzechorzek	Radiographer	
Any AEs?	Y: ; N: ; NA:	AEs followed up?	Y: ; N: ; NA:
Participant reports feeling well?	Y: ; N:	Willingness rechecked?	Y: ; N: ; WD: ; RS:
MRI checklist reviewed and signed?	Y: ; N:	Patch removed?	Y: ; N: ; NA:
Oral temperature (°C)	.	Within range?	Y: ; N: 33.2–38.1 °C women 35.7–37.7 °C men
Prohibited medications or alcohol?	Y: ; N:	Food or caffeine beyond 12-2pm?	Y: ; N:
Vigorous exercise since this morning?	Y: ; N:	Eligibility confirmed?	Y: ; N:
Participant changed into hospital clothing?	Y: ; N:	All MR sequences completed?	Y: ; N:
Reason for withdrawal (if given, otherwise NA)			
Participant reported falling asleep during scan?	Y: ; N: ; NK:	Scan duration (minutes)	
Meal vouchers (dinner) issued?	Y: ; N: ; DC:	Visit duration (minutes)	

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD: ; MMM: ; YYYY: 2019

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IRAS Number:	244533	**REC Number:**	18-HV-045	**R&D Number:**	2019/0133
Sponsor Number:	AC 18038	**Site ID:**	E192051	**Study Acronym:**	CiBraT
NIHR CPMS ID:	42644	**Participant ID:**	CiBraT_	**Participant Initials:**	

Scan Visit 3 (Evening)

Date (DD MMM 2019)		Time (HH MM)	
Participant arrived by 10.45pm (female) or 11.15pm (male)? (Y N NA)		Room temp. (.)	
Location	Edinburgh Imaging (RIE) Facility (BRIC2)		
Data entry	Dr Nina Rzechorzek	Radiographer	
Any AEs? (Y N NA)		AEs followed up? (Y N NA)	
Participant reports feeling well? (Y N)		Willingness rechecked? (Y N WD RS)	
MRI checklist reviewed and signed? (Y N)		Patch removed? (Y N NA)	
Oral temperature (°C) (.)		Within range? (Y N)	33.2–38.1 °C women 35.7–37.7 °C men
Medications, caffeine or alcohol? (Y N)		Food beyond 6-8pm? (Y N)	
Vigorous exercise since last scan? (Y N)		Eligibility confirmed? (Y N)	
Participant changed into hospital clothing? (Y N)		All MR sequences completed? (Y N)	
Reason for withdrawal (if given, otherwise NA)			
Participant reported falling asleep during scan? (Y N NK)		Scan duration (minutes)	
Notes		Visit duration (minutes)	
Participant travel expenses received? (Y N NA)		Expenses reimbursed? (Y N NA)	

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date (DD MMM 2019)	

Academic and Clinical Central Office for Research and Development

IRAS Number:	244533	REC Number:	18-HV-045	R&D Number:	2019/0133
Sponsor Number:	AC 18038	Site ID:	E192051	Study Acronym:	CiBraT
NIHR CPMS ID:	42644	Participant ID:	CiBraT_	Participant Initials:	

Post-scan

Any AEs within 7 days?	Y / N / NA	AEs followed up?	Y / N / NA
Neuroradiology report completed?	Y / N	Any HRFs identified?	Y / N
HRF exclusion reported to CI?	Y / N / NA	HRFs reported to participant?	Y / N / NA
HRFs reported to GP?	Y / N / NA	Clinical radiology report sent to GP?	Y / N

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD / MMM / 2019

Actigraphy chronotype data

Night	Type*	Sleep start	Sleep end	Sleep duration (min)	Sleep midpoint	MSF_{sc}/MSW_{sc}
1	F S	HH MM	HH MM		HH MM	HH MM
2	F S	HH MM	HH MM		HH MM	HH MM
3	F S	HH MM	HH MM		HH MM	HH MM
4	F S	HH MM	HH MM		HH MM	HH MM
5	F S	HH MM	HH MM		HH MM	HH MM
6	F S	HH MM	HH MM		HH MM	HH MM
7	F S	HH MM	HH MM		HH MM	HH MM
8	F S	HH MM	HH MM		HH MM	HH MM
Mean	F S	HH MM	HH MM		Acrophase	HH MM
Mean MSF_{sc}		HH MM	Mean MSW_{sc}	HH MM	SJL_{sc}	HH MM

*F = free; S = scheduled (relating to wake time the following morning). MSF_{sc}/MSW_{sc} = sleep corrected midpoint of sleep on free/work days = sleep onset on free/work days plus half of the average weekly sleep duration (all days). SJL_{sc} = sleep corrected social jetlag (MSF_{sc}-MSW_{sc} = absolute difference between sleep onset on free and work days).

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD / MMM / 2019

*refer to MRS database for blinded analysis and data points from each voxel

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IRAS Number:	244533	**REC Number:**	18-HV-045	**R&D Number:**	2019/0133
Sponsor Number:	AC 18038	**Site ID:**	E192051	**Study Acronym:**	CiBraT
NIHR CPMS ID:	42644	**Participant ID:**	CiBraT_	**Participant Initials:**	

MRS data (post-unblinding)*

MRS data blinded by Study Manager?	Y / N		
Scan visit	v_1	v_2	v_3
MRS data quality			
Mean temperature superficial voxels °C	.	.	.
Temperature thalamic voxel °C	.	.	.
Temperature hypothalamic voxel °C	.	.	.
Time to nearest acrophase	HH MM	HH MM	HH MM
Time since previous corrected sleep midpoint	HH MM	HH MM	HH MM
Time since average MSF_{sc}	HH MM	HH MM	HH MM

*refer to MRS database for blinded analysis and data points from each voxel

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD MMM 2019

Deviations or Non-compliance with Study Protocol and Serious Breaches

Date	DD MMM YYYY	Time	HH MM
Nature of event			
Event requires exclusion?	Y / N		
Event impacts on data quality or integrity?	Y / N		
Event requires follow-up?	Y / N		
Sponsor notified?	Y / N / NA		
GP notified?	Y / N / NA		

I declare that this Section is complete and accurate to the best of my knowledge

Chief Investigator		Date	DD MMM 2019

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Academic and Clinical Central Office for Research and Development

IRAS Number:	244533	**REC Number:**	18-HV-045	**R&D Number:**	2019/0133
Sponsor Number:	AC 18038	**Site ID:**	E192051	**Study Acronym:**	CiBraT
NIHR CPMS ID:	42644	**Participant ID:**	CiBraT_	**Participant Initials:**	

