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Citation for published version:

Brown, R, Lam, AD, Gonzalez-sulser, A, Ying, A, Jones, M, Chou, RC, Tzioras, M, Jordan, CY, Jedrasiak-cape, I, Hemonnot, A, Abou Jaoude, M, Cole, AJ, Cash, SS, Saito, T, Saido, T, Ribchester, RR, Hashemi, K & Oren, I 2018, 'Circadian and Brain State Modulation of Network Hyperexcitability in Alzheimer's Disease', *eNeuro*. <https://doi.org/10.1523/ENEURO.0426-17.2018>

Digital Object Identifier (DOI):

[10.1523/ENEURO.0426-17.2018](https://doi.org/10.1523/ENEURO.0426-17.2018)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

eNeuro

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Research Article: Confirmation | Disorders of the Nervous System

Circadian and Brain State Modulation of Network Hyperexcitability in Alzheimer's Disease

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DOI: 10.1523/ENEURO.0426-17.2018

Received: 5 December 2017

Revised: 8 March 2018

Accepted: 6 April 2018

Published: 27 April 2018

Author Contributions: RB, RRR and IO designed research; RB, ADL, AG-S, MJ, RC-CC, IJ-C, A-LH, TS and TS performed research; ADL, AY, RC-CC, MT, CYJ, A-LH and IO analysed the data; MAJ, AJC, SSC and KH contributed unpublished analytical tools; RB, ADL, KH and IO wrote the paper.

Funding: <http://doi.org/10.13039/501100000320>Alzheimer's Society: PG-2012-208. RS Macdonald Charitable Trust; Muir Maxwell Epilepsy Centre; Euan MacDonald Centre; Patrick Wild Centre; <http://doi.org/10.13039/100005339>American Academy of Neurology (AAN); <http://doi.org/10.13039/501100000295>Epilepsy Research UK (ERUK); <http://doi.org/10.13039/501100000265>Medical Research Council (MRC): MR/M024075/1.

Conflict of Interest: Authors declare no conflict of interest.

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Cite as: eNeuro 2018; 10.1523/ENEURO.0426-17.2018

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Accepted manuscripts are peer-reviewed but have not been through the copyediting, formatting, or proofreading process.

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1 **1. Manuscript Title:** Circadian and brain state modulation of network
2 hyperexcitability in Alzheimer's disease

3 **2. Abbreviated title:** Hyperexcitability modulation in AD

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37 **6. Number of Figures: 7**

38 **7. Number of Tables: 2**

39 **8. Number of Multimedia: 0**

40 **9. Number of words for Abstract: 250**

41 **10. Number of words for Significance Statement: 111**

42 **11. Number of words for Introduction: 656**

43 **12. Number of words for Discussion: 2069**

44 **13. Acknowledgements:** We thank the Gladstone Institute for providing J20

45 mice. We thank Dominic Walsh for supplying APP^{NL/F} mice.

46 **14. Conflict of interest:** The authors declare no conflict of interest.

47 **15. Funding sources:** This work was supported by the grants awarded to IO

48 from the following funders: the Alzheimer's Society (PG-2012-208), the

49 RS Macdonald Charitable Trust, The Muir Maxwell Epilepsy Centre, The

50 Euan MacDonald Centre and The Patrick Wild Centre. ADL was supported

51 by the American Academy of Neurology Institute. AGS was supported by
52 Epilepsy Research UK. RC-CC was supported by an MRC grant
53 (MR/M024075/1) awarded to RRR.
54

55 **16.**

56

57 **Abstract**

58 Network hyperexcitability is a feature of Alzheimer’s disease (AD) as well as
59 numerous transgenic mouse models of AD. While hyperexcitability in AD
60 patients and AD animal models share certain features, the mechanistic overlap
61 remains to be established. We aimed to identify features of network
62 hyperexcitability in AD models that can be related to epileptiform activity
63 signatures in AD patients. We studied network hyperexcitability in mice
64 expressing amyloid precursor protein (APP) with mutations that cause familial
65 AD, and compared a transgenic model that overexpresses human APP (J20), to a
66 knock-in model expressing APP at physiological levels (APP^{NL/F}). We recorded
67 continuous long-term electrocorticogram activity from mice, and studied
68 modulation by circadian cycle, behavioural, and brain state. We report that while
69 J20s exhibit frequent inter-ictal spikes (IIS), APP^{NL/F} mice do not. In J20 mice, IIS
70 were most prevalent during daylight hours and the circadian modulation was
71 associated with sleep. Further analysis of brain state revealed that IIS in J20s are
72 associated with features of rapid-eye movement (REM) sleep. We found no
73 evidence of cholinergic changes that may contribute to IIS-circadian coupling in
74 J20s. In contrast to J20s, intracranial recordings capturing IIS in AD patients
75 demonstrated frequent IIS in non-REM sleep. The salient differences in sleep-
76 stage coupling of IIS in APP overexpressing mice and AD patients suggests that
77 different mechanisms may underlie network hyperexcitability in mice and
78 humans. We posit that sleep-stage coupling of IIS should be an important
79 consideration in identifying mouse AD models that most closely recapitulate

80 network hyperexcitability in human AD.

81

82 **Significance statement**

83 It is increasingly recognized that Alzheimer's disease (AD) is associated with
84 hyperexcitability in brain networks. Brain network hyperexcitability is also
85 reported in several rodent models of AD. We studied the signatures of this
86 hyperexcitability in two rodent models of AD as well as AD patients. Network
87 hyperexcitability was prevalent in a transgenic model of AD, but was absent in a
88 rodent model that is considered to be more physiological. Moreover, while
89 network hyperexcitability was coupled to rapid-eye movement (REM) sleep in
90 transgenic mice, hyperexcitability occurred in non-REM sleep in AD patients. We
91 suggest that brain state coupling of hyperexcitability can be used as a method for
92 screening animal models of AD.

93

94 **Introduction**

95 An increased incidence of seizures in Alzheimer's disease (AD) is indicative of an
96 underlying network hyperexcitability (Hesdorffer et al., 1996; Amatniek et al.,
97 2006; Lozsadi and Larner, 2006; Vossel et al., 2013; Cretin et al., 2016). Inter-
98 ictal spikes (IIS) are also seen in a high proportion of AD patients without a
99 history of clinical seizures (Vossel et al., 2016). Non-ictal network hyperactivity
100 has also been detected by means of fMRI in individuals at risk of developing
101 dementia, for example in people carrying the APOE4 allele (Bookheimer et al.,
102 2000; Filippini et al., 2009), with other genetic predictors of AD (Quiroz et al.,
103 2010) and also in patients with mild cognitive impairment – a diagnosis which is
104 considered to be a prodromal stage of AD (Dickerson et al., 2005). Network
105 hyperexcitability and seizure activity appear at early stages of the disease and
106 have been suggested to be predictors of accelerated cognitive decline (Amatniek
107 et al., 2006; Vossel et al., 2013; Cretin et al., 2016; Vossel et al., 2016).

108

109 Network hyperexcitability has also been reported in numerous mouse models of
110 AD pathology (Palop et al., 2007; Minkeviciene et al., 2009; Busche et al., 2012;
111 Šišková et al., 2014; Kazim et al., 2017), with the aberrant activity being a feature
112 that occurs in advance of plaque deposition (Busche et al., 2012; Bezzina et al.,
113 2015). These phenomenological similarities have led to the suggestion that these
114 animal models can provide a tool by which to study network hyperexcitability in
115 human AD (Palop and Mucke, 2016).

116

117 Aberrant network activity could in itself contribute to neurodegeneration and
118 cognitive dysfunction in AD pathology (Cirrito et al., 2005; Bero et al., 2011;
119 Busche and Konnerth, 2015; Wu et al., 2016). Reducing network
120 hyperexcitability has been shown to ameliorate cognitive dysfunction in both
121 patients and animal models (Bakker et al., 2012; Sanchez et al., 2012; Haberman
122 et al., 2017), and to attenuate A β pathology (Yuan and Grutzendler, 2016). Hence,
123 targeting network hyperexcitability has been suggested as a novel therapeutic
124 avenue for AD. However, studying this therapeutic avenue by means of animal
125 models (Sanchez et al., 2012) requires a deeper understanding of the shared
126 features of network hyperexcitability between AD patients and animal models.

127

128 Expression of epileptiform activity frequently exhibits a circadian pattern and
129 shows preferential activation with specific brain states in a range of epilepsies
130 (Quigg, 2000; Ng and Pavlova, 2013; Sedigh-Sarvestani et al., 2015). Circadian
131 dysfunction and sleep disruption are common features of AD, and are also
132 considered as early features of disease pathogenesis (Musiek et al., 2015;
133 Mander et al., 2016; Musiek et al., 2018). Two recent papers have reported
134 modulation of epileptiform activity by circadian cycle and brain state in
135 transgenic AD models. Epileptiform activity was more prevalent in daylight
136 conditions, and was suggested to occur primarily during REM sleep (Born et al.,
137 2014; Kam et al., 2016). If epileptiform activity is modulated by circadian-cycles
138 and/or brain state in AD patients, it is possible that this might contribute to the
139 reported circadian alterations and sleep dysfunction. In line with this, it has
140 recently been shown that inter-ictal activity in AD patients is highly prevalent
141 during sleep (Vossel et al., 2016; Horváth et al., 2017; Lam et al., 2017). The

142 modulation of ictal related activity by brain state points to a distinguishing
143 feature that could be used to (a) uncover distinct mechanisms underlying
144 hyperexcitability, and (b) ascertain the translational utility of specific animal
145 models in studying network hyperexcitability. To this end, the present study
146 aimed to investigate circadian and brain state modulation of network
147 hyperexcitability in two rodent models of AD of differing aetiology: one in which
148 hAPP is overexpressed and one in which APP is expressed at endogenous levels.
149 In order to shed light on the translational utility of rodent AD models for
150 studying network hyperexcitability in human AD, we further examined sleep-
151 stage modulation of epileptiform activity in two patients with AD, using
152 recordings from intracranial electrodes placed directly adjacent to the
153 hippocampus.

154

155 **Methods**

156 Animals and animal maintenance

157 All animal procedures were performed in accordance with the [Author
158 University] animal welfare committee regulations and were performed under a
159 UK Home Office project license.

160

161 Heterozygous mice expressing hAPP with the KM670/671NL (Swedish) and
162 V717F (Indiana) mutations on a PDGF β promoter (J20; Mucke et al., 2000) were
163 bred by crossing J20 +/- males with C57Bl6J females. Experiments used J20 +/-
164 (n=21) and J20 -/- (n=8) wild-type (WT) littermate controls. The mean age of J20
165 animals was 5 months (range: 3.3 – 6.5 months).

166

167 Homozygous knock-in mice expressing APP KM670/671NL (Swedish) and APP
168 I716F (Iberian) mutations (APP^{NL/F}; Saito et al., 2014) were back-crossed onto
169 C57Bl6J strain for at least 3 generations and were >99.8% cogenic with C57Bl6J.
170 Experiments used APP^{NL/F} +/+ (n=20) and age-matched non-littermate C57Bl6J
171 WT controls (n=15). Animals were either 8 months or 12 months of age.

