This repository relates to the rodent data reported in the manuscript "Circadian and brain state modulation of network hyperexcitability in Alzheimer's disease".

This repository contains the following datasets:

1) J20_control.zip: EEG data from 4-6 month old J20 and wild-type (WT) mice in control conditions
2) APP1Yr_control.zip: EEG data from 12 month old APPNL/F mice in control conditions
3) WT1Yr_control.zip: EEG data from 12 month old WT mice in control conditions
4) APP8mo_control.zip: EEG data from 8 month old APPNL/F mice in control conditions
5) WT8mo_control.zip: EEG data from 8 month old WT mice in control conditions
6) VideoScoring.zip: Wake/sleep video scoring data for a subset of the animals for which video data was acquired during EEG recordings
7) SummaryDataJ20.csv: Information for individual mice in J20_control.zip
8) SummaryDataAPP.csv: Information for individual mice in APP1Yr_control.zip, WT1yr_control.zip, APP8mo_control.zip, WT8mo_control.zip
9) AChEAssay.zip: Data used for determining the activity of Acetylcholinesterase (AChE) in brain homogenates from J20 and WT mice. Some of the mice received oral donepezil treatment prior to death.
10) ChATQuantification.zip: A repository of data used to quantify Choline Acetyl Transferase (ChAT) expression in medial septum (MS) and diagonal band of Broca (DB).
11) ChATInformation.csv: Information for samples contained in ChATQuantfication.zip

This document describes the structure for the data contained in the archives and files within this repository.

1-5) J20_control.zip, APP1Yr_control.zip, WT1yr_control.zip, APP8mo_control.zip, WT8mo_control.zip: Each of these archives are organised into subfolders labelled by mouse ID. Each mouse ID subfolder contains subsubfolders, with each subsubfolder containing EEG data for one hour of acquisition. The subsubfolders are named Mxxxxxxxxxx.ndf, where xxxxxxxxxx indicates the POSIX time stamp (i.e. number of seconds after 00:00:00 on 1/1/1970) for the start of the data acquisition within that subsubfolder. The Mxxxxxxxxxx.ndf subsubfolder contains a file En.txt, where n is a number indicating the channel ID for the transmitter that was implanted in the animal. Hence, n is constant for an animal with several animals sharing a common n.

En.txt files are space delimited text files of EEG data sampled at 512sps using an Opensource Instruments wireless transmitter and electrode implantation as described in the methods section of the manuscript. The En.txt files were created by processing the original Opensource Instruments Neuroarchiver file format (.ndf) with the SCPP4V1.tcl processor:
http://www.opensourceinstruments.com/Electronics/A3018/HTML/SCPP4V1.tcl

The units of En.txt files are arbitrary counts with one count approximately equal to 400nV.

Data samples lost during acquisition are ascribed the same value as the preceding sample. Hence if two or more samples have the same value, it can be assumed that data loss occurred. For more information, see
http://www.opensourceinstruments.com/Electronics/A3018/Recorder.html#Message%20Analysis

Zero count values are indicative of times when the transmitter was switched off.

EEG data was acquired for approximately 3 days for each animal. In some animals, there was a two day break in data acquisition. This is indicated either by a break in the time stamps of the Mxxxxxxxxxx.ndf folders, or zero count values within the En.txt files.

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6) VideoScoring.zip: Archive contains a set of .csv files named VideoScoring_AnimalID_DDMMYY*OptionalSuffix*, where AnimalID is the Animal ID, DDMMYY is the date of video acquisition, and *OptionalSuffix* is an optional additional label for the file.

Each .csv file contains the following variables:

- DateOfEEG: Date in the form dd/mm/yyyy
- TimeOfEEG: Time stamp of video in the form hh:mm:ss
- SleepWake: "S" or "W" to indicate the sleep/wake state of the animal at the specified time. The animal continued in that state until the next specified TimeOfEEG.

For figure 3 and figure 4A and figure 5, we used the subset of EEG data for which video sleep/wake scoring was available (i.e. the times included in the VideoScoring.zip archive). The video time stamps and sleep/wake scoring were used to classify the sleep/wake status in 8s intervals used for analysing EEG data.