Participant withdrawal

Date	DD MMM YYYY
Elected withdrawal or CI-determined?	
Method of notification	
Primary reason for withdrawal (if available)	
GP notified of withdrawal by CI?	
I declare that this Section is complete and accurate to the best of my knowledge	

Chief Investigator		Date	DD MMM 2019

Adverse event (AE) recording

Nature of event			
Start date	DD MMM YYYY		
Stop date	DD MMM YYYY		
Time	HH MM NK		
Location			
Seriousness		Severity	
Expectedness (only if possibly related)		Relatedness	
Outcome			
CI/PI oversight			
MedDRA code			
I declare that this Section is complete and accurate to the best of my knowledge			

Chief Investigator		Date	DD MMM 2019

I declare that this Source Data Document is complete and accurate to the best of my knowledge			
Chief Investigator Final Sign Off		Date	DD MMM 2019

Analytic code for statistical models

The following code was used to generate the key statistical models for Rzechorzek et al. (2021) *'A daily temperature rhythm in the human brain predicts survival after brain injury'*. Generalized linear and linear mixed modelling were performed using R version 3.6.3 (R Core Team, 2020) and the *lme4* (v1.1–23; Bates et al. 2020), *effects* (v4.1-4; Fox et al. 2019), *afex* (Singmann et al. 2020), *Matrix* (v1.2-18; Bates et al. 2019), *Cairo* (v1.5-12.2; Urbanek and Horner 2020), *yarrr* (v0.1.5; Phillips 2017), and *car* (v3.0-8; Fox et al. 2020) packages.

Linear mixed model for oral temperature in healthy volunteers

```
OralTemp = read.table("OralTemp.txt", header=TRUE, sep="",  na.strings="NA", dec=".", strip.white=TRUE)
# loading txt file

LMM = lmer(Toral ~ Time +Sex +Age +BMI +EdTemp + (1 +Time| Subject), data=OralTemp, REML=FALSE)
# coding the model
# Time = time of day normalized for chronotype (this is the 'time distance' between the oral temperature measurement and the subject's MSFsc), a continuous variable specified as the proportion of a linearized unit circle, where 0=MSFsc and 1=24 hours).
# Toral = sublingual temperature measured in each subject at 3 time points in one day
# Sex = male, luteal female, or non-luteal female
# Age = age in years at recruitment
# BMI = BMI on day of scanning
# EdTemp = ground temperature in Edinburgh at time and date of scanning
# Random effects for intercept by Subject, and for slope by Subject with respect to Time
# REML = FALSE (choosing maximum likelihood estimation)

>vif(LMM)
         GVIF Df GVIF^(1/(2*Df))
Time   1.093069  1        1.045499
Sex    1.325030  2        1.072893
Age    1.101824  1        1.049678
BMI    1.245074  1        1.115829
EdTemp 1.128850  1        1.062473
# testing variance inflation factors to check for collinearity – all ok

> summary(LMM)

Linear mixed model fit by maximum likelihood . t-tests use
  Satterthwaite's method [lmerModLmerTest]
Formula: Toral ~ Time + Sex + Age + BMI + EdTemp + (1 + Time | Subject)
   Data: OralTemp

     AIC      BIC   logLik deviance df.resid
   152.8    183.2    -65.4    130.8      106

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.44156 -0.48825  0.01042  0.60551  2.40927

Random effects:
 Groups   Name        Variance Std.Dev. Corr
 Subject  (Intercept) 0.06957  0.2638
          Time        0.02183  0.1478   0.55
 Residual             0.11725  0.3424
Number of obs: 117, groups:  Subject, 40

Fixed effects:
            Estimate Std. Error         df t value Pr(>|t|)
```

(Intercept) 35.034571 0.543976 51.461916 64.405 <2e-16 *** # *predicted minimum OralTemp (at MSF_{sc}) for all subjects is around 35.0°C*
Time -0.210072 0.139268 45.511215 -1.508 0.1384
SexMale -0.303267 0.134295 40.631303 -2.258 0.0294 * # *male oral temp is ~0.3°C below luteal females*
SexNonluteal -0.091260 0.199003 41.491085 -0.459 0.6489
Age 0.003190 0.010572 40.564646 0.302 0.7644
BMI 0.044214 0.018915 40.858005 2.338 0.0244 * # oral temp increases with BMI
EdTemp 0.009742 0.013472 83.672125 0.723 0.4716

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
(Intr) Time SexMal SxNnlt Age BMI
Time -0.208
SexMale 0.165 0.017
SexNonlutel -0.192 0.069 0.330
Age -0.423 -0.027 -0.162 -0.215
BMI -0.702 0.003 -0.273 0.196 -0.176
EdTemp -0.423 0.290 0.050 0.185 0.010 0.027

```
> confint(LMM)
Computing profile confidence intervals ...
                  2.5 %      97.5 %
.sig01        0.086293063  0.50958981
.sig02       -1.000000000  1.00000000
.sig03        0.000000000  0.71967056
.sigma        0.276894519  0.40596712
(Intercept)  33.951564765 36.12679402
Time         -0.487124063  0.07182091
SexMale      -0.575373534 -0.03016486
SexNonluteal -0.491599951  0.30947985
Age          -0.018278499  0.02466916
BMI           0.005646613  0.08278511
EdTemp       -0.017252937  0.03646063
```

```
plot(LMM, col="dimgray", cex=0.4, xlab="Fitted oral temperature", ylab="Residuals", grid=FALSE)
# plot to check residuals

quartz.save("Quartz 2 [*]", type = "tiff", device = dev.cur(), dpi = 300)
# graphics support for transparent colours required for some devices

qqnorm(resid(LMM), col="dimgray", cex=0.4)
qqline(resid(LMM), col="dimgray")
# QQ plot to check residual fits

plot(allEffects(LMM, residuals=TRUE), lwd=4, lines=list(col="blue"), residuals.color=yarrr::transparent("black",
trans.val=.3), residuals.cex=0.8, residuals.pch=16, partial.residuals=list(smooth=TRUE, lty="dashed"),
residuals.smooth.color="yellow", confint=list(alpha=0.3))
# plotting all predictor effects with residuals and partial residuals for summary overview

(plot(predictorEffect("BMI", LMM, residuals=TRUE), lwd=4, lines=list(col="blue"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.8, residuals.pch=16, xlab="BMI", ylab="Oral
temperature", partial.residuals=list(smooth=TRUE, lty="dashed"), residuals.smooth.color="yellow"))
# plotting BMI effect only

(plot(predictorEffect("Sex", LMM, residuals=TRUE), lwd=6, lines=list(col="blue"), confint=list(col="blue",
style="bars"), residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.8, residuals.pch=16, xlab="Sex",
ylab="Oral temperature"))
# plotting Sex effect only
```

```
(plot(predictorEffect("Time", LMM, residuals=TRUE), lwd=4, lines=list(col="blue"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.8, residuals.pch=16, xlab="Time", ylab="Oral
temperature", partial.residuals=list(smooth=TRUE, lty="dashed"), residuals.smooth.color="yellow"))
# plotting Time effect only

(plot(predictorEffect("Age", LMM, residuals=TRUE), lwd=4, lines=list(col="blue"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.8, residuals.pch=16, xlab="Age (y)",
ylab="Oral temperature", partial.residuals=list(smooth=TRUE, lty="dashed"), residuals.smooth.color="yellow"))
# plotting Age effect only

(plot(predictorEffect("EdTemp", LMM, residuals=TRUE), lwd=4, lines=list(col="blue"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.8, residuals.pch=16, xlab="EdTemp",
ylab="Oral temperature", partial.residuals=list(smooth=TRUE, lty="dashed"), residuals.smooth.color="yellow"))
# plotting EdTemp effect only
```