172

173 Both male and female mice were used. Mice were kept on a 7h-19h light-dark
174 cycle in standard, open cages. Mice were group-housed prior to surgery and
175 were housed individually post-surgery and during telemetry data acquisition.

176

177 Surgery and data acquisition

178 A subdural intracranial electrocorticogram (ECoG) recording electrode was
179 positioned in the cortex overlying the hippocampus (co-ordinates x: -2.25 y: -
180 2.46). A reference electrode was implanted either in the skull of the contralateral
181 hemisphere, or above the cerebellum. Electrodes were either bare wire, or skull
182 screws. An EEG transmitter (A3028B, Open Source Instruments) was implanted
183 on the back of the animal subcutaneously. Animals were left to recover for at
184 least 24 hours after surgery before the commencement of telemetry data
185 acquisition. Telemetric ECoG data was acquired for approximately 3 days from
186 each animal. Recording was either carried out continuously between days 1 to
187 day 3 after surgery, or day 1, followed by day 5 to day 6.

188

189 ECoG data was acquired using an Opensource Instruments data acquisition
190 system at 512sps as previously described (Chang et al., 2011).

191

192 Video data was acquired using a Basler aca1300-60gm gigE camera sampling at
193 10fps, or a Logitech C270 HD webcam sampling at 5fps. Video was acquired
194 during the daylight hours.

195

196 ECoG Data processing

197 The raw ECoG data was analysed using custom written Tcl and C processors.

198 ECoG data was analysed in 8s intervals. For each interval we extracted measures
199 of data loss, spike count, delta power (0.1-3.9 Hz) and theta power (4-12 Hz). We
200 defined intervals in which data loss exceeded 20% of samples as “lossy” intervals.

201 Intervals in which delta power exceeded 0.16mV^2 were classified as artifacts.

202 Lossy and artifact intervals were excluded.

203

204 Inter-ictal spikes (IIS) in rodent ECoG were detected as follows. Each 8-s interval
205 of EEG was treated as a two-dimensional path. One dimension is voltage, which
206 was normalized by dividing by the mean absolute step size of the voltage in the
207 8-s interval. The mean absolute step size is the sum of the absolute changes in
208 voltage from one sample to the next, divided by the number of samples. For an 8-
209 s interval, the number of samples would be 4096 and a typical mean absolute
210 step size for mouse EEG is around $12\ \mu\text{V}$. The other dimension is time, which was
211 normalized by dividing by the sample period. The spike-finder proceeds along
212 this EEG path in steps. With each step, it moves to the nearest sample on the path
213 ahead. Whenever the spike-finder steps past one or more samples, it classifies
214 these samples as an aberration in the path. Solitary aberrations larger than 20
215 mean absolute step sizes are classified as IIS. A series of IIS in which single

216 spikes were separated by <78ms (40 samples) were treated as a burst event and
217 counted as one IIS event within the 8s interval.

218

219 For each J20 animal, the false positive rate of IIS detection was determined by
220 randomly hopping through 100 8s intervals identified by the processor as
221 containing IIS, and scoring them as true or false positives. The animal was
222 excluded from analysis if the false positive rate exceeded 10%. One animal was
223 excluded from analysis on this basis. In the remaining animals, the false positive
224 rate ranged from 0 – 6% (mean false positive rate: 1.9%).

225

226 We observed that lossy and artifactual intervals resulted from movement and
227 external sources of interference. We could not exclude the possibility that these
228 events are non-randomly distributed across the 24hr cycle. Non-random
229 exclusion of intervals would impact the evaluation of coupling of IIS. We thus set
230 a criterion: if >5% of all 8s intervals were excluded due loss or artifact, the
231 animal was excluded from calculations of coupling of IIS to circadian cycles,
232 sleep-wake, and θ/δ . Two J20 animals were excluded from data reported in Fig 2-
233 4 on this basis (25% and 16% of 8s intervals excluded in these animals).

234

235 Video analysis

236 Video data was manually scored to classify periods as ‘sleep’ or ‘wake’. Based on
237 previous reports, sustained inactivity ≥ 40 s was classified as ‘sleep’, while
238 stationary periods <40s and periods of movement were classified as ‘wake’ (Pack
239 et al., 2007). Postural shifts during sleep epochs did not break sleep epochs.

240

241 Immunohistochemistry and imaging

242 Animals were killed by transcardial perfusion with N-methyl-D-glucamine
243 (NMDG)-based saline solution (in mM: 92 NMDG, 2.5 KCl, 1.25 NaH₂PO₄, 20
244 HEPES, 30 NaHCO₃, 25 glucose, 10 MgCl₂, 0.5 CaCl₂, sucrose to adjust osmolarity
245 to 315-330mOsm). Brains were post-fixed with 4% paraformaldehyde for 24h
246 then washed and stored in PBS. Samples were put in 50% or 30% sucrose, PBS
247 solution and 50% OCT solution for 24h before cutting, then placed in the same
248 solution and cut using a freezing microtome.

249

250 50µm sections were stored in PBS at 4°C. Slices were presoaked with 5% rabbit
251 normal serum (RNS, Vector S-5000), 0.2% Triton X-100, PBS solution for 30min
252 at room temperature (RT), followed by incubation with 3% RNS, 0.2% Triton X-
253 100, anti-Choline Acetyltransferase (ChAT; 1:500, Millipore #AB144P, RRID:
254 AB_2079751), PBS solution for 48h at 4°C. The sections were washed 3 times
255 with PBS 0.2% Triton X-100 for 5min each and then incubated in 3% RNS, anti-
256 Goat biotinylated (1:200), Dapi (1:5000, Sigma D9542-1MG), PBS solution for 1h
257 at RT. After 3 PBS 0.2% Triton X-100 washings of 5min each, the sections were
258 incubated with ABC reagent (Vectastain PK-6105 kit) prepared half an hour
259 before using and stored in foil at 4°C containing 0.1% of A, 0.1% of B, 0.01%
260 Triton X-100, PBS for 1h at RT. After 6 PBS washings of 10min each, the sections
261 were put in 3 3'-diaminobenzidine (Sigma D5905-50TAB), 0.02% CoCl₂ (1%
262 wt/vol), 0.04% (NH₄)₂Ni(SO₄)₂ (1% wt/vol) dH₂O solution for 30min at 4°C over
263 agitation. Then stained by adding 1.2% of fresh 1% H₂O₂ per slice for 10 to 20s
264 until the slice darkened. The slices were then transferred and washed in PBS, 6
265 times for 10min each, mounted on a slide and dried for 30min at 50°C then

266 finally covered with Mowiol Embedding Medium and coverslips. Slides were
267 stored at RT.

268

269 Imaging was performed on a Zeiss AX10 microscope using StereoInvestigator
270 Software with a 5x/0.16 (420630-9900) apochromat air objective.
271 Quantification was performed using StereoInvestigator Software “Optical
272 Fractioner Workflow” probe with the following settings. Thickness of 50 μ m was
273 manually defined and regions were selected using a 1.25x/0.03 (420310-9900)
274 apochromat air objective for low magnification and then counted with a
275 10x/0.45 (420640-9900) apochromat air objective for high magnification. The
276 border between medial septum (MS) and diagonal band of Broca (DB) was
277 defined as a line between the two major island of Caleja. The regions were
278 separated using different lines. The counting frame used was a square of 75 μ m
279 size and the grid was a square of 150 μ m size. The counter was blind to genotype.

280

281 Oral administration of Donepezil

282 Donepezil hydrochloride (Sigma Aldrich, D6821) was orally administered in a
283 jelly. Mice were trained to voluntarily consume jelly following the protocol
284 described by Zhang (2011). Mice were given placebo jelly or a jelly containing a
285 Donepezil dose of 1.8mg/kg. For experiments studying the effects of Donepezil
286 on acetylcholinesterase (AChE) activity, jelly was given at 8am daily. For
287 experiments studying the effects of Donepezil on IIS, jelly was given daily at
288 either 8am, or 8pm to assess interactions of AChE modulation and circadian
289 cycle. Since there was no effect of AChE on IIS, results were pooled.

290

291 Acetylcholinesterase (AChE) assay

292 Quantitative measurements of AChE enzymic activity were made using a
293 modified Ellman method (Ellman et al., 1961; Rosenfeld et al., 2001). Stock
294 solutions were acetylthiocholine iodide, used as the enzymic substrate (ATH; 1.7
295 mg/ml in PBS, Sigma-Aldrich), 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, 0.8
296 mg/ml in PBS, Sigma-Aldrich). Briefly, brains were rapidly dissected from either
297 WT or J20 mice. Neocortex was isolated, weighed and then homogenised using a
298 Pellet Pestle (Sigma, Z 359971) in 9 volumes of 0.1M sodium phosphate buffer
299 (pH 7.4) (Patel et al., 2014). 5 μ l of brain homogenate was aliquoted into each
300 well of a 96 -well plate, volume made up to 200 μ l with PBS. DTNB (50 μ l from
301 stock) was added, followed by 50 μ l of ATH substrate from stock. Measurement
302 of absorption at 450 nm began immediately (<2 hours from dissection) and was
303 measured every 5 mins for up to 30 mins using a MRX microplate reader (Dynex
304 Technologies, Chantilly, USA). Thiocholine production in the test wells was
305 expressed in units of nmoles/minute, calibrated with reference to the
306 absorbance change over a range of concentrations giving a linear response using
307 glutathione as the DTNB reactant (Eyer et al., 2003). Neostigmine (10 μ M,
308 Sigma-Aldrich) was used to completely inhibit AChE activity and establish there
309 was no baseline drift during the measurements.

310

311 Human scalp EEG and foramen ovale (FO) electrode recordings

312 Human scalp EEG and FO electrode recordings were performed at the
313 Massachusetts General Hospital, as described in detail previously (Lam et al.,
314 2017). Scalp EEG electrodes were placed using the International 10-20 system,
315 with additional T1 and T2 electrodes.

316

317 Sleep staging in patient data was performed by a board-certified clinical
318 neurophysiologist (ADL) based on visual analysis of the full scalp EEG data.
319 While dedicated electrooculogram and electromyogram channels were not
320 recorded for these studies, the frontopolar scalp EEG electrodes allowed
321 assessment of eye movements, while the frontopolar, frontal, and temporal
322 electrodes allowed assessment of myogenic activity. Scalp EEG data was
323 reviewed in 30 second epochs in the longitudinal anterior-posterior bipolar
324 montage, using the Python module *wonambi* ([https://github.com/wonambi-](https://github.com/wonambi-python/wonambi)
325 [python/wonambi](https://github.com/wonambi-python/wonambi)). Each 30 second epoch was classified as awake, NREM1,
326 NREM2, NREM3, or REM, based on the American Academy for Sleep Medicine's
327 manual for sleep scoring.

