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A β /APP-induced hyperexcitability and dysregulation of homeostatic synaptic plasticity in models of Alzheimer's disease

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22 **Abstract**

23 The proper function of the nervous system is dependent on the appropriate timing of
24 neuronal firing. Synapses continually undergo rapid activity-dependent modifications that
25 require feedback mechanisms to maintain network activity within a window in which
26 communication is energy efficient and meaningful. Homeostatic synaptic plasticity (HSP)
27 and homeostatic intrinsic plasticity (HIP) are such negative feedback mechanisms.
28 Accumulating evidence implicates that Alzheimer's disease (AD)-related amyloid precursor
29 protein (APP) and its cleavage product amyloid-beta ($A\beta$) play a role in the regulation of
30 neuronal network activity, and in particular HSP. AD features impaired neuronal activity
31 with regional early hyper-activity and $A\beta$ -dependent hyperexcitability has also been
32 demonstrated in AD transgenic mice. We demonstrate similar hyper-activity in AD
33 transgenic neurons in culture that have elevated levels of both human APP and $A\beta$. To
34 examine the individual roles of APP and $A\beta$ in promoting hyperexcitability we used an APP
35 construct that does not generate $A\beta$, or elevated $A\beta$ levels independently of APP. Increasing
36 either APP or $A\beta$ in wild type (WT) neurons leads to increased frequency and amplitude of
37 calcium transients. Since HSP/HIP mechanisms normally maintain a setpoint of activity, we
38 examined whether homeostatic synaptic/intrinsic plasticity was altered in AD transgenic
39 neurons. Using methods known to induce HSP/HIP, we demonstrate that APP protein
40 levels are regulated by chronic modulation of activity and show that AD transgenic neurons
41 have an impaired response to global changes in activity. Further, AD transgenic compared
42 to WT neurons failed to adjust the length of their axon initial segments (AIS), an adaptation

43 known to alter excitability. Thus, we present evidence that both APP and A β influence
44 neuronal activity and that mechanisms of HSP/HIP are disrupted in neuronal models of AD.

45

46 **Background**

47 Alzheimer's disease (AD) is the leading cause of dementia and the most common
48 neurodegenerative disease. It is characterized by the progressive, age-related accumulation
49 and aggregation of disease-associated proteins, including amyloid-beta (A β) that is cleaved
50 from the amyloid precursor protein (APP). This process is thought to drive the loss of
51 synapses and neurons. However, preceding the massive neurodegeneration, AD features
52 aberrant regional neuronal activity in the form of both hyper- and hypo-excitability, and
53 evidence supports that the occurrence of network hyper-excitability early in the disease
54 process is tied to elevated A β levels (Vossel *et al.*, 2013; Zott *et al.*, 2019). However, the
55 precise mechanisms behind this early A β -induced hyper-excitability remain unclear. In
56 addition, the normal roles of A β /APP in brain physiology and their roles in pathophysiology
57 during AD remain incompletely understood.

58

59 The coordinated firing of neurons across networks is considered to be crucial for cognitive
60 function. To maintain their proper function and levels of activity, neurons employ
61 homeostatic synaptic plasticity (HSP) and homeostatic intrinsic plasticity (HIP), which are
62 means by which neurons can tune their activity to the global tonus of activity. Homeostatic
63 scaling is an example of one such tuning mechanism (Turrigiano *et al.*, 1998), and other

64 mechanisms of regulation are being investigated. These modulatory processes enable
65 neuronal communication to be maintained within an appropriate window, allowing
66 meaningful information transfer (Turrigiano, 2012). Recently, both APP and A β were
67 implicated in the regulation of HSP (Gilbert *et al.*, 2016; Galanis *et al.*, 2021), demonstrating
68 that these proteins play important roles beyond AD pathophysiology. Further, prior in vivo
69 work in AD transgenic mice suggests that HSP mechanisms might be impaired, since
70 chronic hypo-activity or hyper-activity via either long term sleep deprivation or induction,
71 or unilateral whisker removal, conditions in which HSP should be engaged, negatively
72 impacted AD transgenic compared to wild-type (WT) mice (Kang *et al.*, 2009; Tampellini *et*
73 *al.*, 2010).

74
75 In this study, we set out to further elucidate the effects of APP and A β on neuronal activity.
76 To that end, we utilized primary neuronal cultures from APP/PS1 AD transgenic mice and
77 their WT counterparts and live-cell calcium imaging to perform activity analyses of
78 neuronal networks. We demonstrate that a general increase in transient calcium
79 frequencies occurs in the context of elevated APP and A β . Furthermore, we show that
80 specifically CaMKII-positive excitatory neurons from AD transgenic mice exhibit higher
81 amplitude calcium transients. Finally, we demonstrate the impaired ability of AD transgenic
82 compared to WT neurons to properly initiate HSP and HIP mechanisms to adapt to global
83 activity changes.

84

85 **Materials and Methods**

86 Antibodies

87 The antibodies employed in this study were the following: mouse anti-beta-actin (Sigma-
88 Aldrich, Sweden), rabbit anti-OC against high molecular weight A β (Merck Millipore,
89 Sweden), mouse anti-6E10 for human A β /APP (BioLegend, Sweden), APPY188 rabbit anti
90 C-terminal APP (Abcam, Sweden), rabbit anti-somatostatin (Abxexa, UK), mouse anti-
91 GAD67 (Merck Millipore, Sweden), mouse anti-CaMKII (Merck Millipore, Sweden),
92 mouse anti-ankyrin-G (Thermo Scientific, Sweden), guinea pig anti-Vglut1 (Synaptic
93 Systems, Germany), rabbit anti-VGAT (Synaptic systems, Germany), rabbit anti-Gephyrin
94 (Synaptic Systems, Germany), and chicken anti-MAP2 (Abcam, UK).

95 Neuronal cell culture

96 Primary neurons were cultured from the cortices and hippocampi of APP/PS1 AD
97 transgenic mouse (APP^{swe}, PSEN1^{dE9})85Dbo/Mmjax; Jackson Labs, Maine, USA) and APP
98 KO (Jackson labs, Maine, USA, JAX 004133) mouse embryos at embryonic day 15-17 (E15-
99 17). Neurons were cultured as previously described (Willén et al. 2017). Briefly, pregnant
100 mice were anesthetized using isoflurane (MSD Animal Health, Sweden) and sacrificed.
101 Embryos were quickly removed, and biopsies were taken for genotyping. Brains were
102 dissected under constant cooling with chilled (~4 °C) Hanks balanced salt solution (HBSS;
103 Thermo Scientific, Sweden) supplemented with 0.45% glucose (Thermo Scientific, Sweden).
104 Cortices and hippocampi were retrieved and incubated in 0.25% trypsin (Thermo Scientific,
105 Sweden), followed by 2 washes with HBSS. Brain tissue was then triturated in 10% fetal
106 bovine serum (FBS) supplemented Dulbecco's modified Eagle medium (DMEM; Thermo
107 Scientific, Sweden) with 1% penicillin-streptomycin (Thermo Scientific, Sweden) using

108 glass pipettes until neurons were dissociated. Neurons were plated onto 8 well- plates (for
109 calcium imaging; Ibidi), 6 well plates (for Western blot; Sarstedt, Germany) or glass
110 coverslips in 24 well plates (for immunolabeling; Sarstedt, Germany) coated with Poly-D-
111 lysine (Sigma-Aldrich, Sweden). Neurons were plated with 10% FBS and 1% penicillin-
112 streptomycin in DMEM; following 3-5 h incubation, media was exchanged for complete
113 Neurobasal solution, consisting of Neurobasal medium, B27 supplement, penicillin-
114 streptomycin, and L-glutamine (Thermo Scientific, Sweden). One embryo corresponds to
115 one set of cultures. All animal experiments were performed in accordance with the ethical
116 guidelines and were approved by the Animal Ethical Committee at Lund University ethical
117 permit number 5.8.18-05983/2019.

118 Genotyping

119 Genotyping was carried out using the PCRbio Rapid Extract PCR kit (Tectum, Sweden). In
120 brief, biopsies were incubated with 70 μ l distilled H₂O, 20 μ l 5x PCRbio buffer A (lysis
121 buffer) and 10 μ l 10x PCRbio buffer B (protease containing buffer) per vial at 75 °C for 5
122 min, followed by heating to 95 °C for 10 min. The vials were placed on ice and allowed to
123 cool before vortexing for 3-4 s and centrifuged at 10,000 rpm for 1 min to pellet the debris.
124 The DNA supernatant was then transferred to a new vial. The DNA supernatant was either
125 used directly or stored at -20 °C. For PCR, 1 μ l of DNA was incubated with 9.5 μ l distilled
126 H₂O, 12.5 μ l 2x PCRbio rapid PCR mix (containing Taq polymerase for DNA amplification), 1
127 μ l primer-set F (APP knockout) and 1 μ l primer-set G (APP WT; both 10 μ M) for 3 min at 95
128 °C. The temperature was decreased to 55 °C for 15 seconds to allow for the annealing of

129 primers. The temperature was then increased to 72 °C for 5 min to allow for the extension
130 of DNA. DNA bands were detected using agarose gel electrophoresis.

131 Viral vectors

132 We used lentiviral vectors carrying TdTomato, hAPPwt or hAPPmv (mutant APP resistant
133 to BACE cleavage) under a CaMKII promoter; the genes were inserted via Gene synthesis
134 (Thermo Fischer Scientific) into a plasmid compatible with Gateway technology to serve as
135 an entry clone. Production and titration were performed as previously described (Quintino
136 *et al.*, 2013). Primary neurons were transduced at 12-13 days in vitro (DIV) at a multiplicity
137 of infection (MOI) of 5 and analyzed at 19-21 DIV.