Linear mixed model for global brain temperature in healthy volunteers

```
BrainTemp = read.table("BrainTemp.txt", header=TRUE, sep="",  na.strings="NA", dec=".", strip.white=TRUE)
#loading txt file

str(BrainTemp)
'data.frame':         8868 obs. of  8 variables:
 $ Subject    : int  1 1 1 1 1 1 1 1 1 1 ...
 $ Sex        : chr  "Luteal" "Luteal" "Luteal" "Luteal" ...
 $ Age        : num  23 23 23 23 23 23 23 23 23 23 ...
 $ BMI        : num  24.5 24.5 24.5 24.5 24.5 24.5 24.5 24.5 24.5 24.5 ...
 $ Sleep      : chr  "No" "Possible" "No" "No" ...
 $ BrainRegion: chr  "DSuperficial" "DSuperficial" "DSuperficial" "CSuperficial" ...
 $ Time       : num  0.175 0.465 0.755 0.175 0.465 ...
 $ TBrain     : num  38 38.1 38.2 38 38.1 ...

LMM = lmer(TBrain ~ Time +Sex +Age +Sleep +BrainRegion + (1 +Time| Subject), data=BrainTemp, REML=FALSE)
# coding the model
# Time = time of day normalized for chronotype (this is the 'time distance' between the oral temperature measurement
and the subject's MSFsc), a continuous variable specified as the proportion of a linearized unit circle, where 0=MSFsc
and 1=24 hours).
# TBrain = brain temperature measured in each subject at each brain voxel at 3 time points in one day
# Sex = male, luteal female, or non-luteal female
# Age = age in years at recruitment
# Sleep = whether subject reported falling asleep during scan (yes, no, maybe)
# BrainRegion = one of 6 brain MRS regions (Superficial regions A, B, C, D, Thalamus, Hypothalamus)
# Random effects for intercept by Subject, and for slope by Subject with respect to Time
# REML = FALSE (choosing maximum likelihood estimation)

>vif(LMM)
               GVIF Df GVIF^(1/(2*Df))
Time       1.005456  1       1.002724
Sex        1.084022  2       1.020374
Age        1.084473  1       1.041381
Sleep      1.010824  2       1.002695
BrainRegion 1.000027  5       1.000003
#testing variance inflation factors to check for collinearity – all ok

> summary(LMM)

Linear mixed model fit by maximum likelihood . t-tests use
  Satterthwaite's method [lmerModLmerTest]
Formula: TBrain ~ Time + Sex + Age + Sleep + BrainRegion + (1 + Time |
    Subject)
```

```
  Data: BrainTemp

    AIC      BIC   logLik deviance df.resid
 8875.4   8988.8  -4421.7   8843.4     8828

Scaled residuals:
    Min      1Q  Median      3Q     Max
-5.8415 -0.6122  0.0081  0.6359  5.9689

Random effects:
 Groups   Name        Variance Std.Dev. Corr
 Subject  (Intercept) 0.1173   0.3425
          Time        0.2789   0.5281   -0.86
 Residual             0.1543   0.3928
Number of obs: 8844, groups:  Subject, 39

Fixed effects:
                  Estimate Std. Error         df t value Pr(>|t|)
(Intercept)       3.790e+01  1.619e-01  4.566e+01 234.124  < 2e-16 # predicted minimum global BrainTemp (at MSFsc) for all subjects is ~37.9°C
Time             -5.724e-01  8.720e-02  3.836e+01  -6.564 9.21e-08 # diurnal variation of ~0.57°C across unit circle (24h)
SexLutealNon     -3.588e-01  9.517e-02  3.817e+01  -3.770 0.000553 # global BrainTemp ~0.36°C lower in follicular phase females relative to luteal phase females
SexMale          -3.558e-01  6.457e-02  3.824e+01  -5.510 2.63e-06 # global BrainTemp ~0.36°C lower in males relative to luteal phase females
Age               1.502e-02  5.127e-03  3.846e+01   2.930 0.005670 # global BrainTemp increases by ~0.02°C with each unit (year) of age across this cohort
SleepPossible    -4.625e-02  3.151e-02  6.400e+03  -1.468 0.142266 # global BrainTemp not significantly different in those who might have slept versus those that did not sleep
SleepYes          1.125e-01  1.894e-02  5.930e+03   5.940 3.00e-09 # global BrainTemp ~0.11°C higher in those that reported sleeping during the scan versus those that did not
BrainRegionBSuperficial  6.739e-01  1.334e-02  8.765e+03  50.514  < 2e-16 # Layer B ~0.67°C higher than layer A
BrainRegionCSuperficial  8.507e-01  1.279e-02  8.765e+03  66.522  < 2e-16 # Layer C ~0.85°C higher than layer A
BrainRegionDSuperficial  6.528e-01  1.262e-02  8.766e+03  51.734  < 2e-16  # Layer D ~0.65°C higher than layer A
BrainRegionHypothalamus  1.078e+00  3.814e-02  8.765e+03  28.278  < 2e-16 # Hypothalamus ~1.08°C higher than layer A
BrainRegionThalamus      1.642e+00  3.814e-02  8.765e+03  43.049  < 2e-16 # Thalamus ~1.64°C higher than layer A

(Intercept)           ***
Time                  ***
SexLutealNon            ***
SexMale               ***
Age                   **
SleepPossible
SleepYes              ***
BrainRegionBSuperficial ***
BrainRegionCSuperficial ***
BrainRegionDSuperficial ***
BrainRegionHypothalamus ***
BrainRegionThalamus     ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
            (Intr) Time   SxLtlN SexMal Age    SlpPss SlepYs BrnRBS
Time        -0.306
SexLutealNn  0.040 -0.003
SexMale      0.006  0.006  0.422
Age         -0.906  0.011 -0.208 -0.250
```

```
SleepPossbl  0.006  0.019  0.016 -0.026 -0.024
SleepYes     0.038 -0.066  0.034  0.019 -0.055  0.162
BrnRgnBSprf -0.047  0.000  0.000  0.000  0.000  0.000  0.000
BrnRgnCSprf -0.049  0.000  0.000  0.000  0.000  0.000  0.000  0.589
BrnRgnDSprf -0.049  0.000  0.002 -0.001  0.000  0.000 -0.001  0.597
BrnRgnHypth -0.016  0.000  0.000  0.000  0.000  0.000  0.000  0.198
BrnRgnThlms -0.016  0.000  0.000  0.000  0.000  0.000  0.000  0.198
          BrnRCS BrnRDS BrnRgH
Time
SexLutealNn
SexMale
Age
SleepPossbl
SleepYes
BrnRgnBSprf
BrnRgnCSprf
BrnRgnDSprf  0.622
BrnRgnHypth  0.206  0.208
BrnRgnThlms  0.206  0.208  0.069

> confint(LMM)

Computing profile confidence intervals ...
                            2.5 %      97.5 %
.sig01                 0.274283463  0.44046140
.sig02                -0.923902085 -0.73767152
.sig03                 0.422787477  0.67945006
.sigma                 0.387104810  0.39873724