328

329 Spike quantification in patient data was performed by a board-certified clinical
330 neurophysiologist (ADL), using a custom-made GUI in Matlab (Mathworks,
331 Natick, MA). The GUI displayed 15 second epochs of left and right sided FO data,
332 in both bipolar and common reference montages (common reference = C2),
333 along with the EKG trace to allow exclusion of EKG artifact. The reviewer could
334 adjust amplitudes for each trace as needed. For the MCI patient analyzed,
335 contact #3 from the left FO electrode did not record properly and was excluded
336 from analysis. The reviewer marked all spikes in each epoch. Epochs were
337 presented in consecutive order, but the reviewer was otherwise blinded to the
338 sleep stage for each epoch during the review. Instantaneous spike rates were
339 calculated by determining the total number of left FO and right FO spikes
340 detected within all 30 second epochs of the recording (which corresponded to

341 the sleep staging epochs above), and converting these rates to spikes per hour.
342 Average spike rates within each sleep stage were calculated by summing the
343 total number of spikes that occurred during each sleep stage and dividing by the
344 total number of hours the patient spent in each respective sleep stage in the
345 recording.

346

347 Spectral analysis of the FO electrodes was performed in Matlab, using the freely
348 available Chronux toolbox (Mitra and Bokil, 2007). Analysis was performed on
349 the LFO1, LFO2, RFO1, and RFO2 channels, as these were the deepest contacts
350 and thus least prone to noise or artifact. Channels were each normalized to zero-
351 mean, unit-variance. Multi-taper spectrograms were calculated for each
352 normalized channel, using the Chronux script *mtspecgramc* with the following
353 parameters: frequency range: 1-20Hz, window: 30 seconds; step size: 30
354 seconds; time-bandwidth product: 3, tapers: 5. This provided a spectral
355 resolution of 0.2Hz. An average spectrogram across all FO channels was then
356 generated, and the average spectral powers within the δ band (0-4Hz) and θ
357 band (4-12Hz) were then calculated.

358

359 Statistics

360 Statistical data analysis was performed using R (version 3.2.0) including the
361 'dplyr' (Wickham et al., n.d.) and ggplot2 (Wickham, 2009) packages.

362

363 Assumptions for parametric tests were tested using Q-Q plots and residual plots.

364 Data transformations or non-parametric tests were used for two-group

365 comparisons in which test assumptions were violated.

366

367 For evaluating the effects of the fixed effects of age and genotype on the
368 proportion of intervals containing more than one spike in APP^{NL/F} animals, the
369 data first underwent a square-root transformation and then fit using a linear
370 model:

$$\sqrt{IntervalProportion} \sim Age + Genotype + \xi$$

371

372 where ξ is the error term.

373

374 The time of IIS was treated as circular variable. Each interval in which 1 or more
375 IIS were detected was considered an event. The time of each event was evaluated
376 as a phase of a circadian cycle. Circular data was analysed using circular statistics
377 by means of the ‘circular’ package (Agostinelli and Lund, 2013). Circular outliers
378 were identified using ‘CircOutlier’ package (Rambli et al., 2016).

379

380 For tests entailing random variables, linear models were fit using ‘lme4’ (Bates et
381 al., 2015). Significance was tested using a log-likelihood test comparing the full
382 model to a null model without the factor of interest.

383

384 For evaluation of the relationship between spike count and θ/δ , we described
385 each θ/δ value as a member of one of three levels: i) $\theta/\delta \leq 1$; ii) $1 < \theta/\delta \leq 2$, and iii)
386 $\theta/\delta > 2$. We then modelled spike count (Poisson-distributed) as a function of
387 levels of θ/δ , using the R package ‘MCMCglmm’ (Hadfield, 2010). It should be
388 noted that due to poor properties of a single model fitted across all animals

389 (fitting animal as a random effect and θ/δ factor as a fixed effect), separate
390 models were fitted to individual animals without including a random effect. Thus
391 the data do not allow for inference about the population.

392

393 Event-triggered averages of IIS were evaluated by considering each interval in
394 which an IIS was detected as an event. If no intervals within ± 80 s around the
395 event were excluded, then the 160s window was included in the calculation of
396 the event-triggered averages, else the event was excluded from the averaging. An
397 event-triggered average was also evaluated around 2000 randomly sampled
398 points.

399

400 For comparing θ/δ in intervals with IIS to θ/δ in intervals preceding IIS, we
401 considered only interval pairs where the preceding interval did not contain IIS
402 and fit the model

$$\left(\frac{\theta}{\delta}\right)^{1/4} \sim \text{Index} + \text{Subject} + \xi$$

403 where Index was a factor labelling whether the interval contained IIS or the
404 preceding interval and modelled as a fixed effect, and Subject was a random
405 effect with a random intercept.

406

407 For comparison of ChAT+ cells between genotypes, the model used was:

$$\text{EstimatedCount} \sim \text{Genotype} + \text{Region} + \text{Subject} + \xi$$

408

409 where genotype and region were fixed effects and subject was a random effect
410 with a random intercept.

411

412 To study the effect of genotype and treatment of the Thiocholine production rate,
413 the data of Thiocholine production was log-transformed. The model used was

$$\log(\text{ThiocholineRate}) \sim \text{GenotypeTreatment} + \text{RepeatID} + \xi$$

414

415 where GenotypeTreatment was a fixed effect and RepeatID was a random effect
416 with a random intercept. Post-hoc tests for the linear model were performed
417 using package 'multcomp' with the Holm correction method (Hothorn et al.,
418 2008). It should be noted that while the treatment levels of control and
419 donepezil were independent, the neostigmine treatment was applied to a sample
420 of wild-type control tissue and thus was not independent. This repeated factor
421 was not accounted for in the model.

422

423 Significance was tested using $\alpha=0.05$. Two-sided hypothesis testing was used.

424

425 Superscripts following statistical reporting in the results section refer to the
426 statistical table (Table 1).

427

428 Code and data accessibility

429 The processor script used for quantification of IIS, θ and δ power in rodent ECoG
430 data is available from

431 <http://www.opensourceinstruments.com/Electronics/A3018/HTML/SCPP4V1.t>

432 cl

433 Code used for quantifying IIS in human data is available from

434 https://github.com/mauriceaj/GUI-EEG_Spike_Annotation

435 The datasets used for figures 1 – 6 (rodent data) are available from
436 <http://dx.doi.org/10.7488/ds/2319>

437

438 **Results**

439 Network hyperexcitability in mouse models of AD pathology

440 To establish circadian patterns of network hyperexcitability in J20 mice, we
441 recorded ECoG activity from freely-moving J20 and littermate wild-type (WT)
442 mice using wireless telemetry over a period of three days. As network
443 excitability has been suggested to be an early event in AD pathogenesis (Vossel
444 et al., 2013; Sarkis et al., 2015), we focused our study on ages which precede
445 overt plaque pathology in J20s (Mucke et al., 2000).

446

447 As previously reported (Palop et al., 2007), non-seizure, inter-ictal spikes (IIS;
448 Fig 1A) were detected in J20 ECoG (note that while ictal activity was not assessed,
449 we refer to these as inter-ictal events due to the similarity with IIS that have
450 been reported in the literature) . We applied automated event detection (see
451 methods), on 8s intervals of continuous ECoG. The percentage of intervals in
452 which 1 or more spikes were detected was negligible in WTs (mean percentage:
453 0.8%, sd=0.7%, n=8). In contrast, the percentage of intervals with 1 or more
454 spikes was greater in J20s (mean percentage: 11.6%, sd=5.1%, n=18; t=10.6,
455 df=23.98, p<0.0001, t-test on square root transformed data with Welch
456 correction, Figure 1B,C)^a.

457

458 Seizures and IIS have been reported in numerous strains of transgenic mice that
459 express hAPP and that exhibit A β pathology (Del Vecchio et al., 2004; Palop et al.,

460 2007; Minkeviciene et al., 2009; Rasch and Born, 2013). However, it has been
461 suggested that such network hyperexcitability is the result of overexpression of
462 hAPP (Born et al., 2014). To determine whether network hyperexcitability is
463 associated with A β pathology in the absence of hAPP overexpression, we
464 performed telemetric ECoG recordings as above, in mice expressing the
465 humanized A β sequence of APP (APP^{NL/F}; Saito et al., 2014) and age-matched
466 controls. We recorded from mice at ages preceding overt plaque pathology (8
467 months) and at ages where plaques begin to appear (12 months)(Saito et al.,
468 2014; Masuda et al., 2016). We found no significant effect of genotype in the
469 proportion of intervals containing spikes between WT and APP^{NL/F} (Fig 1D; $F(2,$
470 $32)=3.1, R^2=0.11, p=0.06$)^b with a negligible proportion of intervals with one or
471 more spikes detected (mean percentage of intervals with one or more spikes,
472 pooled across genotype and age = 1.2%, 95%CI (0.8%, 1.6%)). A post-hoc power
473 calculation based on the effect size from the J20 group (Cohen's $d=2.5$) and the
474 sample sizes of the APP^{NL/F} and WT groups yielded a power of >0.99 at $\alpha=0.05$
475 for an effect of genotype. Hence, we conclude that APP^{NL/F} mice show no
476 evidence of network hyperexcitability compared to control animals.

477

478 Circadian coupling of IIS

479 It has been suggested that seizure-related activity shows circadian fluctuations
480 in epilepsies (Quigg, 2000). Hence we next asked whether the likelihood of IIS in
481 J20s varies across the day/night cycle. Quantifying the number of IIS per hour
482 revealed that IIS are more frequent during daylight hours (inactive phase; Fig
483 2A). We used circular statistics to extract measures of the phase coupling of IIS
484 to the circadian cycle within individual J20 animals (Fig 2A, see methods). To

485 evaluate the degree of phase coupling of IIS in each animal, we evaluated the
486 mean angular vector length (ρ) from the time of IIS. ρ can vary between 0 (no
487 phase coupling) to 1 (perfect phase coupling). To evaluate the time to which IIS
488 were coupled, we extracted the mean coupling phase off IIS, expressed as a time
489 on a 24-hour cycle (φ_{IIS}).

490

491 The distribution of IIS phases differed significantly from a random distribution in
492 all animals (Rayleigh Test of Uniformity: $p < 10^{-11}$). The extent of phase coupling
493 was variable across the sample of J20s (Fig 2B; mean $\rho_{IIS} = 0.24$, $sd = 0.13$, $n = 16$).

494

495 Evaluating the coupling phase revealed that IIS occurred predominantly in the
496 light condition (Fig 2A). Across the sample of J20s, the mean φ_{IIS} ($\overline{\varphi_{IIS}}$)

497 confirmed this (Fig 2B; $\overline{\varphi_{IIS}} = 15h05$, $\rho = 0.38$, $n = 16$, $p < 0.0001$, Rayleigh's test).

498 Inspection of the φ_{IIS} distribution revealed potential outliers. Testing for outliers
499 on a circular distribution (Rambli et al., 2016) identified four outliers. These four
500 animals were amongst the 5 that showed a cluster of weakest phase coupling as
501 measured by ρ_{IIS} (range: 0.06 – 0.11). We used the upper bound of the range of
502 ρ_{IIS} of the four outlier animals to classify phase-coupling as weak or strong.