138 Treatments

139 Cultured neurons at 19-21 DIV were treated with different compounds before live cell
140 imaging, immunofluorescence or western blot experiments: thiorphan (500 nM, 1 h; Sigma-
141 Aldrich, Sweden), TTX (1 µM, acute (0-1h) or 48 h), bicuculline (20 µM, acute(0-1h) or 48 h),
142 picrotoxin (10 µM, 1 h), CNQX (10 µM, 1 h) (Sigma), and synthetic Aβ1-42 (Tocris, UK) and
143 synthetic reverse Aβ42-1 (Tocris, UK) reconstituted in dimethyl sulfoxide (DMSO) to 250
144 mM, sonicated 10 min and then centrifuged at 10 000g for 15 min before adding the
145 supernatant to cell culture media.

146 Western blot

147 Cell lysates were prepared using modified RIPA buffer containing 50 mM Tris-HCl (pH 7.4),
148 150 mM NaCl, 1 mM EGTA, 1% NP-40, 0.25% sodium deoxycholate with added protease
149 and phosphatase inhibitor cocktail II (Sigma-Aldrich, Sweden). BCA protein assay kit

150 (Thermo Scientific, Sweden) was used to determine protein concentrations. Equal amounts
151 of protein from each sample were loaded into 10-20% Tricine sodium dodecyl sulphate-
152 polyacrylamide gel electrophoresis (SDS-PAGE; Sigma-Aldrich, Sweden), followed by
153 immunoblotting on polyvinylidene difluoride (PVDF) membranes (Sigma-Aldrich, Sweden)
154 and intensity quantification was carried out using Image Lab 5.2.1.

155 Live-cell imaging

156 Cultured neurons at 19-21 DIV were incubated with 3 μ M of the calcium dye Fluo-4 AM
157 (Thermo Scientific, Sweden) in DMSO(Sigma-Aldrich, Sweden) for 30 min before imaging.
158 Cells were imaged under a Nikon Eclipse Ti microscope at 10 x with 1.4 NA. Live cell
159 imaging chamber (Okolab) was kept at 5% CO₂ and 37 °C. Cells were imaged every 100 ms
160 for a duration of 2 min with an iXon Ultra CCD camera (ANDOR Technology).

161 Calcium imaging analysis

162 Time-stacks of calcium imaging files were opened in Fiji; individual Regions of interest
163 (ROIs) were drawn around cell bodies and ROIs were determined to be CaMKII+ or CaMKII-
164 based on TdTomato labeling. Fluorescence intensity over time was extracted, processed
165 and normalized in the MatLab script PeakCaller(Artimovich *et al.*, 2017a; b). Spike detection
166 threshold was set to 10% above baseline; for calculation of amplitude heights and
167 interspike intervals; silent neurons were omitted as these would bias the measurement and
168 underestimate the amplitude heights. Spike frequencies and amplitudes were extracted,
169 and raster plots were generated in MatLab.

170 Immunofluorescence

171 Cultured neurons at 19 -21 DIV were fixed in 4% paraformaldehyde (PFA) in PBS with 0.12
172 M sucrose for 20 min, at room temperature (RT). Cells were then blocked in 0.1% saponin
173 (Sigma-Aldrich, Sweden), 1% bovine serum albumin (BSA; Sigma-Aldrich, Sweden) and 2%
174 normal goat serum (NGS; Thermo Scientific, Sweden) in PBS for 1 h at RT. Cells were
175 incubated in primary antibody (diluted in 2% NGS in PBS) overnight at 4 °C. Cells were
176 rinsed in PBS and incubated with secondary antibodies diluted in 2% NGS in PBS. Cells
177 were rinsed in PBS and counterstained with DAPI diluted at 1:2000 (Sigma-Aldrich,
178 Sweden). Imaging was performed with an inverted Olympus IX70 epifluorescence or an
179 inverted Leica SP8 confocal microscope.

180 Image analysis

181 Neurons were labeled for inhibitory or excitatory pre- and post-synaptic markers; CaMKII
182 and VGLUT1 for excitatory synapses and Gephyrin and VGAT for inhibitory synapses.
183 Images were then processed in the ImageJ plugin: SynaptcountJ. SynaptCountJ is a semi-
184 automated plugin for measuring synapse density (Mata *et al.*, 2017). By colocalization of
185 two different excitatory or inhibitory synaptic markers one can count the number of
186 excitatory and inhibitory synapses per neuron along with other morphological parameters
187 such as dendritic length. For CaMKII cell quantification 19-21 DIV APP/PS1 and WT
188 neuronal cultures were labeled with DAPI, CaMKII and MAP2. Images were sampled at 20x
189 in an inverted Olympus IX70 epifluorescence microscope and analyzed with the “cell-
190 counter” plugin in ImageJ as percent CaMKII positive out of all MAP2 positive neurons. For
191 analysis of axon initial segment length, ankyrin-G positive axon initial segments were traced
192 and measured in ImageJ by a blinded experimenter.

193 Experimental design and statistical analysis

194 All statistical analyses were performed with GraphPad Prism 8.3. Sample size was denoted
195 as n = number of cells analyzed and N = sets of cultures. Data was first tested for normality
196 using D'Agostino-Pearson omnibus K2 normality test to determine the appropriate
197 statistical test. Mann-Whitney or Kruskal Wallis tests were used to compare distribution of
198 data between groups unless otherwise stated. Correction for multiple testing was
199 performed with Dunn's correction unless otherwise stated. Graphs are expressed as mean \pm
200 95% confidence interval with individual values plotted as dots unless otherwise stated in
201 figure legend. Differences were considered significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$;
202 **** $p < 0.0001$, n.s., not significant.

203

204 **Results**

205 **Increased calcium oscillations in APP/PS1 AD transgenic mouse neurons**

206 To study AD-related neuronal activity alterations *in vitro*, primary cortico-hippocampal
207 neurons from APP/PS1 AD transgenic mice and their WT littermates were loaded with the
208 calcium indicator Fluo-4 AM and imaged for calcium transients using a live cell imaging
209 microscope. Representative raster plots and calcium traces showed that neurons from both
210 APP/PS1 transgenic and WT mice were spontaneously active (Supplemental figure 1).
211 APP/PS1 neurons overall had an increased frequency of calcium transients and higher
212 amplitude of calcium spikes compared to WT neurons (Fig. 1a,b). Inter-spike intervals were
213 also altered in APP/PS1 neurons, which had shorter inter-spike intervals than WT neurons

214 (Fig. 1c). Moreover, APP/PS1 cultures had fewer inactive neurons compared to WT cultures
215 (Fig. 1d), consistent with the other signs of increased excitability.

216
217 As glutamate is the main excitatory neurotransmitter, we next assessed whether glutamate
218 signaling was involved in APP/PS1 neuronal hyperactivity. To do this, APP/PS1 cultures
219 were treated with CNQX to block AMPA receptor-mediated signaling. Following treatment
220 with CNQX, APP/PS1 neurons had a sharp decrease in calcium oscillation frequency and
221 amplitude compared to neurons treated with vehicle (Fig. 1e), supporting the conclusion
222 that glutamate signaling contributes to the hyper-activity of the AD transgenic neuronal
223 cultures.

224
225 **Maintained inhibitory GABA signaling and network-level hyperactivity in APP/PS1**
226 **neuronal cultures**

227 During early development, GABA can have excitatory effects in culture (Doshina *et al.*,
228 2017). Normally, GABA signaling shifts to being primarily inhibitory at around 14 days *in*
229 *vitro* (DIV). However, because APP/PS1 neurons may have altered neuronal development
230 due to constitutively over-expressing mutant APP and presenilin (Handler *et al.*, 2000;
231 Rama *et al.*, 2012), the increased activity seen in 19-21 DIV cultures could, in part, have
232 been due to excitatory GABA signaling. Thus, we tested whether GABA signaling had
233 inhibitory or excitatory effects in our presumably mature 19-21 DIV APP/PS1 neurons by
234 treating them with GABA_A blockers bicuculline and picrotoxin. Blocking GABA signaling in

235 the APP/PS1 cultures led to an increased frequency and synchronicity of firing, indicating
236 that GABA had inhibitory effects in our cultures similar to that of WT cultures
237 (Supplemental figure 2a-c).

238
239 To further understand what was driving the hyperactivity in APP/PS1 neurons, we
240 examined whether the hyperactivity was being driven at a network or cellular level. To that
241 end, we treated WT and APP/PS1 cultures with tetrodotoxin (TTX), which reduces global
242 activity by blocking sodium channels. The addition of 1 μ m TTX stopped most calcium
243 transients in both WT and APP/PS1 neurons, indicating that a global response was achieved
244 and that the hyperactivity in APP/PS1 neurons was at the network-level rather than a cell-
245 specific effect (Supplemental figure 2d-f).

246
247 **Hyperactivity of excitatory neurons in APP/PS1 cultures**

248 To parse out the contributions of excitatory and inhibitory neurons in driving the
249 hyperactivity in APP/PS1 cultures, we transduced neurons with a vector to induce the
250 expression of TdTomato under a CaMKII promoter (Fig. 2a), allowing us to distinguish
251 excitatory neurons from GABAergic interneurons. This allowed for the attribution of Fluo-4
252 measurements to CaMKII-positive excitatory neurons or to CaMKII-negative neurons, which
253 mostly include inhibitory neurons. Interestingly, we detected increased activity in CaMKII-
254 positive APP/PS1 neurons compared to CaMKII-positive WT neurons. In contrast, activity
255 levels in CaMKII-negative neurons were similar between APP/PS1 and WT cultures (Fig.