(Intercept)           37.562384575 38.23685713
Time                  -0.747546353 -0.39691142
SexLutealNon          -0.550016709 -0.16709918
SexMale               -0.486451011 -0.22545069
Age                    0.004196089  0.02578471
SleepPossible         -0.108068257  0.01562339
SleepYes               0.074971478  0.15018257
BrainRegionBSuperficial  0.647710310  0.70000779
BrainRegionCSuperficial  0.825612445  0.87574561
BrainRegionDSuperficial  0.628042895  0.67750973
BrainRegionHypothalamus  1.003664525  1.15317024
BrainRegionThalamus      1.566971542  1.71647726

plot(LMM, col="dimgray", cex=0.1, xlab="Fitted brain temperature", ylab="Residuals", grid=FALSE)
# plots residuals - looks ok at high resolution

quartz.save("Quartz 2 [*]", type = "tiff", device = dev.cur(), dpi = 300)
# graphics support for transparent colours required for some devices

qqnorm(resid(LMM), col="dimgray", cex=0.25)
qqline(resid(LMM), col="dimgray")
# QQ plot shows symmetric distribution with fat tails = some degree of kurtosis but not enough to rule out normal
distribution

plot(allEffects(LMM, residuals=TRUE), lwd=4, lines=list(col="red"), residuals.color=yarrr::transparent("black",
trans.val=.3), residuals.cex=0.2, residuals.pch=16, partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2),
residuals.smooth.color="yellow", confint=list(alpha=0.3))
# plotting all predictor effects with residuals and partial residuals for summary overview
```

```
(plot(predictorEffect("Age", LMM, residuals=TRUE), lwd=4, lines=list(col="red"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.2, residuals.pch=16, xlab="Age (y)",
ylab="Brain temperature", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2),
residuals.smooth.color="yellow"))
# plotting Age effect only

(plot(predictorEffect("Sex", LMM, residuals=TRUE), lwd=6, lines=list(col="red"), confint=list(col="red", style="bars"),
residuals.color=yarrr::transparent("black", trans.val=.8), residuals.cex=0.2, residuals.pch=16, xlab="Sex", ylab="Brain
temperature"))
# plotting Sex effect only

(plot(predictorEffect("Time", LMM, residuals=TRUE), lwd=4, lines=list(col="red"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.2, residuals.pch=16, xlab="Time", ylab="Brain
temperature", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow"))
# plotting Time effect only

(plot(predictorEffect("Sleep", LMM, residuals=TRUE), lwd=6, lines=list(col="red"), confint=list(col="red",
style="bars"), residuals.color=yarrr::transparent("black", trans.val=.8), residuals.cex=0.2, residuals.pch=16,
xlab="Sleep", ylab="Brain temperature"))
# plotting Sleep effect only

(plot(predictorEffect("BrainRegion", LMM, residuals=TRUE), lwd=6, lines=list(col="red"), confint=list(col="red",
style="bars"), residuals.color=yarrr::transparent("black", trans.val=.5), residuals.cex=0.2, residuals.pch=16, xlab="Brain
region", ylab="Brain temperature"))
# plotting BrainRegion effect only

LMM = lmer(TBrain ~ Time +Sex +Age +BMI +BrainRegion + (1 +Time| Subject), data=BrainTemp, REML=FALSE)
#replacing fixed effect 'Sleep' with 'BMI'

>vif(LMM)

GVIF Df GVIF^(1/(2*Df))
Time        1.003045  1        1.001521
Sex         1.294049  2        1.066566
Age         1.122437  1        1.059451
BMI         1.255759  1        1.120607
BrainRegion 1.000000  1        1.000000
#testing variance inflation factors to check for collinearity – all ok

> summary(LMM)

Linear mixed model fit by maximum likelihood . t-tests use
  Satterthwaite's method [lmerModLmerTest]
Formula:
TBrain ~ Time + Sex + Age + BMI + BrainRegion + (1 + Time | Subject)
   Data: BrainTemp

     AIC      BIC   logLik deviance df.resid
   506.7    544.4   -242.4    484.7      215

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.1699 -0.6736 -0.0181  0.6044  3.1712

Random effects:
 Groups   Name        Variance Std.Dev. Corr
 Subject  (Intercept) 0.1561   0.3951
          Time        0.2660   0.5158   -0.61
 Residual             0.4174   0.6460
Number of obs: 226, groups:  Subject, 38
```

```
Fixed effects:
                    Estimate Std. Error         df t value Pr(>|t|)
(Intercept)         38.883586   0.559424  40.533015  69.506  < 2e-16 ***
Time               -0.878656   0.197539  37.305782  -4.448 7.54e-05 ***
SexLutealNon        -0.753159   0.223372  37.286577  -3.372 0.001751 **
SexMale             -0.605066   0.154559  37.607107  -3.915 0.000368 ***
Age                 0.025862   0.012093  37.745055   2.139 0.039006 *
BMI                 0.004784   0.021476  38.184096   0.223 0.824901    #Global brain temperature does not vary with BMI
BrainRegionThalamus   0.559667   0.085947 153.246981   6.512 1.01e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
          (Intr) Time   SxLtlN SexMal Age    BMI
Time       -0.150
SexLutealNn -0.134  0.025
SexMale      0.196 -0.003  0.341
Age         -0.435 -0.048 -0.242 -0.171
BMI         -0.754  0.002  0.209 -0.261 -0.199
BrnRgnThlms -0.077  0.000  0.000  0.000  0.000  0.000

>confint(LMM)

Computing profile confidence intervals ...
                        2.5 %      97.5 %
.sig01              0.023080184  0.70715187
.sig02             -1.000000000  1.00000000
.sig03              0.000000000  1.10756097
.sigma              0.579867609  0.72543846
(Intercept)         37.746597713 40.02491753
Time               -1.275906313 -0.48075480
SexLutealNon        -1.212342177 -0.29571036
SexMale             -0.922751101 -0.28816908
Age                 0.001462829  0.05016401
BMI                -0.038820467  0.04825773
BrainRegionThalamus  0.390153101  0.72918141

(plot(predictorEffect("BMI", LMM, residuals=TRUE), lwd=4, lines=list(col="red"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.2, residuals.pch=16, xlab="BMI", ylab="Brain
temperature", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow"))
```