503 Henceforth, we refer to the five animals with $\rho_{IIS} \leq 0.11$ as showing weak phase-
504 coupling, and the other 11 animals as showing strong phase-coupling ($\rho_{IIS} > 0.17$).

505

506 Sleep/wake modulation of IIS

507 Since IIS predominantly occurred in the normal inactive phase of the circadian

508 cycle, we next asked whether this circadian modulation of IIS could be accounted

509 for by the sleep/wake state of the animals. In a subset of J20s, we acquired
510 simultaneous video recordings while recording ECoG data (n=4). We manually
511 scored the video and classified periods as 'sleep' or 'wake' (see Methods). Two of
512 these four J20 animals showed strong circadian phase-coupling of IIS, and two
513 showed weak phase-coupling. For the two animals that showed strong phase-
514 coupling of IIS, IIS occurred more frequently in sleep than during waking (Fig 3A,
515 B). In contrast, the modulation of IIS probability did not show a consistent
516 pattern in animals showing weak phase-coupling (Fig 3B). This suggests that the
517 strong phase-coupling of IIS may be accounted for by differences in behavioural
518 state across the circadian cycle.

519

520 Brain state modulation of IIS in J20 mice

521 Sleep-related ictal and inter-ictal activity is differentially modulated by REM and
522 NREM sleep in different forms of epilepsy (Bazil and Walczak, 1997; Herman et
523 al., 2001; Sedigh-Sarvestani et al., 2014; Ewell et al., 2015). REM and NREM can
524 be distinguished by the relative power in the δ (defined here as 0.1 – 3.9 Hz) and
525 θ (4- 12 Hz) frequency bands, with high θ/δ associated with REM (Ewell et al.,
526 2015) as well as waking exploration (Buzsáki, 2002). Thus, we next asked
527 whether IIS are more likely to occur in particular brain states. To this end, we
528 performed spectral analysis of the ECoG data from a subset of the mice (n=5
529 J20s) in which a reference electrode was implanted at cerebellar coordinates (a
530 non-cortical reference for detection of cortical rhythms). ECoG recordings from
531 J20 mice, exhibited periods showing a peak in θ -band power when animals were
532 either awake (i.e. moving) or asleep, while periods of elevated δ -band power
533 were seen during sleep (Fig 4A). We evaluated the θ/δ ratio for each 8s interval

534 and related it to the number of IIS in the interval. Transient increases in θ/δ were
535 observed during sleep and were associated with increased occurrences of IIS
536 (Fig 4B).

537

538 To quantify whether IIS were more likely in particular brain states, we next
539 investigated the relationship between θ/δ and IIS count/8s interval. As we were
540 interested in discriminating between REM and NREM sleep, we limited the
541 analysis to daylight hours when animals are more likely to be asleep. We used a
542 value of $\theta/\delta < 1$ and > 2 to classify periods as NREM-like and REM-like respectively
543 (Ewell et al., 2015). This revealed significantly higher spike counts during REM-
544 like vs NREM-like periods in all 5 animals (Fig 4B, $p < 0.0005$ for all 5 animals,
545 Markov Chain Monte Carlo generalised linear model)^c. Interestingly, IIS were
546 associated with increased θ/δ in animals showing both weak and strong phase-
547 coupling (Fig 4C). Since sleep and wake are not predictive of IIS in animals with
548 weak phase-coupling, this suggests that there is a mismatch between θ/δ and
549 behavioural state in animals with weak phase-coupling. Moreover, high
550 θ/δ states are predictive of IIS, regardless of behavioural state.

551

552 To examine the temporal dynamics of θ/δ around IIS, we evaluated the IIS-
553 triggered average of θ/δ (Sedigh-Sarvestani et al., 2014) for 160s window around
554 each interval in which at least one IIS was identified. In all animals, θ/δ was
555 increased around the time of IIS relative to θ/δ averaged around randomly
556 sampled points (Fig 4D). In three strongly phase-coupled animals, θ/δ returned
557 to baseline levels within the 160s window around the event. However, in the

558 weakly phase coupled animals, θ/δ remained elevated above baseline levels in
559 this window. The peak in the θ/δ IIS-triggered average did not occur at $t=0$ in any
560 of the animals. Since intervals neighbouring the IIS-containing interval show
561 increased θ/δ , this suggests that the IIS contribution to spectral power did not
562 underlie the association between increases in θ/δ and IIS probability. To further
563 examine whether IIS could directly contribute to the increased θ/δ , we compared
564 θ/δ in intervals with IIS to θ/δ in the preceding intervals only in cases where the
565 preceding interval contained no IIS. We found no significant difference in
566 θ/δ between intervals with IIS and the preceding interval (Linear mixed model,
567 $\chi^2(1) = 0.35, p=0.56$, data not shown)^d.

568

569 To determine whether the spectral ECoG patterns in J20 mice are a reflection of
570 normal sleep or a result of pathology, we performed similar analysis of video-
571 scored ECoG data from 3 wild-type mice. As in the J20, intervals of strong
572 θ power were evident during wake and sleep, while periods of prominent
573 δ -band activity were seen in sleep. Transient increases in θ/δ during sleep akin
574 to those seen in J20s were also observed in all WT animals, suggesting that such
575 increases are a feature of normal sleep, and not pathological (Fig 5). To compare
576 the distribution of θ/δ during sleep between genotypes, we calculated the range
577 and 90th percentile of θ/δ while animals were asleep (using data for which we
578 had video-scoring). Group sizes were too small for statistical comparison but
579 suggested that θ/δ values spanned a narrower range in J20 mice than in WT mice
580 (J20 mean range=(0.02, 10.0), 90th percentile=2.4, SD(1.1), n=4; WT mean
581 range=(0.04, 19.3), 90th percentile =5.4, SD(1.4), n=3; data not shown).

582

583 No evidence of cholinergic changes in J20 mice

584 Cholinergic levels exhibit a circadian modulation (Hut and Van der Zee, 2011),

585 and high cholinergic tone is implicated in generating θ oscillatory states (Buzsáki,

586 2002). In addition, cholinergic dysfunction has been suggested to be a key

587 feature of AD pathogenesis (Craig et al., 2011). Recently, it has been suggested

588 that cholinergic alterations may contribute to network excitability in the Tg2576

589 model of AD (Kam et al., 2016). Hence, we hypothesized that cholinergic changes

590 might underlie the brain-state dependent modulation of IIS in the J20 mice. We

591 used immunohistochemistry to quantify the number of ChAT+ cells in the MS

592 and DB, and asked whether the number of ChAT+ cells differs between J20 (n=7)

593 and WT (n=5) mice. Fitting a linear mixed model to the data, we found no effect

594 of genotype on the estimated number of ChAT+ cells in the MS or DB (Fig 6A;

595 Linear mixed model, $\chi^2(1)=0.0002$, $p=0.99$)^e.

596

597 AChE activity is reduced in AD (García-Ayllón et al., 2011). We assayed

598 cholinergic function by measuring AChE activity. AChE activity was quantified by

599 estimating the rate of thiocholine production in neocortical brain homogenates

600 (see methods). There was no significant difference in the rate of thiocholine

601 production in brain homogenates prepared from WT and J20 mice (V=15, $p=0.06$,

602 $n=5$ WT/J20, Wilcoxon signed rank test, matched by day of assay, Fig 6B)^f. We

603 also wanted to directly test the effect of modulation of ACh levels on IIS.

604 However, using oral administration of Donepezil at a dose previously suggested

605 to achieve clinically relevant drug plasma levels (Dong et al., 2009) was

606 ineffective at altering AChE activity in brain homogenates. In contrast, a positive

607 control treatment of direct application of neostigmine to brain homogenate led
608 to a significant reduction in AChE activity (Fig 6B; Linear mixed model:
609 $\chi^2(4)=75.3$, $p<0.0001$. Post-hoc using Tukey paired comparisons: $p<0.0001$ for
610 neostigmine vs. each of the treatment and genotypes. $p>0.05$ for all other group
611 comparisons)^g. Two days of oral Donepezil administration at this dose did not
612 affect the IIS rate in J20 mice ($t(11)=0.8$, $p=0.43$, paired t-test, data not shown)^h.
613

614 Sleep stage modulation of IIS in human AD

615 The first intracranial recordings in humans with AD were recently reported and
616 demonstrated marked activation of mesial temporal lobe (mTL) IIS during sleep
617 compared to the awake state (Lam et al., 2017). We further analyzed the
618 combined scalp EEG and intracranial electrode recordings from these two
619 patients to better understand the relationship between sleep stage and mTL IIS
620 rate in AD patients. One patient with advanced AD did not achieve REM sleep but
621 showed mTL IIS preferentially during NREM sleep as opposed to waking states
622 (Table 2, Patient 1). The second patient was a 67-year-old woman with amnesic
623 mild cognitive impairment (aMCI), an early stage of AD that is thought to
624 correspond to the early stage of AD modelled in our young J20 mice. The data
625 from this patient was used to compare the frequency of IIS in wake, NREM and
626 REM states.

627

628 We analyzed 14.25 consecutive hours of combined scalp EEG and FO recordings
629 from the aMCI patient, which spanned from ~ 7PM on the first day of FO
630 recording (FOD1) to 9:15AM the following morning (FOD2). Further recordings
631 were not analyzed, as the patient was initiated on treatment with the

632 anticonvulsant levetiracetam on the afternoon on FOD2. Of note, the patient
633 underwent implantation with FO electrodes on FOD1 from ~ 12:40PM – 1:50 PM
634 and received sevoflurane, propofol and midazolam during the procedure. She
635 was awake and answering questions appropriately by 2:15PM on FOD1.

636

637 We performed sleep staging of the recording using the full scalp EEG data, and
638 measured mTL spike rates using the bilateral FO electrode data (Figure 7A,B).
639 As described previously, we found that mTL spiking in the aMCI patient was
640 largely activated during sleep. In contrast to what we found in the young J20
641 mice, mTL spiking in the aMCI patient occurred with highest frequency during
642 NREM sleep stages, particularly during NREM3, and were lowest during REM
643 sleep (Figure 7 and Table 2). mTL IIS rates during REM sleep were also
644 markedly lower than during wakefulness (Table 2). We also calculated spectral
645 power in the θ and δ bands, as well as the θ/δ ratio, in the FO electrodes across
646 sleep states (Figure 7C-E). Increases in both θ and δ power were seen with
647 deepening stages of NREM sleep, while a reduction was seen with REM sleep. In
648 contrast to what we observed in the J20 mice, the θ/δ ratio was reduced during
649 periods of highest spike frequency (Figure 7E).