256 2b). Furthermore, CaMKII-positive neurons had higher amplitude calcium transients
257 compared to CaMKII-negative neurons in APP/PS1 but not WT cultures (Fig. 2c).

258
259 We next sought to investigate whether an imbalance in the proportion of excitatory to
260 inhibitory neurons and synapses could underlie the increased levels of activity in APP/PS1
261 neurons. To do this, we evaluated the relative levels of select proteins known to be localized
262 in either excitatory or inhibitory neurons. Immunoblotting against CaMKII and GAD67,
263 markers expressed by nearly all excitatory and inhibitory neurons, respectively, showed no
264 differences in the levels of CaMKII and GAD67 between APP/PS1 and WT neurons (Fig. 3a-
265 c). Likewise, analyses of neurons immunolabelled for glutamatergic (VGluT and CaMKII)
266 and GABAergic (vGAT and gephyrin) synaptic markers using the image analysis plugins
267 NeuronJ and Synapcount (Mata *et al.*, 2017) did not show a significant difference between
268 APP/PS1 and WT cultures (Fig. 3d-f). Similarly, counting CaMKII positive cells per culture
269 did not show a significant difference between the percentage of CaMKII neurons in WT and
270 APP/PS1 neuronal cultures (Fig. 3g). However, consistent with prior work (Siskova *et al.*,
271 2014), our analyses did show decreased dendritic length in APP/PS1 compared to WT
272 neurons (Fig. 3h-i).

273

274 **Individual contributions of APP and A β to neuronal hyperactivity**

275 To dissect out the individual role of APP on neuronal activity, we investigated whether APP
276 over-expression alone without a concomitant elevation in A β could cause hyper-activity by

277 transducing WT neurons with constructs encoding either mutant human APP resistant to
278 BACE cleavage (hAPP^{mv}) (Kamenetz *et al.*, 2003) or WT human APP (hAPP^w), both under a
279 CaMKII promoter. Remarkably, the expression of either APP construct in WT neurons led to
280 a robust increase in the frequency and amplitude of calcium transients (Fig. 4a,b),
281 indicating an A β -independent effect of APP on neuronal activity in excitatory neurons.

282
283 Likewise, we sought to investigate whether increased A β levels alone could induce hyper-
284 activity. We wanted to increase A β levels in WT cultures without affecting APP and PS1 as
285 this could confound our results, since we provide evidence that overexpression of APP has
286 A β -independent effects on neuronal activity, while PS1 has been shown to alter calcium
287 signaling (Lerdkrai *et al.*, 2018). Therefore, we utilized an inhibitor of the A β degrading
288 enzyme neprilysin, which increases A β levels (Abramov *et al.*, 2009), primarily at synapses,
289 as neprilysin is highly expressed pre-synaptically (Iwata *et al.*, 2004; Abramov *et al.*, 2009).
290 After 1 h of treatment with the neprilysin inhibitor thiorphan (500 nM), calcium transient
291 frequencies (Fig. 4c) and amplitudes (Fig. 4d) were increased in WT primary neurons.
292 Interestingly, thiorphan led to a greater increase in firing frequency in CaMKII-positive
293 compared to CaMKII-negative neurons (Fig. 4e). As neprilysin also degrades other peptides,
294 such as substance P and neurokinin A, as a control, we assessed the effect of thiorphan
295 treatment on APP knockout (KO) neurons, which lack APP and, thus, the capacity to
296 generate A β . Indeed, calcium transient frequencies and amplitudes from APP KO neurons
297 treated with thiorphan did not significantly differ from APP KO neurons treated with
298 vehicle alone (Fig. 4f), supporting the conclusion of elevated A β levels as driving the
299 hyperactivity in WT neurons treated with thiorphan. However, APP KO neurons have been

300 shown to have altered synaptic composition (Martinsson *et al.*, 2019) and calcium
301 transients (Yang *et al.*, 2009), which could potentially mask effects by thiorphan. Therefore,
302 as an alternative to investigate elevated A β levels and hyperactivity, we added exogenous
303 synthetic A β peptide to WT cultures.

304
305 Immunolabeling WT neuronal cultures treated with 0.5 μ M of synthetic human A β 1-42 for 2
306 hours with the human-specific A β /APP antibody 6E10 showed that the added exogenous
307 A β 1-42 localized to the dendritic spines of CaMKII-TdTomato expressing neurons (Fig. 5a,
308 b). This was consistent with prior findings showing that exogenous A β 1-42 preferentially
309 binds to synapses of CaMKII-immunoreactive neurons (Willén *et al.*, 2017). Interestingly,
310 we detected marked colocalization of the added human A β 1-42 with the fibril and fibrillar
311 oligomer-specific antibody OC that detects amyloid structures (Hatami *et al.*, 2014),
312 consistent with aggregation of the exogenous A β 1-42 at synapses, which is consistent with
313 prior work (Willén *et al.*, 2017). While for these experiments we added supraphysiological
314 levels of A β 1-42 (0.5 μ M), in order to more readily visualize its localization we next assayed
315 what effect more physiological A β 1-42 increases would have on calcium oscillations. Given
316 that physiological levels of A β are in the picomolar range and that picomolar levels of
317 exogenous A β were reported to increase LTP (Puzzo *et al.*, 2008), we added 200 pM of
318 synthetic human A β 1-42 acutely to mouse WT neuronal cultures expressing CaMKII-driven
319 TdTomato. Addition of 200 pM synthetic A β to WT cultures led to modest increases in the
320 firing frequencies of both CaMKII-positive and CaMKII-negative neurons (Fig. 5c). In
321 addition, these picomolar levels of A β 1-42 led to increased amplitudes of calcium

322 oscillations in CaMKII-positive neurons but not CaMKII-negative neurons (Fig. 5d).
323 However, higher concentrations of synthetic A β 1-42 (500 nM) did not lead to significantly
324 increased activity (Fig. 5e), though 500 nM of synthetic A β 1-40 did lead to a robust increase
325 in firing frequency. In summary, our results support the concept that APP and A β can
326 independently induce increases in synaptic activity, which likely plays a role under
327 physiological and pathological conditions.

328

329 **Dysregulated homeostatic plasticity in APP/PS1 neurons**

330 Since we showed that elevating either APP or A β in WT neurons can increase neuronal
331 activity, we hypothesized that the continuously high levels of APP and A β in APP/PS1
332 neurons disrupt neuronal network activity and function. We speculated that HSP and HIP
333 mechanisms, which help maintain synaptic firing within the boundaries of meaningful
334 communication (Turrigiano, 2008), may be impaired in AD transgenic neurons and, as a
335 result, may no longer be effective at returning activity levels back to a baseline.
336 Dysfunctional HSP/HIP could thus explain part of the sustained hyperexcitability observed
337 in neurons from AD transgenic mouse models. We therefore hypothesized that long-term
338 high levels of A β /APP might impair homeostatic plasticity. This can be tested by
339 manipulating neuronal firing outside of a network's set point, leading to plasticity changes
340 that maintain the baseline activity level. We therefore initially treated APP/PS1 and WT
341 neurons with TTX and bicuculline, which should immediately decrease network activity and
342 increase network activity, respectively. Indeed, acute treatment of WT and APP/PS1
343 neurons with TTX and bicuculline led to the expected decreased and increased activity,

344 respectively (Supplementary figure 2e-f). Next we treated WT and APP/PS1 neurons with
345 TTX or bicuculline for a longer period of time (48 hours), which are established methods for
346 inducing HSP/HIP (Turrigiano *et al.*, 1998). Interestingly, 48 hours of TTX treatment led to
347 a sharp decrease by western blot in total APP protein levels in both WT and APP/PS1 (Fig.
348 6a-b), which was consistent with immunolabeling of cultures treated with TTX or
349 bicuculline (Fig. 6c). There was also a trend for a decrease in CaMKII α , which was
350 previously reported to be downregulated with TTX induced homeostatic scaling
351 (Thiagarajan *et al.*, 2002). To determine whether HSP and HIP mechanisms were induced
352 by the long-term treatments, we measured the calcium transients after HSP induction.
353 Treating WT neurons with TTX or bicuculline for 48 hours led to the expected changes in
354 excitability; most TTX treated WT neurons recover their ability to fire and while the
355 distribution of firing rates is altered (Fig. 6d) the mean firing frequency does not differ
356 significantly from WT vehicle treated neurons after 48 hours (Fig. 6f). In response to the 48
357 hours of treatment with bicuculline, which acutely elevates activity, most WT neurons
358 significantly decreased their firing frequency. Of note, the extended bicuculline treatment in
359 WT neurons appears to lead to two types of firing, with one group of neurons maintaining a
360 high firing frequency and another group that is silent (Fig. 6d,f). In contrast, the firing
361 frequency of APP/PS1 neurons treated for 48 h with bicuculline remained increased, while
362 the firing frequency of APP/PS1 neurons after chronic TTX treatment remained low (Fig.
363 6e,g). Thus, APP/PS1 neurons did not respond to the prolonged bicuculline or TTX
364 exposure as the WT neurons, indicating that the APP/PS1 neurons were unable to
365 compensate to these pharmacologically-induced changes in neuronal activity.