Linear mixed model for global brain temperature amplitude in healthy volunteers

```
BrainTempAmp = read.table("BrainTempAmp.txt", header=TRUE, sep="",  na.strings="NA", dec=".",
strip.white=TRUE)
#loading txt file

>str(BrainTempAmp)

'data.frame':         2956 obs. of  8 variables:
 $ Subject    : int  1 1 1 1 1 1 1 1 1 1 ...
 $ Sex        : chr  "Luteal" "Luteal" "Luteal" "Luteal" ...
 $ Age        : num  23 23 23 23 23 23 23 23 23 23 ...
 $ BMI        : num  24.5 24.5 24.5 24.5 24.5 24.5 24.5 24.5 24.5 24.5 ...
 $ Sleep      : chr  "No" "No" "No" "No" ...
 $ BrainRegion: chr  "BSuperficial" "CSuperficial" "DSuperficial" "CSuperficial" ...
 $ Time       : num  0.755 0.755 0.755 0.755 0.755 ...
 $ TBrainAmp  : num  0.0262 0.0433 0.0507 0.0696 0.1124 ...
```

LMM = lmer(TBrainAmp ~ Time +Sex +Age +BMI +BrainRegion + (1 +Time| Subject), data=BrainTempAmp, REML=FALSE)
#replacing absolute brain temperature with brain temperature amplitude over time (temporal range across the day)

>vif(LMM)

```
               GVIF Df GVIF^(1/(2*Df))
Time       1.656332  1        1.286986
Sex        1.508782  2        1.108298
Age        1.562454  1        1.249982
BMI        1.287352  1        1.134615
BrainRegion 1.000049  5        1.000005
```

> summary(LMM)

```
Linear mixed model fit by maximum likelihood . t-tests use
  Satterthwaite's method [lmerModLmerTest]
Formula: TBrainAmp ~ Time + Sex + Age + BMI + BrainRegion + (1 + Time |
    Subject)
   Data: BrainTempAmp

     AIC      BIC   logLik deviance df.resid
  -759.4   -669.5    394.7   -789.4     2941

Scaled residuals:
    Min      1Q  Median      3Q     Max
-4.9971 -0.5695 -0.0675  0.4387  7.8182

Random effects:
 Groups   Name        Variance Std.Dev. Corr
 Subject  (Intercept) 0.81404  0.9022
          Time        0.86191  0.9284   -1.00
 Residual             0.04276  0.2068
Number of obs: 2956, groups:  Subject, 38

Fixed effects:
                    Estimate Std. Error         df t value Pr(>|t|)
(Intercept)         -3.675e-02  3.743e-01  2.906e+01  -0.098    0.922
Time                 3.317e-01  4.911e-01  3.610e+01   0.675    0.504
SexLutealNon         7.173e-03  9.154e-02  5.006e+01   0.078    0.938
SexMale              4.398e-02  5.118e-02  2.657e+01   0.859    0.398
Age                  3.903e-03  4.644e-03  3.063e+01   0.840    0.407
BMI                  4.272e-03  6.959e-03  2.267e+01   0.614    0.545
BrainRegionBSuperficial -1.257e-02  1.216e-02  2.920e+03  -1.034    0.301
BrainRegionCSuperficial  1.033e-02  1.166e-02  2.920e+03   0.886    0.376
BrainRegionDSuperficial  5.733e-02  1.149e-02  2.920e+03   4.992 6.33e-07
BrainRegionHypothalamus  7.349e-01  3.477e-02  2.920e+03  21.136  < 2e-16
BrainRegionThalamus      4.930e-01  3.477e-02  2.920e+03  14.180  < 2e-16

(Intercept)
Time
SexLutealNon
SexMale
Age
BMI
BrainRegionBSuperficial
BrainRegionCSuperficial
BrainRegionDSuperficial ***
BrainRegionHypothalamus ***
```

```
BrainRegionThalamus     ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
            (Intr) Time   SxLtlN SexMal Age    BMI    BrnRBS BrnRCS
Time        -0.869
SexLutealNn -0.301  0.266
SexMale      0.236 -0.119  0.262
Age          0.211 -0.486 -0.247 -0.192
BMI         -0.284 -0.090  0.145 -0.254 -0.124
BrnRgnBSprf -0.019  0.000  0.000  0.000  0.000  0.000
BrnRgnCSprf -0.019  0.000 -0.001  0.000  0.000  0.000  0.589
BrnRgnDSprf -0.018 -0.001  0.003 -0.001  0.000 -0.001  0.598  0.623
BrnRgnHypth -0.007  0.000  0.000  0.000  0.000  0.000  0.198  0.206
BrnRgnThlms -0.007  0.000  0.000  0.000  0.000  0.000  0.198  0.206
            BrnRDS BrnRgH
Time
SexLutealNn
SexMale
Age
BMI
BrnRgnBSprf
BrnRgnCSprf
BrnRgnDSprf
BrnRgnHypth  0.209
BrnRgnThlms  0.209  0.069
convergence code: 0
boundary (singular) fit: see ?isSingular

>confint(LMM)

Computing profile confidence intervals ...
                              2.5 %     97.5 %
.sig01                    0.877384844 0.93626871
.sig02                   -1.000000000 1.00000000
.sig03                    0.807360811 0.95781837
.sigma                    0.201594024 0.21220870
(Intercept)              -0.798999336 0.72294354
Time                     -0.655490296 1.32758198
SexLutealNon             -0.177191135 0.19263988
SexMale                  -0.059603628 0.15075616
Age                      -0.005453221 0.01338030
BMI                      -0.011808158 0.01828080
BrainRegionBSuperficial -0.036419840 0.01127112
BrainRegionCSuperficial -0.012530144 0.03318714
BrainRegionDSuperficial  0.034814684 0.07985178
BrainRegionHypothalamus  0.666703486 0.80304028
BrainRegionThalamus      0.424853486 0.56119028
There were 50 or more warnings (use warnings() to see the first 50)

(plot(predictorEffect("Age", LMM, residuals=TRUE), lwd=4, lines=list(col="red"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.2, residuals.pch=16, xlab="Age", ylab="Brain
temperature amplitude", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow"))
```