650

651

652 **Discussion**

653 Network hyperexcitability is a feature of AD. Here we compared patterns of
654 network hyperexcitability in two rodent models of AD, as well as in two AD
655 patients, in order to reveal shared phenomenological features with the disease,
656 We show that while J20, (hAPP overexpressing) mice exhibit frequent IIS as

657 previously reported, APP^{NL/F} mice (that express APP at physiological levels) do
658 not show evidence of network hyperexcitability. Moreover, IIS in J20s occur
659 primarily during daylight hours, and this circadian fluctuation is accounted for
660 by an increased probability of IIS during sleep. Interestingly, we found that IIS in
661 J20 mice are modulated by brain state, with increased likelihood of IIS in brain
662 states with high θ/δ activity, a marker of REM sleep. In contrast, patients with AD
663 showed prevalent IIS during NREM sleep. Moreover, in the one AD patient who
664 exhibited REM sleep, IIS frequency was lowest in REM compared to other sleep
665 states.

666

667 Circadian dysfunction and network hyperexcitability in AD

668 Brain network hyperexcitability in the form of IIS and seizures has now been
669 reported in numerous models of AD pathology (reviewed in Scharfman, 2012;
670 Born, 2015). Our data, along with those reported by others (Born et al., 2014;
671 Kam et al., 2016) reveal that network hyperexcitability in animals models of AD
672 can be modulated by the circadian cycle. Circadian disturbances in AD include
673 sleep fragmentation, increased daytime somnolence, and sundowning, the
674 phenomenon in which neuropsychiatric symptoms are heightened late in the day
675 (Peter-Derex et al., 2015). Animal models of AD have also been reported to show
676 disturbances in the circadian cycle, some of which overlap with patterns of
677 circadian alterations seen in patients (Huitrón-Reséndiz et al., 2002; Vloeberghs
678 et al., 2004; Wisor et al., 2005; Jyoti et al., 2010; Sterniczuk et al., 2010; Duncan et
679 al., 2012; Roh et al., 2012). Our findings of circadian modulation of network
680 hyperexcitability in AD raise the question of whether IIS might causally
681 contribute to the alterations in circadian-coupled behaviour observed in AD.

682 Future work investigating the effects of anti-epileptic drugs on circadian
683 alterations in AD would go towards answering this.

684

685 Brain state modulation of network excitability

686 Here we report that IIS in J20 animals are modulated by θ/δ , with higher IIS rates
687 seen in states of high θ/δ during sleep. The spectral patterns of ECoG that we
688 report here are in line with previous reports in WT mice, that have shown
689 increases in cortical EEG θ power in REM sleep relative to wake and NREM
690 (Brankačk et al., 2010). We also report transient increases in θ/δ in sleep in both
691 WT and J20 mice. Since these increases in θ/δ occur in both WT and J20s, they
692 are likely to be indicative of REM sleep periods (Ewell et al., 2015). Given that
693 J20 animals with strong circadian phase-coupling show highest IIS rates during
694 sleep this suggests that IIS in these animals are associated with REM sleep.

695 An alternative explanation for the association between IIS and high θ/δ during
696 sleep may be that IIS occur during ectopic θ in sleep, in the absence of a
697 concomitant drop in muscle tonus. A phenomenon of ictal activity during ectopic
698 θ has been reported in a mouse model of Huntington's disease (Pignatelli et al.,
699 2012). Without simultaneous EMG recordings, the present data cannot
700 conclusively distinguish between REM states and ectopic θ . In the human data,
701 analysis of θ/δ ratios showed that these were lowest during periods of highest IIS
702 frequency. This argues against the idea of IIS coupled to ectopic θ in humans,
703 though a more definitive assessment will require data from more AD subjects as
704 well as healthy elderly controls.

705 Our finding of an association between IIS and high θ/δ is in line with recent
706 reports that young Tg2576 model of AD as well as mice overexpressing WT-
707 hAPP also demonstrate IIS predominantly during states of high θ which the
708 authors suggest is indicative of REM sleep (Kam et al., 2016).

709 The findings that IIS in multiple mouse models of AD are most likely to occur in
710 REM-like states begs the question of what makes REM a pro-ictal state in these
711 models. Both REM sleep and the awake state share common features of high
712 θ/δ activity and high cholinergic tone (Vazquez and Baghdoyan, 2001; Lee et al.,
713 2005), yet IIS occur much less frequently in the awake state in these models.
714 There are several potential explanations for this. Firing rates of hippocampal
715 neurons increase during REM (Grosmark et al., 2012), which might contribute to
716 the propensity to seize. In addition, systems that normally show distinct activity
717 in REM sleep vs. waking and NREM sleep might contribute to the pro-ictal REM
718 state in these models (Sedigh-Sarvestani et al., 2014; Ewell et al., 2015; Kam et al.,
719 2016). Unlike cholinergic neurons, which increase their activity in both REM and
720 waking, monoaminergic neurons in brainstem nuclei (including the locus
721 coeruleus and the tuberomammillary nucleus) as well as the dorsal raphe
722 nucleus of the hypothalamus, show differential activity between these brain
723 states. These neurons are highly active in waking, exhibit low firing rates in
724 NREM sleep, and are quiescent during REM sleep (Lee and Dan, 2012). It may be
725 that brain state modulation of one or more of these systems is disrupted in these
726 mouse AD models, and other forms of epilepsy which show REM-coupling
727 (Sedigh-Sarvestani et al., 2014; Ewell et al., 2015).

728 The present study quantified cholinergic neurons in MS and DB. Cholinergic

729 neurons in laterodorsal tegmental and pedunculopontine tegmental nuclei of the
730 pontomesencephalic tegmentum have been suggested to control REM onset (Van
731 Dort et al., 2015). In the rat, these neurons have been shown to be active during
732 both wake and REM, however, firing rates are higher in REM, and correlate with
733 θ/δ (Boucetta et al., 2014). Thus, changes to these neurons are also potential
734 candidates for mediating the pro-ictal nature of REM sleep in J20 mice.

735 Kam et al.,(2016) reported that MS-DB cholinergic neuron number was
736 unchanged in young Tg2576 mice. However, they found evidence to support the
737 notion that overactivity of cholinergic neurons might contribute to IIS by
738 showing that antagonism of muscarinic receptors reduced IIS in these animals.
739 Hence they concluded that IIS during REM might be the result of cholinergic
740 hyperfunction. We did not find evidence for cholinergic changes in J20 mice as
741 quantified by the number of cholinergic neurons in MS-DB, or AChE activity. If
742 cholinergic activity is indeed unaltered in J20 mice, future experiments using
743 muscarinic antagonism in J20 mice could be used to investigate whether
744 atropine can act to reduce IIS by reducing overall neuronal excitability, rather
745 than by reversing cholinergic hyperfunction.

746 Our assay of cholinergic function was based on measurements of AChE enzymic
747 activity in brain homogenate. There was no significant difference between AChE
748 levels in WT and J20, or with donepezil treatment. While it is possible that post-
749 mortem degradation of AChE could have masked differences in AChE levels, the
750 robust effect of neostigmine supports the conclusion that the tissue contained
751 functional AChEs.

752 In a subset of our animals, IIS were weakly-coupled to the circadian cycle and the
753 sleep-wake pattern, but were still modulated by θ/δ . This suggests that the
754 relationship between θ/δ and behavioural state might be disturbed in these
755 animals. It is possible that these animals also exhibited greater disturbances in
756 other elements of the circadian-cycle, such as a circadian decoupling of sleep
757 quantity/quality.

758 During both REM and NREM, hippocampal neurons have been shown to replay
759 firing patterns that were experienced prior to sleep (Skaggs and McNaughton,
760 1996; Louie and Wilson, 2001), and such precisely timed sequences are likely to
761 be involved in the memory facilitation role of sleep. IIS are thought to arise from
762 depolarization and synchronous firing of neurons. This firing is followed by an
763 inhibition and reduction of firing (Holmes and Lenck-Santini, 2006). Thus, IIS
764 during sleep are likely to interfere with the coordinated replay of firing
765 sequences, and consequently, would be expected to contribute to memory
766 impairments. In support of this, it has recently been shown that reducing IIS by
767 treatment with anti-epileptic drugs, rescues memory deficits in J20s (Sanchez et
768 al., 2012).

769 Relationship between IIS and AD pathology in mouse models

770 Here we report that while IIS are prevalent in hAPP overexpressing mice,
771 APP^{NL/F} mice that exhibit A β pathology without APP overexpression, do not
772 exhibit IIS at two ages preceding widespread plaque deposition (8 and 12
773 months). This finding is in line with other reports that it is overexpression of
774 hAPP that is causal in generating network hyperexcitability in these animal

775 models (Born et al., 2014; Xu et al., 2015; Kam et al., 2016). An alternative
776 explanation of the presence of IIS in J20 but not APP^{NL/F} mice may be differences
777 in the levels of A β between the two models. However, levels of soluble A β in 6
778 month old J20 and 12 month old APP^{NL/F} are comparable, and levels of total A β
779 are higher in APP^{NL/F} (Shankar et al., 2009; Saito et al., 2014). Thus it is unlikely
780 that higher levels of A β in the J20s are a cause of IIS in this model.

781 Interestingly, APP^{NL/F} mice begin to exhibit cognitive deficits at 8 months of age
782 (Masuda et al., 2016), which suggests that cognitive deficits at these ages are not
783 the result of IIS, as has been suggested for J20s (Sanchez et al., 2012). Moreover,
784 differences in the types of memory affected in J20 and APP^{NL/F} at ages preceding
785 overt plaque deposition have been reported. Specifically, 4 – 6 month old J20s
786 show impairments in hippocampal dependent spatial memory (Sanchez et al.,
787 2012). In contrast, in 8-month old APP^{NL/F} mice, spatial memory as assayed by a
788 place preference task is intact. However, place-avoidance memory, which is also
789 dependent on amygdala-circuits (Wilensky et al., 2000), is impaired (Masuda et
790 al., 2016). It may be that hippocampus dependent processes are susceptible to
791 interference by IIS while the disturbances in the non-hippocampal circuits result
792 from processes independent of IIS.

793 Differential sleep-stage coupling between mouse models of AD and human AD

794 Lam et al., (2017) recently used intracranial electrode recordings to detect mTL
795 IIS in two AD patients without a history of epilepsy. Here, we report that in these
796 patients, IIS were predominantly associated with NREM sleep (ie. low θ/δ). In the
797 patient with aMCI, IIS occurred most frequently in N3 sleep and were least

798 frequent in REM, with a greater than 4.5-fold difference in spike rates between
799 N3 and REM. In the AD patient, frequent IIS were seen during NREM sleep,
800 though REM sleep was absent from this patient's brief recording, in line with
801 previous reports of REM deficits in AD (Vitiello et al., 1984). Our findings from
802 intracranial electrodes in AD patients are consistent with prior scalp EEG studies
803 by Vossel et al., (2016), who reported that epileptiform discharges are highly
804 prevalent in sleep stages >2 (although the authors did not differentiate between
805 REM and NREM sleep). Although the means of characterising sleep differed
806 between rodents and patients, combined, these results point to important
807 differences in sleep stage coupling of epileptiform activity between rodent AD
808 models and humans with AD and suggest that the specific mechanisms that
809 underlie hyperexcitability in AD may differ between certain mouse models and
810 humans.