366

367 To explore a mechanistic aspect of these HSP/HIP alterations we investigated axon initial
368 segment (AIS) modifications in the chronic TTX and bicuculline treated cultures, as
369 lengthening or shortening of the AIS modifies the excitability of neurons (Hedstrom *et al.*
370 (2008). By immunolabeling for ankyrin-G, a protein involved in linking voltage-gated
371 channels to the AIS, we could identify the AIS and measure its length (Hedstrom *et al.*,
372 2008). In WT neurons, 48 hours of treatment with either bicuculline or TTX led to the
373 expected decreased and increased AIS lengths, respectively (Fig. 6h, i). In contrast, APP/PS1
374 neurons did not display adjustments of the AIS length upon either of these HSP/HIP-
375 inducing treatments. Interestingly, the AIS lengths of APP/PS1 neurons were already
376 shorter than those of WT neurons at baseline. Together these experiments demonstrate an
377 inability of APP/PS1 neurons to use HSP/HIP mechanisms to adapt to extrinsic changes in
378 activity.

379

380 **Discussion**

381 Here we present evidence that homeostatic plasticity mechanisms are disrupted in
382 APP/PS1 AD transgenic neurons. While A β and APP have recently been implicated in
383 normal HSP (Gilbert *et al.*, 2016; Galanis *et al.*, 2021), we present evidence of dysfunctional
384 homeostatic plasticity in AD transgenic neurons. This could help explain why AD transgenic
385 mice are more susceptible to pharmacologically-induced and spontaneous seizures
386 (Minkeviciene *et al.*, 2009; Reyes-Marin & Nuñez, 2017) and could provide a framework for

387 explaining the increased sensitivity to seizures in AD patients (Pandis & Scarmeas, 2012).
388 Furthermore, we present data consistent with hyperexcitability in APP/PS1 compared to
389 WT neurons, which we show occurs mainly in excitatory neurons. The calcium oscillation
390 hyper-activity that we observed appeared to be network-driven rather than cell intrinsic.
391 Moreover, part of the hyper-activity in AD transgenic neurons may be caused by the over-
392 expression of APP independent of A β . However, elevated levels of A β alone also increased
393 activity.

394
395 Similar to previous studies, we found that exogenously added A β 1-42 primarily targeted
396 synapses of CaMKII-positive neurons, specifically in a dendritic rather than axonal pattern
397 (Lacor *et al.*, 2004; Willen *et al.*, 2017). However, this does not exclude the possibility of A β
398 binding to presynaptic terminals proximal to dendrites. Still, the binding of A β 1-42 to
399 CaMKII-positive synapses might explain why excitatory neurons, in particular, are affected.

400
401 The increased frequencies and amplitudes of calcium oscillations that we observed with
402 neprilysin inhibition as a means to elevate endogenous A β are consistent with results from
403 Abramov *et al.* (2009), showing increased release probability with thiorphan treatment in
404 cultured hippocampal neurons. However, since neprilysin also degrades various
405 enkephalins and peptide neurotransmitters, such as substance P, which can influence
406 calcium stores (Heath *et al.*, 1994), we additionally showed a lack of effect on calcium
407 oscillations with neprilysin inhibition in APP KO neurons.

408

409 As the calcium oscillations were increased in APP/PS1 compared to wild type neurons, we
410 also considered whether this might be due to decreased inhibitory interneurons/synapses.
411 However, we did not see significant differences in the protein levels of GAD67 and CaMKII,
412 markers of GABAergic interneurons and excitatory neurons, respectively. Further, we did
413 not detect significant differences in the synaptic density of either excitatory or inhibitory
414 synapses. While neuropathological studies have emphasized the vulnerability of select
415 classes of excitatory projection neurons (Stranahan & Mattson, 2010), the relative
416 involvement of different inhibitory and excitatory neurons in early A β -induced
417 hyperactivity remains poorly defined. Increasing evidence suggests that alterations in
418 inhibitory neuron connectivity lead to changes in network functions in AD. For example,
419 increased parvalbumin and gephyrin labeling perisomatically in CA1 neurons of young
420 APP/PS1 transgenic mice was shown (Hollnagel *et al.*, 2019), which might represent an
421 adaptation to increased A β /APP and, therefore, increased activity in these mice.

422

423 The function of neuronal networks is highly dependent on maintaining homeostatic set
424 points and keeping activity within functional windows. Deviations from these set-points
425 lead to network dysfunction. Structural homeostatic synaptic plasticity is known to occur
426 at three main locations: 1. at the post-synapse involving reduced or increased levels of
427 surface receptors (Turrigiano *et al.*, 1998); 2. at the axon initial segment (AIS) by either
428 increasing or decreasing its length or by shifting the AIS further out into the axon
429 (Wefelmeyer *et al.*, 2016); or 3. at the pre-synapse by modifying how much

430 neurotransmitter is stored in synaptic vesicles or through homeostatic maintenance of
431 presynaptic exocytosis (Delvendahl *et al.*, 2019). Of note, A β was suggested to overshoot
432 normal homeostatic scaling in response to sensory deprivation *in vivo* or TTX-mediated
433 inhibition *in vitro* (Gilbert *et al.*, 2016), and A β was recently shown to regulate homeostatic
434 synaptic upscaling after activity blockade in dentate gyrus *in vivo* (Galanis *et al.*, 2021). Our
435 findings that APP/PS1 neurons do not increase their activity after 48 hours of TTX
436 treatment nor decrease firing rate after 48 hours of bicuculline treatment suggest that
437 A β /APP play an important roles not only in adjusting to activity blockade but also have
438 roles in homeostatic downscaling in response to excessive activity. Interestingly, we found
439 that 48 hours of bicuculline treatment led to a strong reduction in firing in the majority of
440 neurons in WT cultures and another population of neurons that maintained a high firing
441 frequency; a related observation was made in visual cortex homeostatic plasticity following
442 visual deprivation (Barnes *et al.*, 2015), where differential adaptations by excitatory and
443 inhibitory neuron populations was described. Interestingly, the mean firing rates are
444 similar between TTX, vehicle and bicuculline treated WT neurons after 48 hours consistent
445 with findings suggesting that while single unit firing is unstable, networks maintain
446 surprisingly stable firing frequencies (Slomowitz *et al.*, 2015). We previously showed in
447 primary neurons from Tg2576 AD transgenic mice, which overexpress human APP with the
448 Swedish mutation, reduced AMPA receptor levels in culture (Almeida *et al.*, 2005). Whether
449 this is the result of adaptation to higher basal activity levels or a consequence of
450 synaptotoxicity remains to be determined. Moreover, we demonstrated that APP protein
451 levels decrease with chronic TTX treatment. This reduction of APP protein levels could
452 potentially be involved in increasing excitability of excitatory neurons as it has been

453 reported that conditional APP family triple knockout increases excitability of excitatory
454 neurons (Lee *et al.*, 2020) and we previously showed that APP knockout increased GluA1
455 protein levels in cultured neurons (Martinsson *et al.*, 2019). While TTX decreased APP, the
456 bicuculline treatment did not significantly alter the APP levels. Neuronal activity can also be
457 modulated by modifying the AIS. We show evidence that the AIS is lengthened with TTX
458 treatment and shortened after treatment with bicuculline in WT neurons, which, however,
459 did not occur in APP/PS1 neurons. Shortening the AIS is a way to decrease intrinsic
460 excitability and previous work in slice cultures has shown that 1 hour of bicuculline
461 treatment was sufficient to decrease the length of the AIS (Jamann *et al.*, 2021). Since the
462 average lengths of the AIS in APP/PS1 neurons are shorter at baseline than in WT neurons,
463 and are not altered by chronic treatments known to induce HSP, our findings could indicate
464 that APP/PS1 neurons have attempted to adapt to reduce excitability (reduced baseline
465 AIS) but are unable to do so to treatments that normally would induce HSP.

466

467 Cortical neurons in proximity to plaques were reported to have higher basal calcium levels
468 in spines and dendrites (Kuchibhotla *et al.*, 2008). A β oligomers of different varieties have
469 been reported to bind various cell surface receptors such as PrP (Lauren *et al.*, 2009),
470 alpha7 nicotinic receptor (Sadigh-Eteghad *et al.*, 2014) and Ephrins (Vargas *et al.*, 2018),
471 leading to an influx of calcium. Yet another hypothesis proposes that A β increases the cell
472 membrane permeability for calcium (Kawahara & Kuroda, 2000; Kagan *et al.*, 2002). To
473 complicate matters, presenilins have also been implicated in the handling of Ca²⁺ stores
474 independently of γ -secretase in AD transgenic mouse models (Lerdkrai *et al.*, 2018). A

475 recent study suggested that A β dimers could cause hyperactivity by inhibiting glutamate
476 reuptake (Zott *et al.*, 2019). Further, it was reported that A β oligomers can impair synaptic
477 activity by repressing P/Q calcium channels (Nimmrich *et al.*, 2008). While we prepared
478 synthetic A β in DMSO, which prevents the formation of fibrils, A β forms amyloids with time
479 in culture (Takahashi *et al.*, 2004) and progressively aggregates at synapses (Takahashi *et*
480 *al.*, 2002; Takahashi *et al.*, 2004; Willén *et al.*, 2017), consistent with A β aggregation at
481 synaptic compartments (Bilousova *et al.*, 2016). Moreover, while A β and APP influence
482 synaptic activity, neuronal activity also regulates APP cleavage and A β generation
483 (Kamenetz *et al.*, 2003); increased neuronal activity can increase both the generation and
484 degradation of A β (Kamenetz *et al.*, 2003; Tampellini *et al.*, 2009). Thus, converging data
485 indicate that both APP and A β are important for regulating neuronal activity. Among
486 questions that remain to be answered are which specific aspects of neuronal activity APP
487 and A β regulate/influence. Many transgenic models of AD exhibit epileptic seizures and
488 hyperactivity (Scharfman, 2012; Born *et al.*, 2014), and even models overexpressing wild-
489 type human APP develop seizures, which could be consistent with our data. Importantly,
490 hyper-synchrony in AD transgenic mice could be rescued by genetic suppression of APP
491 over-expression (Born *et al.*, 2014). A better understanding of the neuron subtypes and
492 molecular mechanisms involved in early A β /APP-induced hyperexcitability and synapse
493 dysfunction might provide not only new insights into the disease, but also to new treatment
494 strategies for AD.