Linear mixed model for deep brain temperature in healthy volunteers

```
BrainTemp = read.table("BrainTempDeep.txt", header=TRUE, sep="",  na.strings="NA", dec=".", strip.white=TRUE)
# loading txt file
```

```
str(BrainTemp)
'data.frame':         226 obs. of  8 variables:
 $ Subject    : int  1 1 1 1 1 1 2 2 2 2 ...
 $ Sex        : chr  "Luteal" "Luteal" "Luteal" "Luteal" ...
 $ Age        : num  23 23 23 23 23 ...
 $ BMI        : num  24.5 24.5 24.5 24.5 24.5 24.5 29.5 29.5 29.5 29.5 ...
 $ Sleep      : chr  "No" "Possible" "No" "No" ...
 $ BrainRegion: chr  "Thalamus" "Thalamus" "Thalamus" "Hypothalamus" ...
 $ Time       : num  0.175 0.465 0.755 0.175 0.465 ...
 $ TBrain     : num  39 38.7 39.6 40.3 39.8 ...

LMM = lmer(TBrain ~ Time +Sex +Age +Sleep +BrainRegion + (1 +Time| Subject), data=BrainTemp, REML=FALSE)
```

 # coding the model

 # Time = time of day normalized for chronotype (this is the 'time distance' between the oral temperature measurement and the subject's MSF_{sc}), a continuous variable specified as the proportion of a linearized unit circle, where 0=MSF_{sc} and 1=24 hours).

 # TBrain = brain temperature measured in each subject at each deep brain voxel at 3 time points in one day

 # Sex = male, luteal female, or non-luteal female

 # Age = age in years at recruitment

 # Sleep = whether subject reported falling asleep during scan (yes, no, maybe)

 # BrainRegion = one of two deep brain MRS voxels (Thalamus, Hypothalamus)

 # Random effects for intercept by Subject, and for slope by Subject with respect to Time

 # REML = FALSE (choosing maximum likelihood estimation)

```
>vif(LMM)
              GVIF Df GVIF^(1/(2*Df))
Time       1.040578  1        1.020087
Sex        1.108491  2        1.026084
Age        1.106316  1        1.051815
Sleep      1.092063  2        1.022261
BrainRegion 1.000000  1        1.000000
```

 # testing variance inflation factors to check for collinearity – all ok

```
> summary(LMM)

Linear mixed model fit by maximum likelihood . t-tests use
  Satterthwaite's method [lmerModLmerTest]
Formula: TBrain ~ Time + Sex + Age + Sleep + BrainRegion + (1 + Time |
    Subject)
   Data: BrainTemp

     AIC      BIC   logLik deviance df.resid
   505.6    546.7   -240.8    481.6      214

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.1642 -0.6011 -0.0333  0.6194  3.1716

Random effects:
 Groups   Name        Variance Std.Dev. Corr
 Subject  (Intercept) 0.1950   0.4416
          Time        0.2438   0.4938   -0.71
 Residual             0.4110   0.6411
Number of obs: 226, groups:  Subject, 38

Fixed effects:
                 Estimate Std. Error       df t value Pr(>|t|)
(Intercept)       38.94911    0.37156  43.38210 104.825  < 2e-16 ***
```