811 Analysis of ictal and inter-ictal activity in epilepsy patients has led the view that
812 NREM sleep is a generally pro-ictal state, whereas REM sleep is an anti-ictal state
813 (Sammaritano et al., 1991; Herman et al., 2001; Minecan et al., 2002; Ng and
814 Pavlova, 2013). Many animal models of epilepsy have also shown that seizures
815 are more frequent in NREM and rarely occur in REM (Shouse et al., 2000) .
816 Interestingly, rodent models of the same type of epilepsy can still exhibit
817 differences in the sleep-stage coupling of epileptiform activity. For example, in
818 both the kindling as well as the pilocarpine models of temporal lobe epilepsy in
819 rats, IIS are most common during NREM sleep (Colom et al., 2006; Gelinis et al.,
820 2016). In contrast, rats with either the tetanus toxin or the low-dose kainate
821 models of temporal lobe epilepsy have seizures that occur most commonly

822 during REM sleep (Sedigh-Sarvestani et al., 2014; Ewell et al., 2015). Based on
823 this, we hypothesize that different mouse models of AD may have specific
824 mechanisms underlying their network hyperexcitability, which could be
825 differentially expressed through sleep-stage coupling of IIS. We propose that
826 sleep-stage coupling of IIS should be an important factor for identifying mouse
827 AD models that more closely resemble the EEG signature of network
828 hyperexcitability in human AD.

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838 **Figures**

839 **Figure 1:** Inter-ictal spikes (IIS) are prevalent in J20 mice, but not in APP knock-
840 in mice. (A) ECoG trace recorded from a J20 mouse showing IIS. Inset is 250ms
841 expansion around IIS event marked by *. (B) Empirical cumulative distribution
842 frequency plots for individual animals quantifying the number of detected IIS in
843 8s intervals across 3 days of recording in WT and J20s. Colours represent
844 distributions for individual animals. (C) Plot showing the proportion of intervals
845 with one or more detected IIS in WT and J20. (D) Plot showing the proportion of
846 intervals with one or more detected IIS in WT and APP^{NL/F} at 8 months and 12
847 months. Bars represent medians. Whiskers extend to 1.5 IQR. *** p<0.001.

848

849 **Figure 2:** Circadian modulation of IIS. (A) Circular histogram of IIS counts over
850 three days of recording in an individual J20 mouse plotted on 24hr cycle. Light
851 condition indicated by shading. For the animal shown, $\phi_{IIS} = 14\text{h}38$ and $\rho = 0.43$.
852 (B) Summary data for ϕ_{IIS} vs ρ for all animals, shown on circular plot. Solid
853 symbols are strongly-coupled animals. Weakly coupled animals are shown with
854 orange fill.

855

856 **Figure 3:** The probability of IIS is modulated by behavioural state in strongly
857 phase-coupled animals. (A) IIS count/8s interval versus time over 2 hours of
858 ECoG recording in a J20 mouse, with sleep and wake indicated by shading. (Bi)
859 Mean spike rate in sleep and wake condition for strongly and weakly phase
860 coupled animals. Error bars: 95% CI. (ii) Circular histograms for a strongly (left)
861 and weakly (right) phase coupled animals using conventions as in Fig 2A.

862

863 **Figure 4:** IIS occur during high θ/δ states. (A) 8s ECoG signals (left) and
864 corresponding power spectra (right) during different behavioural states
865 recorded from a J20 mouse. A single IIS is seen in the sleep high θ state (ii). (B)
866 Time series of δ power, θ power, θ/δ and spike count per 8s intervals across 2
867 hours of ECoG recorded from the same J20 mouse as shown in A. Black/grey
868 symbols indicate sleep/wake as classified by simultaneous video data. Red
869 symbols and vertical dotted lines indicate the 8s intervals for which the ECoG
870 signal is shown in panel A (C) Spike number per 8s interval as a function of θ/δ in
871 5 animals (represented by different colours and connected by lines). The
872 increase spike count in intervals with high θ/δ was seen in animals with both
873 strong (filled symbols) and weak (open symbols) circadian phase-coupling. ***
874 $p < 0.001$ (D) IIS-triggered averages of θ/δ for 5 individual animals (black) and
875 windowed averages triggered around 2000 randomly sampled points (grey)
876 show an increased θ/δ around IIS. Strong/weak coupling shown in filled/open
877 symbols. Error bars in B and C represent 95% CI.

878

879 **Figure 5.** Transient increases in θ/δ are non-pathological features of sleep. (A) 8s
880 ECoG signals (left) and corresponding power spectra (right) during different
881 behavioural states recorded from a WT mouse. (B) Time series of δ power, θ
882 power and θ/δ per 8s interval across 2 hours of ECoG recorded from same WT
883 mouse as shown in A. Black/grey symbols indicate sleep/wake as classified by
884 simultaneous video data. Red symbols and vertical dotted lines indicate the 8s
885 intervals for which the ECoG signal is shown in panel A.

886

887 **Figure 6:** No evidence of cholinergic alterations in J20s. (A) Immunostained
888 brain section showing ChAT+ cells in medial septum (MS) and diagonal band of
889 Broca (DB). Lower panel shows zoomed in region of upper panel (left) and
890 corresponding regions of a negative control stained section (right). Upper right:
891 quantification of stereological estimates of ChAT+ cell count in MS and DB in WT
892 and J20. (B) AChE activity was assayed by the rate of thiocholine production in
893 brain homogenate from WT and J20 in control conditions and following oral
894 administration of donepezil (DPZ). The AChE activity was compared to a positive
895 control of direct application of neostigmine (10 μ M) to the brain homogenate.
896 Experimental repeat groups are indicated by different colours and connected
897 lines. *** p<0.001.

898

899 **Figure 7:** Sleep stage coupling of mesial temporal lobe (mTL) spiking in a human
900 with aMCI, a suspected early stage of AD. (A) Hypnogram showing the patient's
901 sleep architecture, spanning from ~ 7PM on FOD1 to 9:15AM on FOD2. (B) Bar
902 plot showing instantaneous mTL lobe spike rates over the course of the
903 recording. Bars are colored by sleep stage, with light green for Wake, light blue
904 for NREM (includes NREM1, NREM2, and NREM3), and dark blue for REM. The
905 patient had three brief subclinical seizures (SZ) from the left FO electrodes
906 during this recording, the timing of which is depicted by red vertical bars. (C-E):
907 Plots showing (C) δ power (0-4Hz), (D) θ power (4-12Hz), and (E) θ/δ ratio of
908 bilateral mTL activity, based on FO electrodes recordings. Dots represent the
909 spectral power for each non-overlapping 30 second window of the recording.
910 Power is measured in arbitrary units.

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912 **Table 1:** Statistical table

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914 **Table 2:** Average mTL spike rates were evaluated from foramen ovale electrodes
915 and related to sleep stage as assayed by scalp EEG in two patients with AD.