495

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722 **Figure legends**

723 **Figure 1. Increased spontaneous activity in APP/PS1 primary neurons. a)** Frequency
724 of firing (spikes per minute) is increased in APP/PS1 compared to WT neurons (APP/PS1
725 mean = 6.072, CI = 4.948-7.197, n = 90 compared to WT mean = 3.184, CI = 2.25-4.118, n =
726 142, p < 0.0001). **b)** Amplitudes of spikes are increased in APP/PS1 compared to WT

727 neurons (APP/PS1 mean = 0.1682, CI = 0.1532-0.1833, n = 76, compared to WT mean =
728 0.1352, CI = 0.1266-0.1437, n = 80, p = 0.0003). **c)** Inter-spike interval distributions differ
729 between APP/PS1 compared to WT neurons with more of the APP/PS1 neurons having low
730 inter-spike intervals (p = 0.0011 using Kolmogorov-Smirnoff test). **d)** Frequency
731 distribution of firing frequencies of APP/PS1 compared to WT neurons shown in a graph;
732 note higher percentage of WT neurons in the inactive bins; N = 4 cultures. **e)** Graph
733 depicting decrease of firing frequency in APP/PS1 neurons treated with AMPA receptor
734 antagonist CNQX (10 μ M; vehicle mean = 3.276, CI = 2.758-3.794, n = 329 compared to
735 CNQX mean = 0.2423, CI = 0.1265-0.3581, n = 172, p = 0.0001); N = 3. Kruskal-Wallis test
736 with Dunn's correction for multiple comparisons; * p < 0.05, **, p < 0.01 ***, p < 0.001.

737 **Figure 2. Increased amplitude and frequency of spontaneous calcium transients in**
738 **CaMKII positive excitatory neurons. a)** Micrograph provides an example of an image of
739 Fluo-4 (green) and CaMKII TdTomato (red) neurons in culture. **b)** Graph showing increase
740 in spike frequency (spikes per minute) of APP/PS1 CaMKII positive compared to WT
741 CaMKII positive neurons (APP/PS1 mean = 5.176, CI = 4.456-5.896, n = 133 compared to
742 WT mean = 3.479, CI = 2.9-4.057, n = 205, p = 0.0001). In contrast, spike frequency of
743 CaMKII negative APP/PS1 neurons did not significantly differ from WT CaMKII negative
744 neurons (APP/PS1 mean = 6.094, CI = 4.898-7.291, n = 108 compared to WT mean = 5.083,
745 CI = 3.837-6.328, n = 114, p = 0.124). **c)** Amplitude of transients is increased specifically in
746 APP/PS1 compared to WT CaMKII positive excitatory neurons (APP/PS1 mean = 0.171, CI
747 0.1566-0.1856, n = 115, compared to WT mean = 0.1375, CI = 0.1304-0.1446, n = 138, p =
748 0.0024). In contrast, the amplitude of transients in CaMKII negative neurons did not differ

749 between APP/PS1 and WT neurons (APP/PS1 mean = 0.1505, CI = 0.1385-0.1625, n = 85,
750 compared to WT mean = 0.1391, CI = 0.1275-0.1506, n = 78, p = 0.302); N = 3. Kruskal-
751 Wallis test with Dunn's correction for multiple comparisons. ; * p < 0.05, ** p < 0.01 *** p <
752 0.001; scale bar: 50 μ m.

753 **Figure 3. No evidence for gross imbalances in excitatory/inhibitory**
754 **neurons/synapses in APP/PS1 compared to WT neurons. a)** Representative western
755 blot of CaMKII and GAD67 protein levels with actin as loading control. **b)** Quantification of
756 western blot of CaMKII in a) (APP/PS1 mean = 1.164, CI = 1.030-1.298, n = 15 and WT mean
757 = 1.181, CI = 1.080-1.282, n = 21, p = 0.8270). **c)** Quantification of western blot of GAD67 in
758 a) (APP/PS1 mean = 0.7359, CI = 0.6129-0.8589, n = 15 and WT mean = 0.6620, CI =
759 0.5774-0.7466, n = 21, p = 0.2860, unpaired t-test). **d)** Representative micrograph showing
760 WT neuron labeled with VGAT (red), Gephyrin (green) and MAP2 (magenta). Lower panels
761 show an enlarged view of neuron both with (left) and without (right) deconvolution. Scale
762 bars = 20 μ m and 10 μ m. **e)** Graph depicting excitatory synaptic density from
763 VGLUT/CAMKII overlap divided by neurite length (APP/PS1 mean = 16.64., CI = 12.53-
764 20.74, n = 35 and WT mean = 15.28, CI = 11.74-18.83, n = 38, p = 0.6129, unpaired t-test). **f)**
765 Graph depicting inhibitory synaptic density from VGAT/Gephyrin overlap divided by
766 neurite length (APP/PS1 mean = 12.00, CI = 9.160-14.84, n = 29 and WT mean = 11.87, CI =
767 9.243-14.50, n = 29 p = 0.9458). **g)** Graph depicting quantification of percentage CaMKII
768 neurons in WT and AD cultures. (APP/PS1 mean = 49.61% CaMKII positive neurons CI =
769 41.67-57.54% compared to WT mean = 54.64% CI = 47.08-62.14%, p = 0.35, unpaired t-
770 test). **h)** Representative binary images of WT and APP/PS1 neurons labeled for MAP2; scale

771 bar = 20 μ m. **i)** Graph showing decreased dendrite length in APP/PS1 compared to WT
772 neurons (APP/PS1 mean = 1518, CI = 1355-1682, n = 64, and WT mean = 1744, CI = 1588-
773 1900. N = 67, p = 0.045, unpaired t-test).

774 **Figure 4. APP and A β increase calcium oscillation frequency and amplitude. a)** Graph
775 depicts firing frequencies in WT neurons transduced with a viral vector harboring hAPPwt
776 or hAPPmv (BACE cleavage resistant) under a CaMKII promoter. Note that both hAPPwt
777 and hAPPmv increase the firing frequency compared to control (CTRL), with hAPPmv
778 having a stronger effect (hAPPmv mean = 5.087, CI = 4.542-5.633, n = 436; hAPPwt mean =
779 4.112, CI = 3.585-4.640, n = 375 and CTRL mean = 3.757, CI = 2.925-4.588, n = 292, p values
780 respective to CTRL = hAPPmv = 0.0001, hAPPwt = 0.0001, p value hAPPmv compared to
781 hAPPwt (p = 0.0259), Kruskal Wallis Dunn's correction). **B)** Graph showing increased
782 amplitude in neurons expressing hAPPwt and hAPPmv under CaMKII promoter (hAPPmv
783 mean = 0.3444, CI = 0.3197-0.3691, n = 344 and hAPPwt mean = 0.3021, CI = 0.2808-
784 0.3433, n = 278 and CTRL mean = 0.2256, CI = 0.2034-0.2479, n = 67, , N = 3, p values
785 respective to CTRL; hAPPmv = 0.0001, hAPPwt = 0.0004, p value, Kruskal Wallis Dunn's
786 correction.). Kruskal-Wallis test with Dunn's correction for multiple comparisons. ; * p <
787 0.05, ** p < 0.01 *** p < 0.001. **c)** WT neurons treated with 500 nM of the neprilysin
788 inhibitor thiorphan for 1 hour show increased firing frequency (thiorphan mean = 6.814, CI
789 = 5.283-8.344, n = 133, compared to vehicle mean = 5.783, CI = 3.749-7.817, n = 125, N = 3,
790 p = 0.0101). **d)** The graph depicts increased spike amplitudes after 1 hour of treatment of
791 WT neurons with thiorphan (thiorphan mean = 0.1605, CI = 0.1436-0.1774, n = 76,
792 compared to vehicle mean = 0.1198, CI = 0.1036-0.136, n = 42, p = 0.0012). **e)** Graph

793 showing increase in spike frequency of thiorphan treated CaMKII positive compared to
794 vehicle treated CaMKII positive neurons (thiorphan mean = 3.905, CI = 2.942-4.869, n = 128
795 compared to vehicle mean = 2.432, CI = 1.934-2.931, n = 332, N = 3, p = 0.0089). In contrast,
796 spike frequency of CaMKII negative thiorphan treated neurons did not significantly differ
797 from vehicle treated CaMKII negative neurons (thiorphan mean = 2.566, CI = 1.726-3.406, n
798 = 173 compared to vehicle mean = 3.018, CI = 2.243-3.794, n = 218, p = 0.0894). **f)** Graph
799 showing how thiorphan was ineffective at inducing increased frequency in APP KO neurons
800 compared to vehicle (APP KO thiorphan mean = 3.329, CI = 2.692-3.967, n = 601 compared
801 to APP KO vehicle mean = 2.196, CI = 1.689-2.704, n = 514, N = 3, p = 0.8604, Mann-Whitney
802 U-test).