 # predicted minimum for deep regions at MSF_{sc} is ~38.9°C

```
Time                -0.85561    0.19844  38.12657  -4.312 0.000110 *** # diurnal variation of ~0.86°C in deep regions across a unit circle (24h)
SexLutealNon        -0.82053    0.22092  38.00242  -3.714 0.000653 *** # deep brain temp ~0.82°C higher in luteal phase females than follicular phase females
SexMale             -0.60253    0.15079  38.31558  -3.996 0.000283 *** # deep brain temp ~0.60°C higher in luteal phase females than males
Age                 0.02920     0.01204  39.01803   2.426 0.019985 *  # ~0.03°C increase in deep brain temp per year of age in this cohort
SleepPossible       -0.29299    0.21706 157.70181  -1.350 0.179021    # no significant effect of reported possible sleep on deep brain temp
SleepYes            -0.18707    0.12875 164.22369  -1.453 0.148135    # no significant effect of reported sleep on deep brain temp
BrainRegionThalamus  0.55967    0.08529 152.87677   6.562 7.77e-10 *** # thalamus ~0.56°C higher than hypothalamus
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) Time   SxLtlN SexMal Age    SlpPss SlepYs
Time        -0.247
SexLutealNn  0.042  0.013
SexMale      0.007 -0.019  0.413
Age         -0.905 -0.027 -0.218 -0.237
SleepPossbl  0.008  0.075  0.045 -0.103 -0.060
SleepYes     0.099 -0.162  0.092  0.032 -0.150  0.145
BrnRgnThlms -0.115  0.000  0.000  0.000  0.000  0.000  0.000

> confint(LMM)

Computing profile confidence intervals ...
                        2.5 %      97.5 %
.sig01              0.094841077  0.75856893
.sig02             -1.000000000  1.00000000
.sig03              0.000000000  1.09278289
.sigma              0.575332896  0.71964104
(Intercept)        38.200358244 39.69442448
Time               -1.257067569 -0.45790353
SexLutealNon       -1.276551404 -0.36850999
SexMale            -0.917553794 -0.28941499
Age                 0.005027576  0.05347177
SleepPossible      -0.747267324  0.15518136
SleepYes           -0.442360067  0.07043526
BrainRegionThalamus  0.391449742  0.72788477

plot(LMM, col="dimgray", cex=0.4, xlab="Fitted brain temperature", ylab="Residuals", grid=FALSE)
# plots residuals to check

quartz.save("Quartz 2 [*]", type = "tiff", device = dev.cur(), dpi = 300)
# graphics support for transparent colours required for some devices

qqnorm(resid(LMM), col="dimgray", cex=0.4)
qqline(resid(LMM), col="dimgray")
# QQ plot shows symmetric distribution with fat tails = some degree of kurtosis but not enough to rule out normal distribution

plot(allEffects(LMM, residuals=TRUE), lwd=4, lines=list(col="red"), residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.2, residuals.pch=16, partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow", confint=list(alpha=0.3))
# plotting all predictor effects with residuals and partial residuals for summary overview
```

```
(plot(predictorEffect("Age", LMM, residuals=TRUE), lwd=4, lines=list(col="red"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.4, residuals.pch=16, xlab="Age (y)",
ylab="Brain temperature", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2),
residuals.smooth.color="yellow"))
# plotting Age effect only

(plot(predictorEffect("Sex", LMM, residuals=TRUE), lwd=6, lines=list(col="red"), confint=list(col="red", style="bars"),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.4, residuals.pch=16, xlab="Sex", ylab="Brain
temperature"))
# plotting Sex effect only

(plot(predictorEffect("Time", LMM, residuals=TRUE), lwd=4, lines=list(col="red"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.4, residuals.pch=16, xlab="Time", ylab="Brain
temperature", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow"))
# plotting Time effect only

(plot(predictorEffect("Sleep", LMM, residuals=TRUE), lwd=6, lines=list(col="red"), confint=list(col="red",
style="bars"), residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.4, residuals.pch=16,
xlab="Sleep", ylab="Brain temperature"))
# plotting Sleep effect only

(plot(predictorEffect("BrainRegion", LMM, residuals=TRUE), lwd=6, lines=list(col="red"), confint=list(col="red",
style="bars"), residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=0.4, residuals.pch=16, xlab="Brain
region", ylab="Brain temperature"))
# plotting BrainRegion effect only
```

Generalized linear mixed model for outcome in TBI patients

```
Outcome = read.table("Outcome.txt", header=TRUE, sep="\t", na.strings="NA", dec=".", strip.white=TRUE)

str(Outcome)

'data.frame':        114 obs. of  15 variables:
 $ Subject   : int  1 2 3 4 5 6 7 8 9 10 ...# Patient ID number
 $ Age       : int  57 65 62 25 42 21 31 52 64 56 ...# Patient age in years
 $ Sex       : chr  "Female" "Male" "Female" "Male" ...# Patient sex (menstrual cycle phase not known)
 $ Outcome   : int  0 1 0 0 0 0 0 1 0 0 ...# Patient outcome in intensive care: 0=alive, 1=dead
 $ BrainMean : num  38.7 37.9 38.5 40.1 39 ...# Absolute mean brain temperature for patient across processed data set
(after excluding artefacts etc)
 $ BrainRange: num  3 4.66 3.52 3.64 2.96 4.68 5.02 NA NA 4.7 ...# Range in patient brain temperature across
processed data set
 $ BodyRange : num  1.3 4.3 3.7 2.7 NA 3.1 NA 2.5 2.2 2.6 ...# Range in patient body temperature across processed
data set
 $ BrainMax  : num  40.3 39.8 39.9 41.7 40.5 ...# Maximum patient brain temperature across processed data set
 $ BrainMin  : num  37.3 35.1 36.4 38.1 37.6 ...# Minimum patient brain temperature across processed data set
 $ BodyMax   : num  37.7 38.3 39.6 39 NA 39.1 NA 39.3 39 38.6 ...# Maximum patient body temperature across
processed data set
 $ BodyMin   : num  36.4 34 35.9 36.3 NA 36 NA 36.8 36.8 36 ...# Minimum patient body temperature across processed
data set
 $ Daily     : chr  "Yes" "No" "Yes" "Yes" ...# Was there evidence of daily rhythmicity in temperature across processed
data set? Yes or No
 $ PLR       : int  2 1 2 2 2 2 2 2 2 NA ...# Pupillary light reflex present in both eyes (2),one eye (1), or no eyes (0)
 $ GCS       : int  6 3 9 5 4 6 11 3 9 9 ...# Glasgow Coma Scale score
 $ GCSM      : int  4 1 5 1 2 4 6 1 5 5 ...# Glasgow Coma Scale Motor response score

GLMM = glmer(Outcome ~ Age +Sex + BrainMean +BrainRange +Daily + (1|Subject), data=Outcome,
family=binomial, nAGQ=0)
# coding the model
```