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7) SummaryDataJ20.csv: Spreadsheet of information for individual mice included in J20_Control.zip. The variables are:

- AnimalID: The mouse ID which is the name of the corresponding data subfolder in J20_Control.zip
- Genotype: "J20" or "WT"
- Channel: The channel number of the wireless transmitter. This specifies n in the En.txt data files
- TimeZone: Europe/London or UTC. This information was used when converting POSIX time to local time
- ReferenceElectrode: The location of the reference electrode. The reference electrode was placed either above cortex ("CTX") or cerebellum ("CBLM")
- Fig1_IISRate: Variable specifying whether the data from the animal was included in Figure 1 of the manuscript
- Fig2_CircadianCoupling: Variable specifying whether the data from the animal was included in Figure 2 of the manuscript
- Fig3_BehaviouralState: Variable specifying whether the data from the animal was included in Figure 3 of the manuscript
- Fig4_BrainState: Variable specifying whether the data from the animal was included in Figure 4 of the manuscript
- Fig5_WT: Variable specifying whether the data from the animal was included in Figure 5 of the manuscript

- FalsePositiveRate: False positive rate of processor which was used to identify intervals with IIS. The false positive rate of the processor was determined by randomly inspecting 100 intervals with IIS and visually judging whether the interval contained IIS.
- LossIntervalPercentage: The percentage of total intervals in which data loss exceeded a threshold of 20% of samples within the interval
- Protocol: The experimental protocol. This was either 3 days of continuous EEG recordings ("C"), or 1 day followed by a break of 2 days, followed by 2 further days of recording ("B")
- Age: Age of the animals at commencement of the recording (units: days)
- Outliers: A 0 or 1 to indicate whether the data from the animal was identified as a circular outlier as described in the manuscript. 1 indicates a circular outlier.

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8) Spreadsheet of information for individual mice included in APP1Yr_Control.zip, APP8mo_Control.zip, WT1Yr_Control.zip , WT8mo_Control.zip.

The variables are:

- AnimalID: The mouse ID which is the name of the corresponding data subfolder in the .zip archive
- Genotype: "APPNLF" or "WT"
- Channel: The channel number of the wireless transmitter. This specifies n in the En.txt data files
- TimeZone: Europe/London or UTC. This information was used when converting POSIX time to local time
- ReferenceElectrode: The location of the reference electrode. The reference electrode was placed either above cortex ("CTX") or cerebellum ("CBLM")
- Age: Variable specifying whether animal was in 8month or 12 month group

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9) AChEAssay.zip: This acetylcholinesterase (AChE) assay was modified from the Ellman method (Elman et al, Biochem. Pharmacol. 7:88-95;1961). With this assay, AChE hydrolyses acetylthiocholine (ATH) to produce acetate and thiocholine. The thiocholine reacts with DTNB (5,5-dithio-bis-(2-nitrobenzoic acid)) to split the DTNB molecule, thereby producing a thiolate anion that absorbs 412 nm light.

The archive contains 5 .csv files. Each .csv file contains data from one run of the AChE assay experiment. The files are named by the date of the experiment. Each run of the AChE assay experiment comprised applying the AChE assay across the following 6 conditions:

- Blank wells
- Cortical homogenate from wild-type (WT) mouse not treated with donepezil before killing
- Cortical homogenate from WT mouse treated with donepezil before killing
- Cortical homogenate from J20 mouse not treated with donepezil before killing
- Cortical homogenate from J20 mouse treated with donepezil before killing
- Cortical homogenate from WT mouse either with or without donepezil treatment and neostigmine applied directly to brain homogenate

Each experiment consisted of replicating the AChE assay 3 times (i.e. in 3 wells) for each of the 6 conditions giving a total of 18 samples per experiment.

The variables in .csv file are as follows:

- Mouse: ID variable for mouse. “Blank” for blank wells
- Genotype: “J20”, “WT”. “NA” for blank wells
- Donepezil: “YES” or “NO” specifying whether mouse was treated with 2 days of orally administered Donepezil prior to killing
- Neostigmine: “YES” or “NO” specifying whether neostigmine was added to the well
- Well: Well number
- Before ATH: 450nm absorption of well prior to addition of ATH. Arbitrary units
- 0min – 30min: 450nm absorption measurements commencing at time of ATH application and every 5 minutes after that. Arbitrary units.
- Notes: Additional variable specifying whether sample needs special consideration/exclusion