803 **Figure 5. A β preferentially binds synaptic compartments on CaMKII positive neurons**
804 **and appears to have a dose dependent effect on spike frequency. a)** Micrograph
805 showing that synthetic human A β 1-42 preferentially binds to dendrites of CaMKII positive
806 murine WT neurons; Td Tomato expressed through CaMKII promoter, human specific anti-
807 A β antibody 6E10 (green) and conformation specific anti-amyloid antibody OC (magenta);
808 scale bar = 50 μ m. **b)** Insert from a) showing A β 1-42 targeting synapses; note that antibody
809 6E10 labels the added synthetic human A β and that this is also labeled by the fibrillar
810 oligomer antibody OC; scale bar = 5 μ m. **c)** WT neurons treated with 200 pM synthetic A β 1-
811 42 show increased spike frequency compared to DMSO treated vehicle control neurons
812 both in CaMKII positive neurons (A β 1-42 mean = 0.7554, CI = 0.5724-0.9383, n = 477,
813 compared to vehicle mean = 0.367, CI = 0.1636-0.5704, n = 401, p < 0.0001) and in CaMKII
814 negative neurons (A β 1-42 mean = 3.829, CI = 3.033-4.685, n = 214, compared to vehicle

815 mean = 3.346, CI = 2.508-4.183, n = 218). **d)** Graph depicting increased amplitude in CaMKII
816 positive neurons treated with 200 pM A β 1-42 compared with DMSO vehicle control (A β 1-
817 42 mean = 0.1256, CI = 0.119-0.1321, n = 128, compared to vehicle mean = 0.1087, CI =
818 0.09495-0.1225, n = 24, p = 0.045). However, CaMKII negative neurons did not show a
819 significant increase in amplitude with A β treatment (A β 1-42 mean = 0.1272, CI = 0.1209-
820 0.1334, n = 133, compared to vehicle mean = 0.1235, CI = 0.114-0.133, n = 94, p = 0.2103).
821 **e)** Graph shows WT neurons treated with 500 nM A β 1-42 or 500 nM A β 1-40. While 500 nM
822 A β 1-42 leads to a slight but not significantly different distribution of activity, A β 1-40
823 strongly increases activity (A β 1-40 mean = 6.344, CI = 5.503-7.186, n = 235 and A β 1-42
824 mean = 3.901, CI = 3.439-4.363, n = 287 compared to vehicle mean = 3.194, CI = 2.752-
825 3.635, n = 255; p = 0.0001 and p = 0.14.); Kruskal Wallis test, N = 3.

826 **Figure 6. Neurons from AD transgenic mice are unable to initiate homeostatic**
827 **synaptic/intrinsic plasticity. a)** Representative western blot of APP and CaMKII α in WT
828 and APP/PS1 neurons treated with TTX or bicuculline for 48 hours; α Tubulin is used as a
829 loading control. **b)** Graph depicts quantification of fold change from western blots in a),
830 APPY188 upper band (WT TTX mean = 0.2865, CI 0.1287-0.4244, n = 5, WT control mean
831 =1.000, CI 0.5298-1.470, n = 5, WT bicuculline mean = 0.9782, CI 0.3574-1.599, n = 3,
832 APP/PS1 TTX mean = 0.4251, CI 0.2893-0.5609, n = 6, APP/PS1 control mean = 1.137, CI
833 0.8116-1.462, n = 6, APP/PS1 bicuculline mean = 0.9038, CI 0.6975-1.110, n = 3) and
834 APPY188 lower band (WT TTX mean = 0.4605, CI 0.1990-0.7220, n = 5, WT control mean
835 =1.000, CI 0.6601-1.340, n = 5, WT bicuculline mean = 1.386, CI -0.5278-3.299, n = 3,
836 APP/PS1 TTX mean = 0.8590, CI 0.4258-1.292, n = 6, APP/PS1 control mean = 2.239, CI

837 1.493-3.290, n = 6, APP/PS1 bicuculline mean = 1.602, CI 0.6279-2.576, n = 3). Note that
838 upper APP band is lower in TTX treated WT compared to WT control p = 0.0006 and TTX
839 treated APP/PS1 compared to APP/PS1 control p = 0.0002. For APP lower band APP/PS1
840 control was significantly higher than WT control p = 0.0017 while APP/PS1 TTX was lower
841 than APP/PS1 control p = 0.0003 (one-way ANOVA, Šidak correction). **c)** Micrograph
842 showing APPY188 and CaMKII α labelling after 48 hours of TTX or Bicuculline treatment.
843 Note that APPY188 labeling is relatively weaker in TTX treated neuron consistent with the
844 results from western blot. Scale bar = 20 μ m. **d)** Graph demonstrating HSP adaptations in
845 WT cultures treated with TTX or bicuculline for 48 hours. Note that the distribution of
846 oscillation frequency in bicuculline treated neurons is different compared to vehicle treated
847 (TTX mean = 4.265, CI 3.397-5.134, n = 442; vehicle mean = 4.467, CI = 3.741-5.192, n =
848 413; bicuculline mean = 4.593, CI = 3.574-5.611, n = 303, p values compared to vehicle: TTX
849 = 0.0001 and bicuculline = 0.0001, Kruskal-Wallis test, N = 4). **e)** Graph showing absence of
850 adaptation in APP/PS1 neurons in response to 48 hours of TTX or bicuculline treatment.
851 Note how both TTX and bicuculline treated APP/PS1 are largely opposite from WT neurons
852 treated with TTX bicuculline after 48 hours. (TTX mean = 0.272, CI 0.0154-0.527, n = 210;
853 vehicle mean = 4.368, CI = 3.729-5.008, n = 464; bicuculline mean = 10.990, CI = 9.835-
854 12.140, n = 368, p values compared to vehicle: TTX = 0.0001 and bicuculline = 0.0001,
855 Kruskal-Wallis test, N = 4). **f)** bar graph showing mean firing frequency and CI from d) note
856 that mean firing frequencies are similar between the groups. (WT TTX vs WT vehicle p =
857 0.9203, WT bicuculline vs WT vehicle, p = 0.9733, one-way ANOVA, dunnett's test). **g)** bar
858 graph showing mean firing frequency and CI from e) note that mean firing frequencies are
859 do not recover as in WT. (APP/PS1 TTX vs APP/PS1 vehicle p = 0.0001, APP/PS1

860 bicuculline vs APP/PS1 vehicle, $p = 0.0001$, one-way ANOVA, dunnet's test). **h)** Micrograph
861 depicting WT neuron labeled with MAP2 (red) and ankyrin-G (green), which labels the AIS.
862 White arrow points to AIS; scale bar = 20 μm . **i)** Graph shows quantification of AIS lengths
863 after treatment with TTX or bicuculline for 48 hours (WT TTX mean = 47.76, CI = 42.36-
864 53.17, $n = 32$; WT vehicle mean = 39.44, CI = 35.87-43.02, $n = 37$; WT bicuculline mean =
865 28.07, CI = 25.75-30.39, $n = 57$; APP/PS1 TTX mean = 27.52, CI = 25.01-30.04, $n = 35$;
866 APP/PS1 vehicle mean = 27.20, CI = 24.85-29.55, $n = 49$; APP/PS1 bicuculline mean = 21.57,
867 CI = 18.86-24.27, $n = 26$); ordinary one way ANOVA, Tukey's multiple comparisons test.

868
Supplemental figure 1. Live-cell imaging method for assaying spontaneous calcium
869 **transients. a-b)** Representative traces of spontaneous activity over a 2 min period in
870 neurons from WT and APP/PS1 mice, respectively. **c-d)** Raster plots showing overall
871 calcium transients (spikes represented as green dots) in WT and APP/PS1 neuron field of
872 views (FOVs); ROI# or neuron number on the Y-axis and time in minutes on the X-axis.

874 **Supplemental figure 2. APP/PS1 neurons respond appropriately to both GABA**
875 **inhibitors and tetrodotoxin. a)** Representative Raster plot of APP/PS1 neurons treated
876 with vehicle (sterile milliQ water); neurons were imaged every 100 ms for 5 min with
877 detected spikes represented as green dots. **b)** Representative raster plot of APP/PS1
878 neurons treated with 10 μM of picrotoxin. **c)** Representative raster plot of APP/PS1
879 neurons treated with 20 μM of bicuculline. **d)** Representative raster plot of APP/PS1
880 neurons treated with 1 μM of tetrodotoxin (TTX). **e)** Graph shows frequency from WT
881 neurons treated with 1 μM TTX or 20 μM bicuculline. Note that TTX significantly reduces

882 calcium oscillations whereas bicuculline significantly increases them compared to vehicle
883 treated CTRL (TTX mean = 0.01429 CI = -0.01421-0.04278, n = 70; Vehicle mean = 2.923, CI
884 = 2.094-3.752, n = 209; bicuculline mean = 25.00, CI = 23.04-26.97, n = 104, p values
885 compared to vehicle: TTX = 0.0001 and bicuculline = 0.0001, Kruskal-Wallis test). **f)** Graph
886 demonstrating acute effects of TTX and bicuculline in APP/PS1 neurons. Note that similar to
887 in WT neurons, TTX blocks activity while bicuculline raises activity (TTX mean = 0.1644 CI -
888 0.07614-0.4050, n = 118; vehicle mean = 5.164, CI = 4.140-6.188, n = 188; bicuculline mean
889 = 13.77, CI = 10.49-17.06, n = 97, p values compared to vehicle: TTX = 0.0001 and
890 bicuculline = 0.0379, Kruskal-Wallis test).