 # model includes several fixed effects plus random effects for intercept by Subject
 # there were no significant relationships between maximum BrainTemp, minimum BrainTemp, maximum BodyTemp, minimum BodyTemp, PLR, GCS or GCSM and Outcome and including these fixed effects did not improve the model fit

```
>summary(GLMM)

Generalized linear mixed model fit by maximum likelihood (Adaptive Gauss-Hermite Quadrature, nAGQ =
  0) [glmerMod]
 Family: binomial  ( logit )
Formula: Outcome ~ Age + Sex + BrainMean + BrainRange + Daily + (1 | Subject)
   Data: Outcome

     AIC      BIC   logLik deviance df.resid
    83.6    101.6    -34.8     69.6       91

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.8846 -0.4212 -0.1789 -0.0231  4.3176

Random effects:
 Groups  Name        Variance Std.Dev.
 Subject (Intercept) 0.02809  0.1676
Number of obs: 98, groups:  Subject, 98

Fixed effects:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) 26.06901   15.56630   1.675 0.093992 .
Age          0.09914    0.02696   3.677 0.000236 ***
SexMale      0.33892    0.80702   0.420 0.674510
BrainMean   -0.87808    0.41734  -2.104 0.035378 *
BrainRange   0.39958    0.25779   1.550 0.121141
DailyYes    -3.03024    1.25685  -2.411 0.015910 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
            (Intr) Age    SexMal BranMn BrnRng
Age         0.218
SexMale     0.157  0.159
BrainMean  -0.991 -0.328 -0.210
BrainRange -0.081  0.338  0.022 -0.005
DailyYes   -0.173 -0.248  0.211  0.189 -0.236

plot(GLMM, col="dimgray", cex=0.8, xlab="Mortality", ylab="Residuals", grid=FALSE)
# plots residuals – as expected

quartz.save("Quartz 2 [*]", type = "tiff", device = dev.cur(), dpi = 300)
# graphics support for transparent colours required for some devices

qqnorm(resid(GLMM), col="dimgray", cex=0.8)
qqline(resid(GLMM), col="dimgray")
# QQ plot

plot(allEffects(GLMM, residuals=TRUE), lwd=4, lines=list(col="purple"), residuals.color=yarrr::transparent("black",
trans.val=.3), residuals.cex=0.2, residuals.pch=16, partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2),
residuals.smooth.color="yellow", confint=list(alpha=0.3))
# plots all predictor effects for summary overview
```

```
(plot(predictorEffect("Age", GLMM, residuals=TRUE), lwd=4, lines=list(col="purple"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=1.6, residuals.pch=16, xlab="Age",
ylab="Mortality", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow"))
# plots Age effect only

(plot(predictorEffect("Daily", GLMM, residuals=TRUE), lwd=4, lines=list(col="purple"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=1.6, residuals.pch=16, xlab="Daily?",
ylab="Mortality"))
# plots Daily effect only

(plot(predictorEffect("BrainRange", GLMM, residuals=TRUE), lwd=4, lines=list(col="purple"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=1.6, residuals.pch=16, xlab="BrainRange",
ylab="Mortality", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow"))
# plots BrainRange effect only (not significant)

(plot(predictorEffect("BrainMean", GLMM, residuals=TRUE), lwd=4, lines=list(col="purple"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=1.6, residuals.pch=16, xlab="BrainMean",
ylab="Mortality", partial.residuals=list(smooth=TRUE, lty="dashed", lwd=2), residuals.smooth.color="yellow"))
# plots BrainMean temperature effect only

(plot(predictorEffect("Sex", GLMM, residuals=TRUE), lwd=4, lines=list(col="purple"), confint=list(alpha=0.3),
residuals.color=yarrr::transparent("black", trans.val=.3), residuals.cex=1.6, residuals.pch=16, xlab="Sex",
ylab="Mortality"))
# plots Sex effect only (not significant)

exp(0.09914) # calculating Age odds for death
[1] 1.104221

exp(-0.87808) # calculating BrainMean temperature odds for death
[1] 0.4155801

exp(-3.03024) # calculating Daily odds for death
[1] 0.04830404

1/0.04830404 # calculating Daily odds for survival
[1] 20.7022

# calculate 95% confidence intervals using estimate +/- (1.96*standard error) for each of the significant predictors

LogAgeCI = 1.96*0.02696
print(LogAgeCI)
[1] 0.0528416 # so 95% CI for Age in log odds = 0.09914+/- 0.0528416

0.09914+LogAgeCI
[1] 0.1519816

0.09914-LogAgeCI
[1] 0.0462984 # now convert to CI on the odds

exp(c(0.0462984, 0.1519816))
[1] 1.047387 1.164139 # good – it doesn't include 1
# CIs are numerically asymmetric once turned back into odds

LogBrainMeanCI = 1.96* 0.41734
print(LogBrainMeanCI)
[1] 0.8179864# so 95% CI for BrainMean in log odds = -0.87808+/- 0.8179864

-0.87808+LogBrainMeanCI
[1] -0.0600936
```

-0.87808-LogBrainMeanCI
[1] -1.696066 # *now convert to CI on the odds*

exp(c(-1.696066, -0.0600936))
[1] 0.1834036 0.9416764 # *good – it doesn't include 1*
 # CIs are numerically asymmetric once turned back into odds

LogDailyCI = 1.96* 1.25685
print(LogDailyCI)
[1] 2.463426 # *so 95% CI for Daily in log odds = -3.03024+/- 2.463426*

-3.03024+LogDailyCI
[1] -0.566814

-3.03024-LogDailyCI
[1] -5.493666 # *now convert to CI on the odds*

exp(c(-5.493666, -0.566814))
[1] 0.004112739 0.567330076 # *good – it doesn't include 1*
 # CIs are numerically asymmetric once turned back into odds

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***
Relevant page numbers highlighted yellow in brackets

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (1)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (1-2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (3-4)
Objectives	3	State specific objectives, including any prespecified hypotheses (4-11)
Methods		
Study design	4	Present key elements of study design early in the paper (4-13)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (4-10,14-15)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (4-10, Table 1; Supplementary Table 2)
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed (N/A)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (10-13; Supplementary Methods)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (4-13; Supplementary Methods, Supplementary Text)
Bias	9	Describe any efforts to address potential sources of bias (6; Supplementary Methods)
Study size	10	Explain how the study size was arrived at (7-8)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (10-13; Supplementary Methods)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (10-13; Supplementary Methods)
		(*b*) Describe any methods used to examine subgroups and interactions (Table 1, 10-13)
		(*c*) Explain how missing data were addressed (13-15)
		(*d*) If applicable, explain how loss to follow-up was addressed (14-15, Figure 2A)
		(*e*) Describe any sensitivity analyses (N/A)
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (13-15, Figure 2A)
		(b) Give reasons for non-participation at each stage (Table 1, Table S2
		(c) Consider use of a flow diagram (Figure 2A)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (13-18, Figures 1 to 5, Table 2, Table 3)
		(b) Indicate number of participants with missing data for each variable of interest (13-18, Figure 2A)
		(c) Summarise follow-up time (eg, average and total amount) (10)

Outcome data	15*	Report numbers of outcome events or summary measures over time (13-18, Figures 1-5, Tables 2-3)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (95% confidence intervals reported)
		(*b*) Report category boundaries when continuous variables were categorized (Figure 3A)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (N/A)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (13-18, Figures 1-5, Tables 2-3)
Discussion		
Key results	18	Summarise key results with reference to study objectives (18-19)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (19-23; Supplementary Text)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (19-23; Supplementary Text)
Generalisability	21	Discuss the generalisability (external validity) of the study results (19-23; Supplementary Text)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (24)

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.