For each well/sample, a thiocholine production rate was evaluated as follows:

1) The difference between absorption at 0min and 20min was calculated
2) The difference was converted into thiocholine production rate with reference to a glutathione calibration curve, which plots absorbance against concentration, which was linear up to an absorbance of 3 arbitrary units, giving us the slope (Gs in units per nanomole) and y-intercept (Gi). The concentration of reagents in the experiment was adjusted so that the absorbance reading never exceeded 3 units. We then converted the absorbance change A over time t (mins) was converted to thiocholine production (TCP) in nmoles per minute using the formula:

   TCP = A*(1-Gi)/(Gs*t)*0.3

   Where, Gi=0.06053, Gs = 0.006404. The multiplier of 0.3 was a volume correction to bring measurements in line with measurements made by colleagues using a spectrophotometric method and a different volume of reagents.

The average of the thiocholine production rate in the three replications of each condition was evaluated and used for statistical comparisons.

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10) ChATQuantification.zip: This repository contains

- Folder “Controls” :

  - "Negative_Control" folder with raw images of the ChAT immunostaining protocol without primary antibody. Images saved as .tif

  - "Positive_Control" folder with raw mages of the full ChAT immunostaining protocol showing nice neurons. Images saved as .tif

- Folder named "Samples" containing :

  - “#n” subfolders, where n is a number. #n is the BrainID label used in ChATInformation.csv (see below). This subfolder contains:

* all the raw images for the sample. Each image is labelled as: BrainID_SlideNumber_SliceNumber_Staining_Magnification_ExposureTime.tif

* 2 results spreadsheets:

- BrainID_MS.xls: StereoInvestigator output for MS stereology

- BrainID_DB.xls: StereoInvestigator output for DB stereology.

Details for stereology image acquisition and quantification are below.

Images acquisition

Images were acquired using a Zeiss AX10 with a 5X magnification and the exposure time was conserved between slices and animals at 5ms. Images were saved as .tif files.

StereoInvestigator counting

1. Cut thickness set at 50µm

2. ROIs drawn at 1.25X

* Line 1 for the Medial Septum (see manuscript for description of ROI)

* Line 2 for the Diagonal Band (see manuscript for description of ROI)

3. Counting at 10x

4. Thickness manually set at 50µm

5. Counting frame set at 75/75

6. Grid size set at 150/150

7. Neurons were counted in the sampled counting area. Neurons were counted if more of the half cell body is within the counting square. Counting was repeated for each counting sampled counting square across sections.

8. The stereological count results were saved in BrainID_MS.xls or BrainID_DB.xls spreadsheets within each folder.

Results

The spreadsheets obtained as output from the StereoInvestigator analysis are organised in multiple tabs.

For each sample, it was ensured that the "Estimated CE (Scheaffer)" in the "Coefficient of Error" tab is less than or equal to 0.05. This was used to ascertain that there are sufficient slices and regions analysed as required for a robust population estimate.

The "Estimated Population Using User Defined Section Thickness" in the "Summary" tab was used for statistical analysis. This parameter used the count an incorporates slice thickness and size of the brain to extrapolate an Estimated population for this animal.

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11) ChATInformation.csv: Information relating to each of the samples contained in ChatQuantification.zip "Samples" subfolder. ChATInformation.csv contains the following variables

- DoB: The date of birth of the animal in format dd/mm/yy

- DateOfPerfusion: The date of perfusion of the animal in format dd/mm/yy

- Age: Age in months

- Sex: Male ("M") or Female ("F")

- Genotype: "WT" or "J20"

- DonepezilTreatment: Whether Donepezil was administered prior to killing the animal

- BrainID: Brain ID label as relating to label in "Samples" folder

- NoSectAll: Number of brain slices in the set, variable between animals

- NoSectMS: Number of brain slices with the MS

- NoSectDB: Number of brain slices with the DB

- PopEstAll: Estimated population of cholinergic neurons in both regions (MS and DB). As determined from StereoInvestigator.

- PopEstMS: Estimated population of cholinergic neurons only in the MS. As determined from StereoInvestigator.

- PopEstDB: Estimated population of cholinergic neurons in the DB. As determined from StereoInvestigator.

- EstCE: Estimated Coefficient of Error (Scheaffer). As determined from StereoInvestigator.

- Notes: Any comments regarding the slices