891

Figure 1

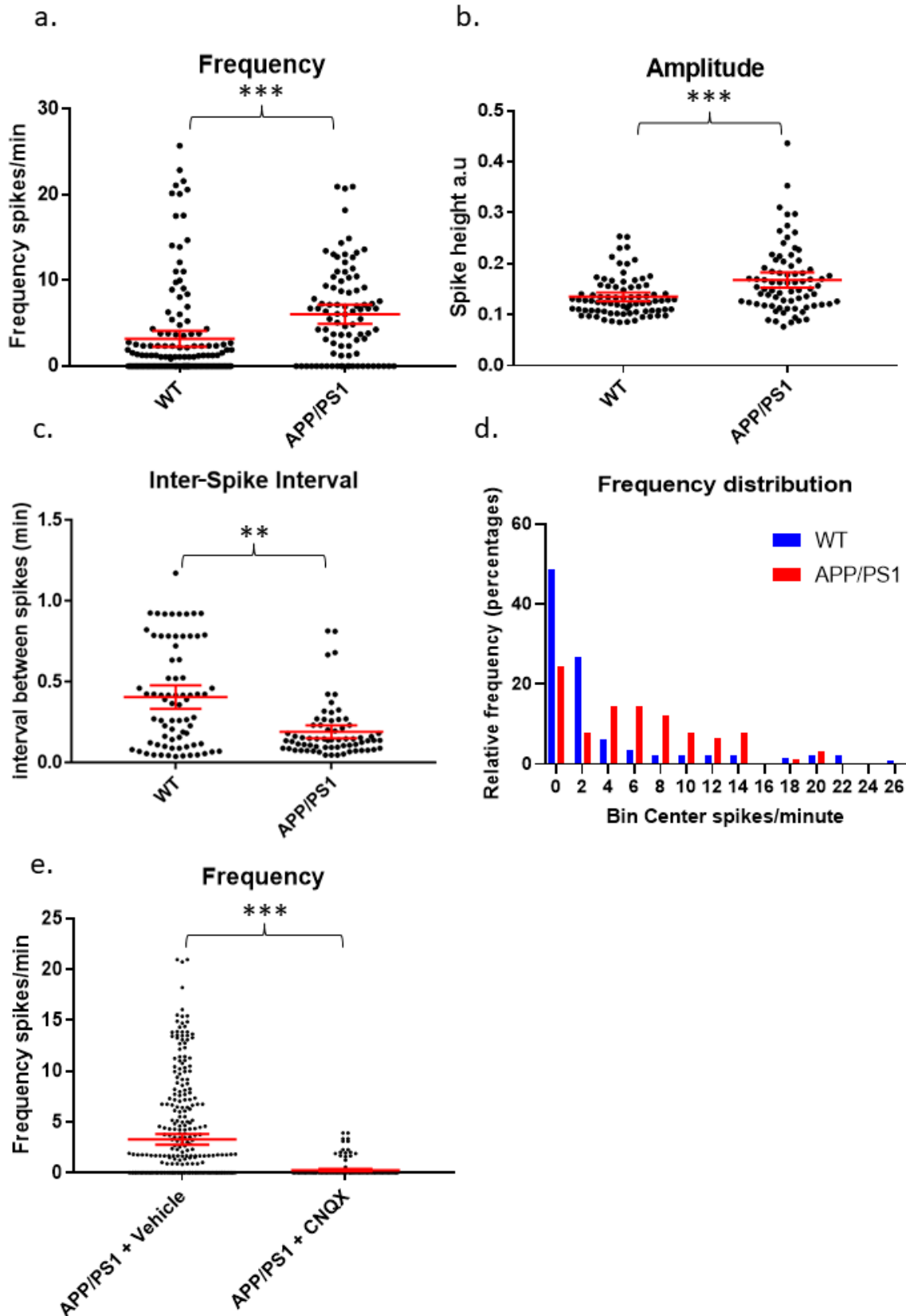


Figure 2

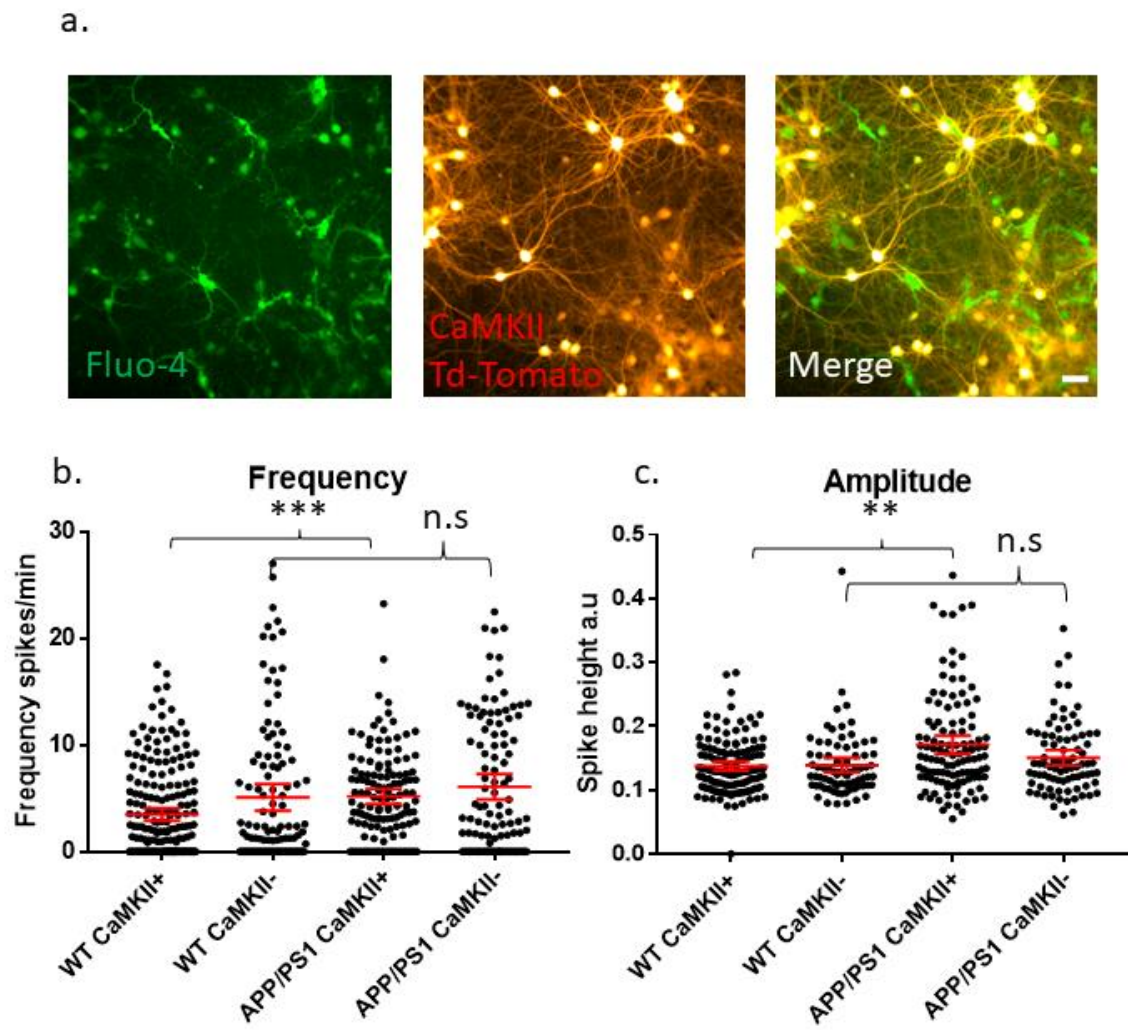


Figure 3

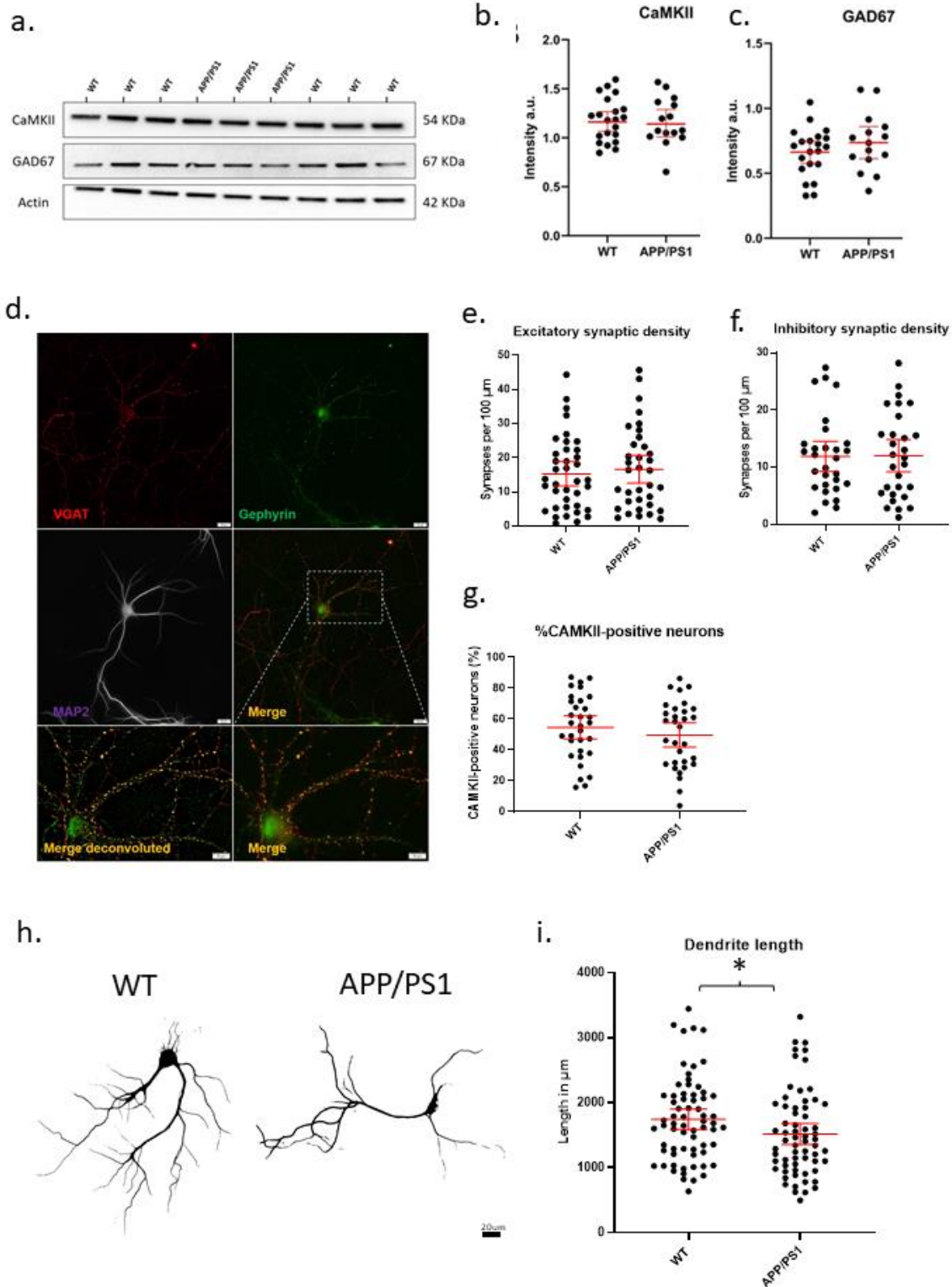


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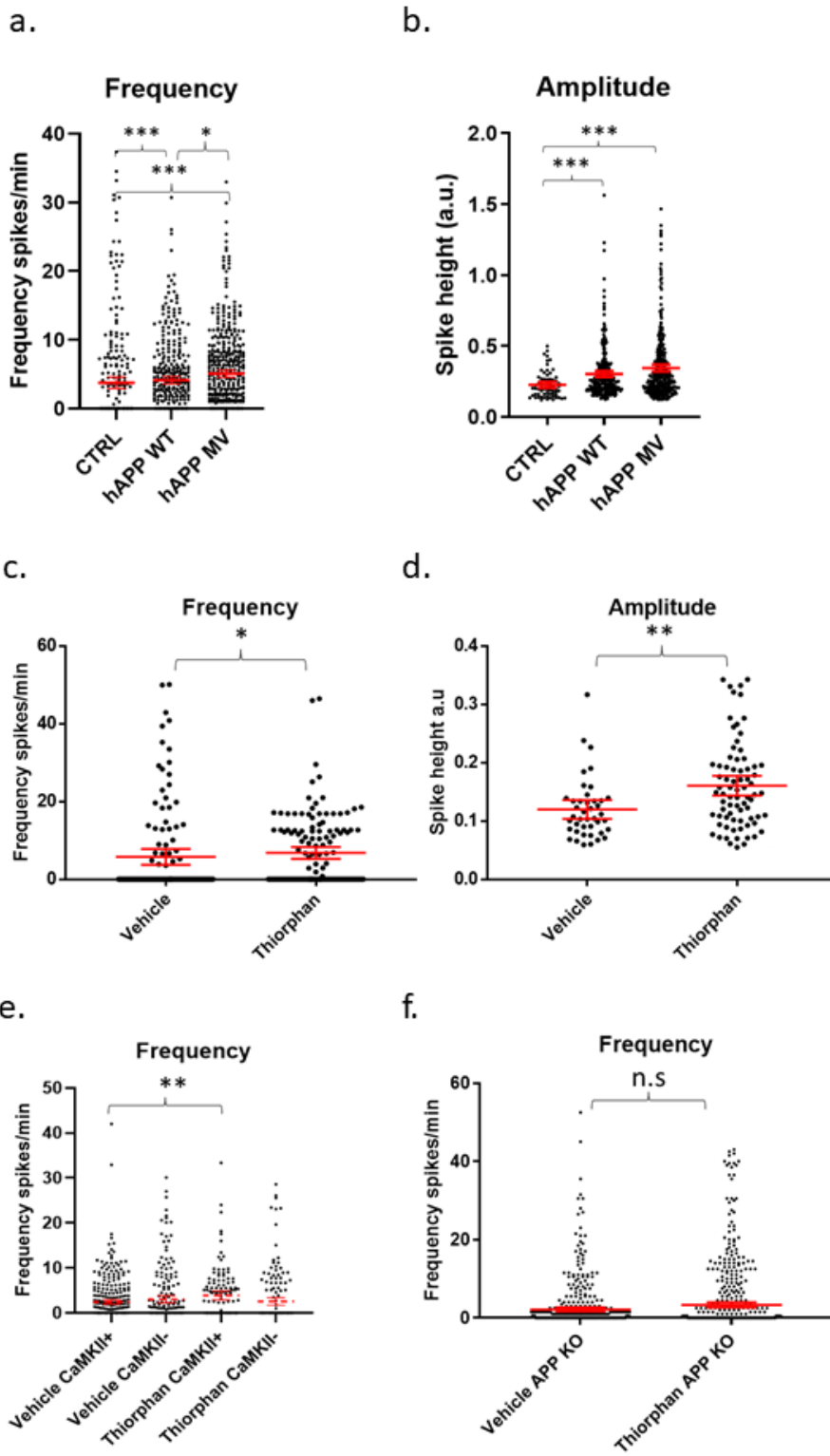
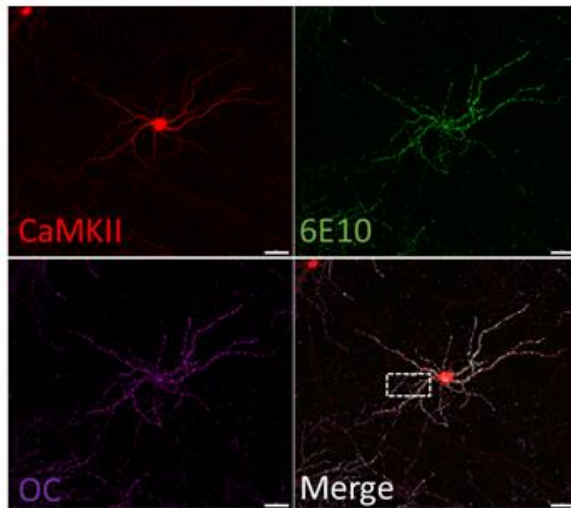