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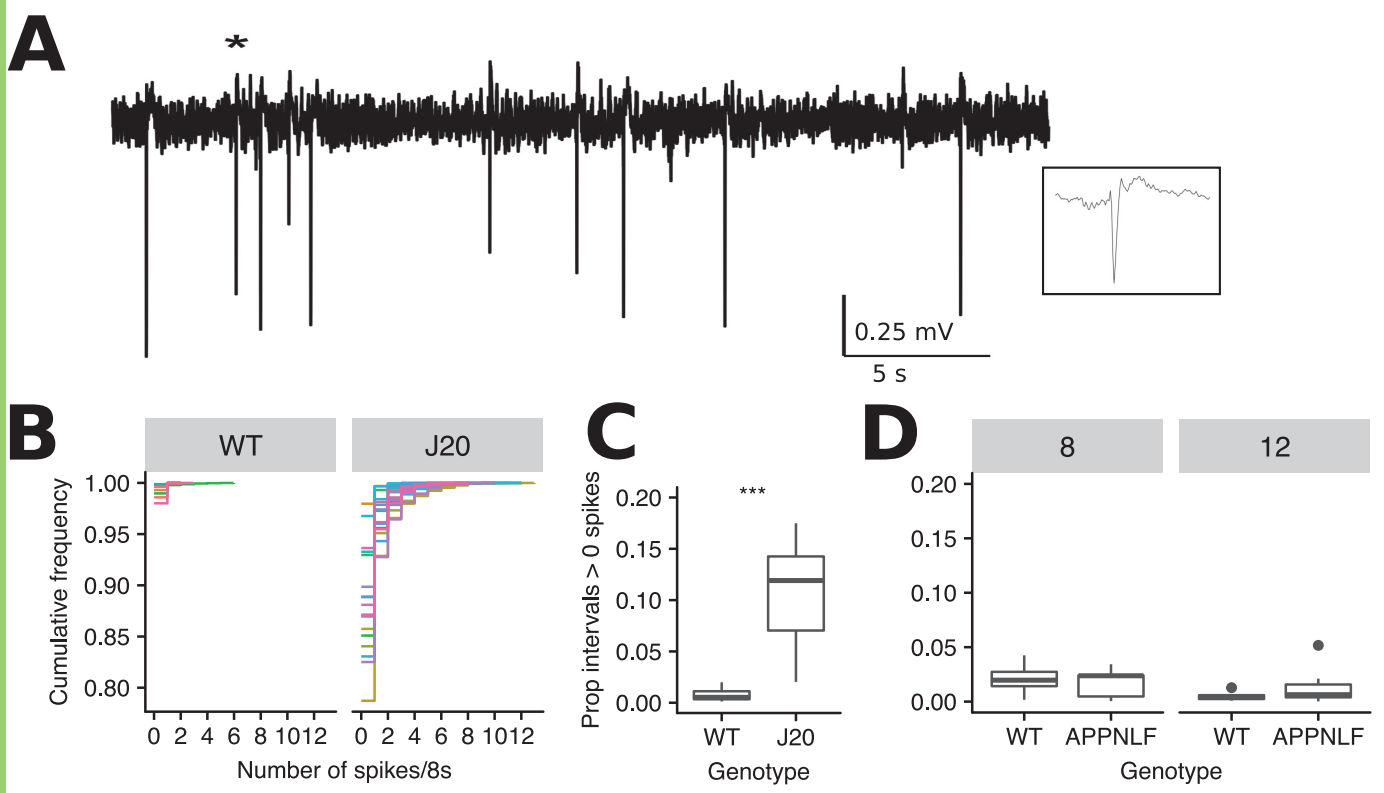
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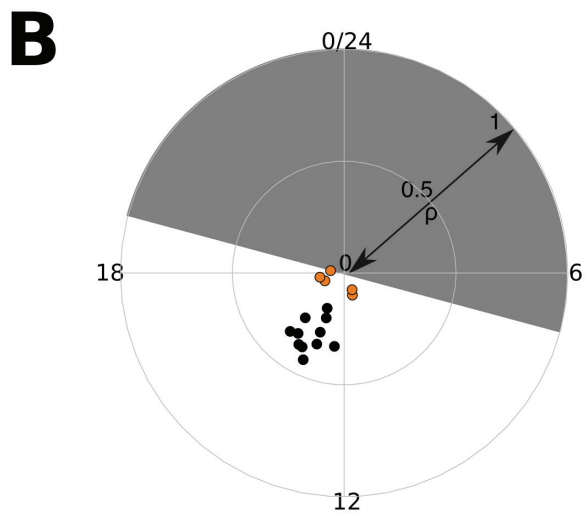
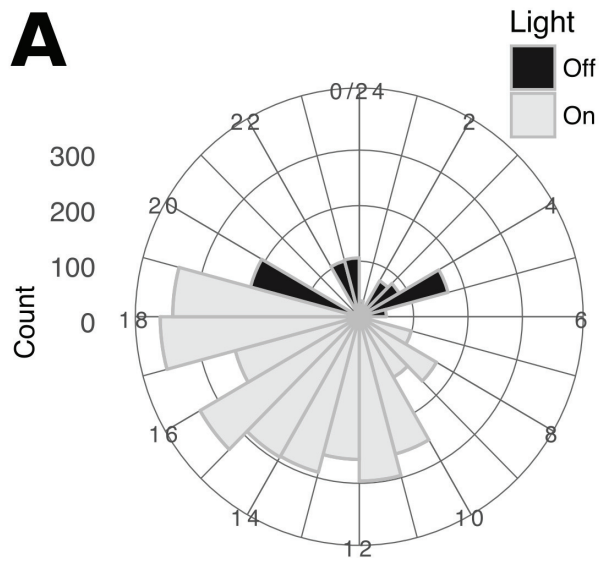
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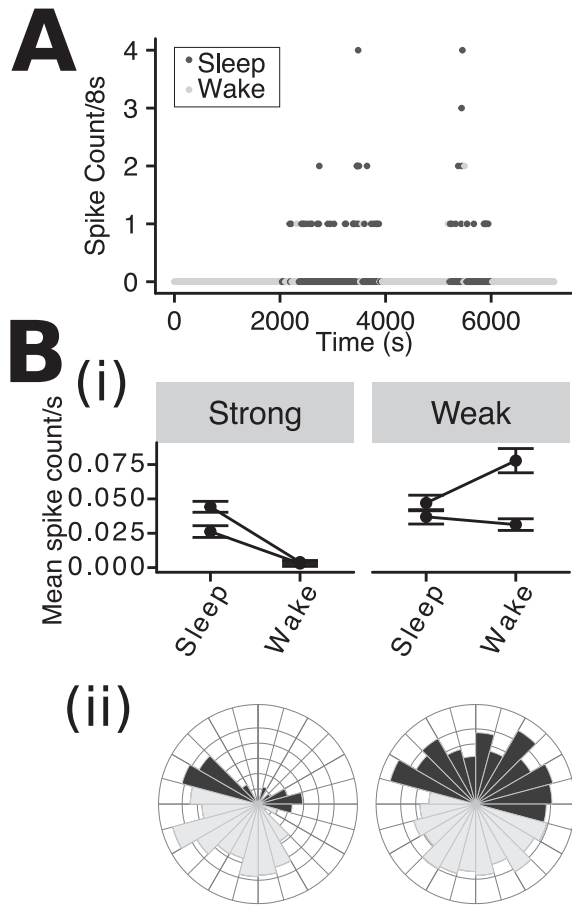
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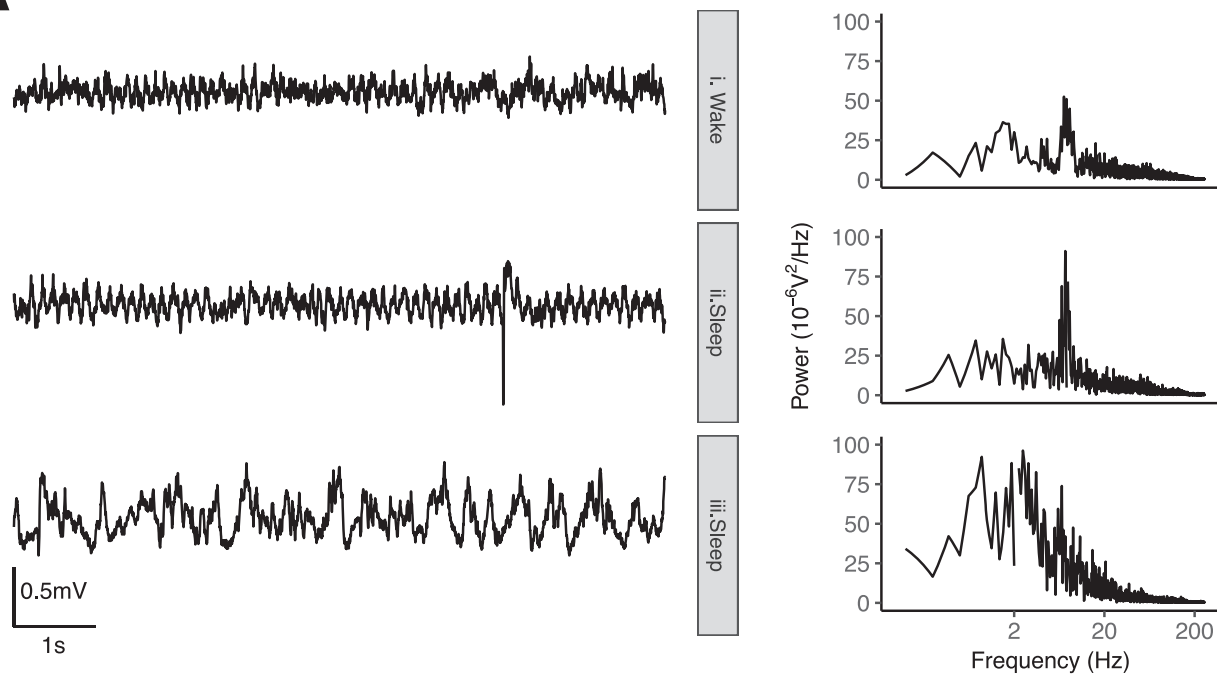
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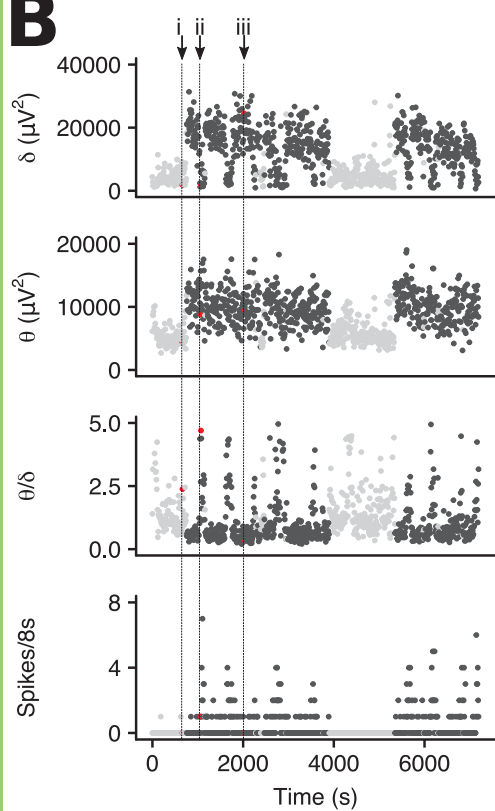




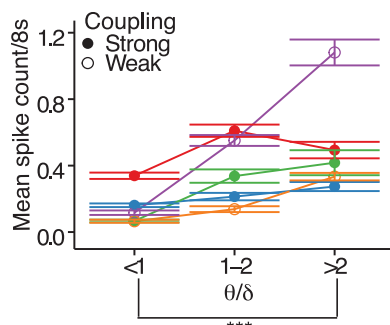
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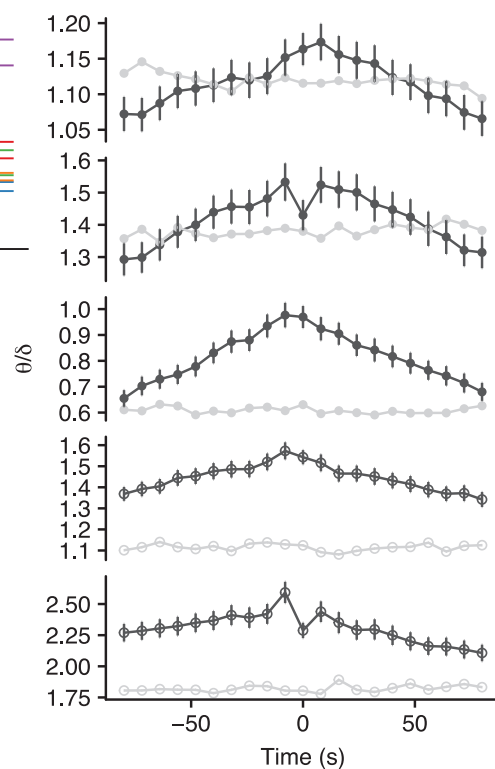
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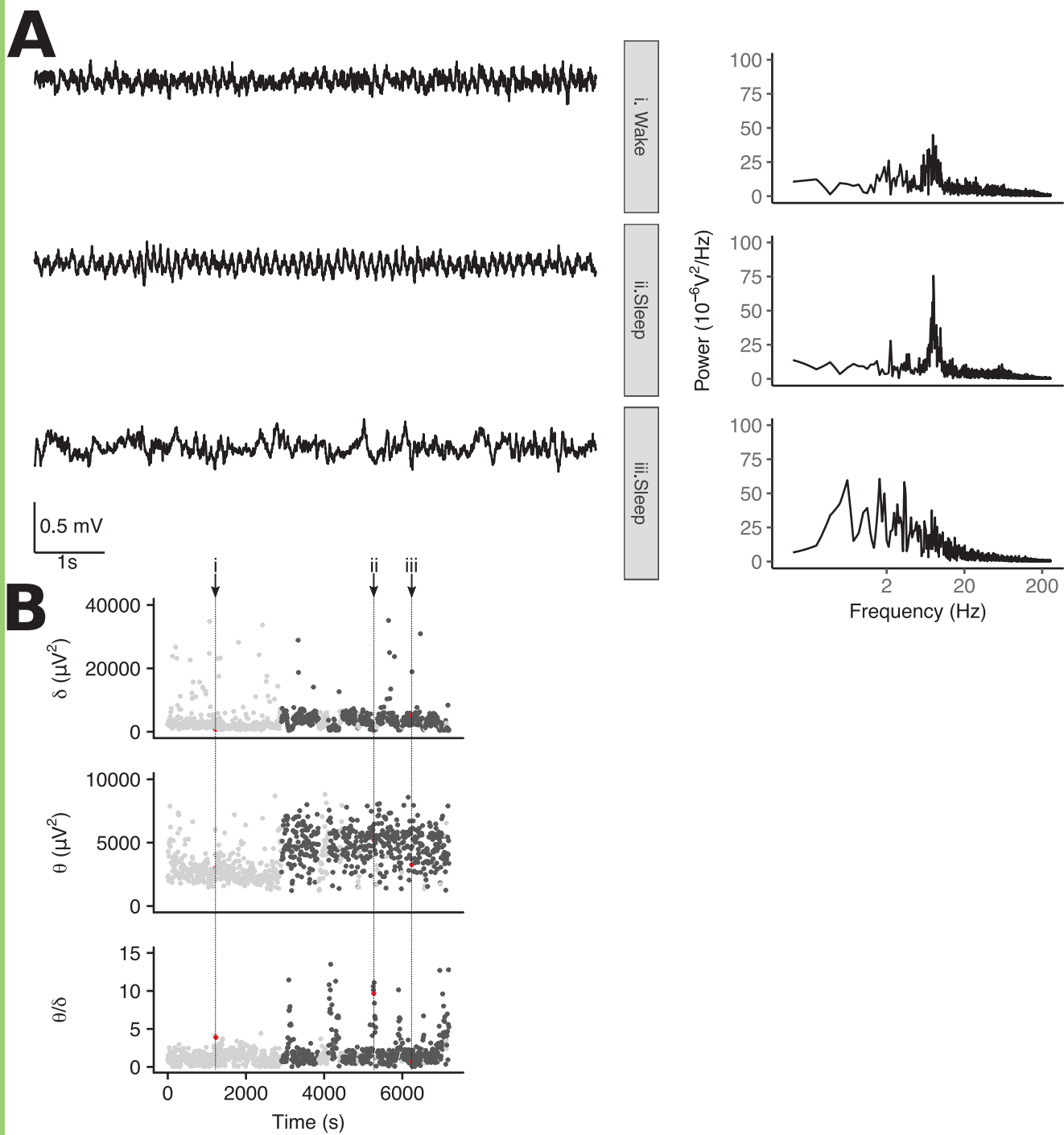