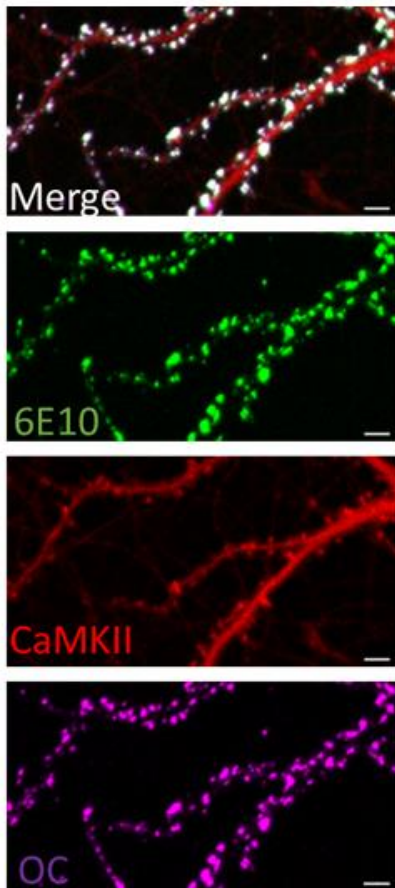


Figure 5

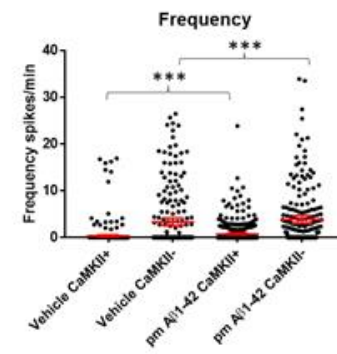
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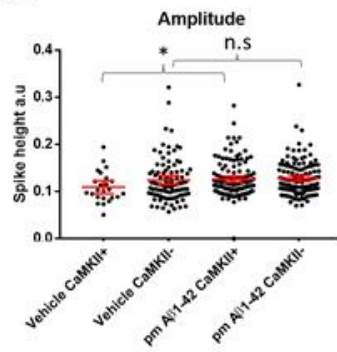
b.



c.



d.



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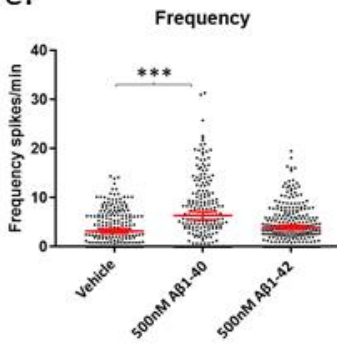


Figure 6