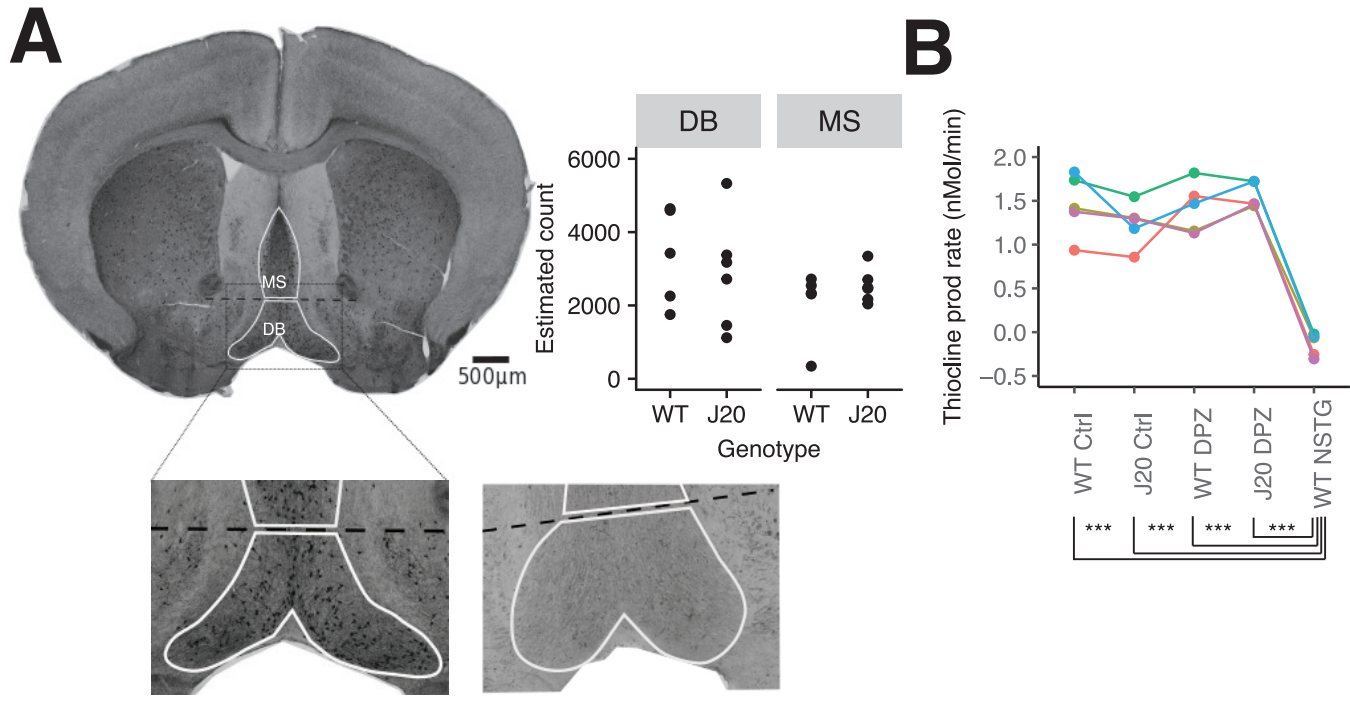
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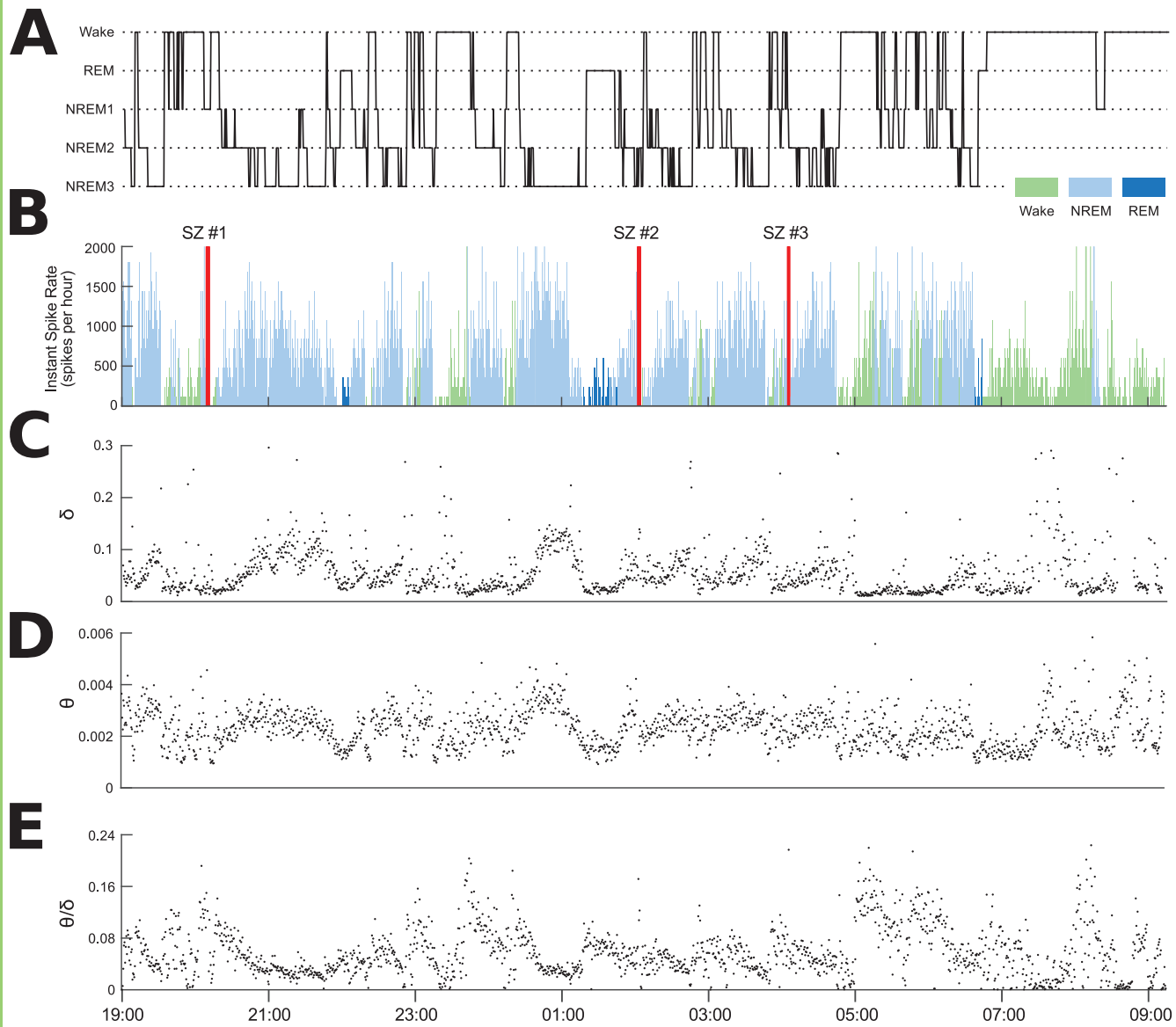


Table 1: Statistical table

	Data Structure	Type of test	Confidence/ credible interval parameter	95% CI
a	Normal (square root transformed)	t-test	Difference of means of square root data	(0.20, 0.30)
b	Normal (square root transformed)	Linear mixed model	β -Genotype β -Age	(-0.01, 0.03) (-0.02, -0.002)
c	IIS count data - (analysed with log-link function)	MCMC generalized model	Difference between estimates of $\theta/\delta < 1$ vs. $\theta/\delta > 2$. Provided for animals JF221, JF220, JF218, J0460, J0456, respectively	(1.619, 2.122) (0.261, 0.471) (0.254, 0.478) (1.166, 1.392) (2.128, 2.372)
d	Normal (fourth root transformed)	Linear mixed model	β -[?][?][?][?]	(-0.004, 0.008)
e	Normal	Linear mixed model	β -Genotype	(-1015.7, 1029.0)
f	Non-normal	Wilcoxon signed rank test	Difference of medians	(0.08, 0.65)

g	Normal (log transformed)	Tukey contrasts	J20_Ctrl - WT_Ctrl WT_DPZ - WT_Ctrl J20_DPZ - WT_Ctrl WT_NSTG - WT_Ctrl WT_DPZ - J20_Ctrl J20_DPZ - J20_Ctrl WT_NSTG - J20_Ctrl J20_DPZ - WT_DPZ WT_NSTG - WT_DPZ WT_NSTG - J20_DPZ	(-0.24, 0.03) (-0.15, 0.12) (-0.08, 0.19) (-1.50, -1.23) (-0.04, 0.23) (0.02, 0.29) (-1.40, -1.13) (-0.07, 0.21) (-1.49, -1.22) (-1.56, -1.29)
h	Normal	Paired t-test	Difference of mean IIS rate	(-0.01, 0.03)

Sleep Stage	Patient #1 (AD dementia)		Patient #2 (aMCI)	
	Total Hours in Record	Average spike rate (spikes/hour)	Total Hours in record	Average spike rate (spikes/hour)
Wake	4.7	11	5.2	329
NREM1	0.7	31	1.5	670
NREM2	2.1	80	3.8	739
NREM3	1.4	62	3.1	903
REM	0	n/a	0.7	159