

Title

Glucocorticoids activate a synapse weakening pathway culminating in tau phosphorylation in the hippocampus

Authors

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1 **Abstract**

2 Evidence suggests that the stress hormones glucocorticoids (GCs) can cause
3 cognitive deficits and neurodegeneration. Previous studies have found GCs facilitate
4 physiological synapse weakening, termed long-term depression (LTD), though the
5 precise mechanisms underlying this are poorly understood. Here we show that GCs
6 activate glycogen synthase kinase-3 (GSK-3), a kinase crucial to synapse
7 weakening signals. Critically, this ultimately leads to phosphorylation of the
8 microtubule associated protein tau, specifically at the serine 396 residue, and this is
9 a causal factor in the GC-mediated impairment of synaptic function. These findings
10 reveal the link between GCs and synapse weakening signals, and the potential for
11 stress-induced priming of neurodegeneration. This could have important implications
12 for our understanding of how stress can lead to neurodegenerative disease.

13 **Keywords: Glucocorticoids; Long-term potentiation; GSK-3; Tau**

14 **1. Introduction**

15 Repeated and chronic stress can cause acceleration of brain ageing, memory
16 deficits and various mental illnesses [1,2]. Rising levels of the stress hormone
17 cortisol are strongly correlated with temporal lobe atrophy and cognitive deficits
18 during ageing in humans [2]. These effects are prevalent in patients with
19 hypercortisolism [3,4] and can even be mirrored in otherwise healthy individuals by
20 repeated exposure to environmental stressors such as jet lag [5]. It has been
21 reported that prolonged secretion of glucocorticoids (GCs) during chronic stress, or
22 exogenous application of GCs, disrupts memory performance in both humans [6,7]
23 and rodents [8-10]. Understanding the precise mechanisms underpinning how
24 elevated-GCs cause both cognitive deficits and structural atrophy in the brain
25 remains a major scientific challenge with numerous translational implications.

26 Several rodent studies have demonstrated that stress and GCs inhibit long-term
27 potentiation (LTP) but enhance long-term depression (LTD) [11,12]. Given the
28 postulated role for these synaptic phenomena in learning and memory [13], it is likely
29 that their dysregulation is central to the cognitive impairments induced by GCs.
30 Although the activation of glucocorticoid receptors (GRs) triggers a wide variety of
31 intracellular signals [14-16], the synaptic signalling pathways that underlie aberrant
32 synapse weakening by GCs remain to be elucidated.

33 Based on the aforementioned findings, one possibility is that GCs regulate the
34 direction of plasticity and/or the fate of synapses via modulation of certain signalling
35 pathways involved in LTD, or synapse weakening. A key molecule linking synapse
36 weakening and disruption of LTP expression is glycogen synthase kinase-3 (GSK-3)
37 [17]. Our previous studies also implicate caspase-3-induced cleavage of Akt1 as a

38 critical factor for the expression of physiological LTD [18], and suggest that it acts as
39 an upstream regulator of GSK-3 to enhance LTD in amyloid-beta (A β)-mediated
40 pathophysiological conditions [19]. Furthermore, GSK-3 orchestrates this synapse
41 weakening mechanism through regulation of tau phosphorylation (pTau) [20,21].
42 Given the key role of aberrant pTau in the pathophysiology of Alzheimer's disease
43 and stress-mediated neurodegeneration [22-24], we hypothesise that GSK-3-
44 mediated signalling pathways leading to pTau could be a synapse weakening
45 mechanism common to these diseased states.

46 Here we provide evidence that GCs activate a synapse weakening signal pathway
47 centred around the cleavage of Akt1 by caspase-3 and subsequent activation of
48 GSK-3, which causes aberrant pTau and abolishes LTP expression in the rat
49 hippocampus. We identify a single pTau residue (Ser396) as a critical downstream
50 signal in this mechanism, resulting in GC-mediated aberrant synaptic weakening.
51 These findings could provide further translation to our understanding of how GCs
52 prime progressive neurodegeneration through aberrant functional plasticity in the
53 hippocampus.

54 **2. Materials and Methods**

55 **2.1. Animals**

56 Experiments involving animals were conducted in accordance with the UK Animals
57 Scientific Procedures Act, 1986 and associated guidelines. All experimental
58 protocols were approved by the University of Bristol Animal Welfare & Ethical
59 Review Body. Male Wistar rats (Charles River, UK) were used to prepare
60 organotypic (7-day-old rats) and acute hippocampal slices (4- to 5-week-old rats).
61 Rats were housed four or five per cage, and were allowed access to water and food

62 *ad libitum*. The cages were maintained at a constant temperature ($23 \pm 1^\circ\text{C}$) and
63 relative humidity ($60 \pm 10\%$) under a 14/10 hour light-dark cycle (lights on from 8:00
64 A.M.).

65 **2.2. Electrophysiology**

66 *2.2.1. Acute hippocampal slice field recording*

67 Animals were sacrificed from 9:00 - 10:00AM by cervical dislocation. Brains were
68 quickly removed into ice-cold artificial cerebrospinal fluid (ACSF; 124mM NaCl, 3mM
69 KCl, 26mM NaHCO_3 , 1.25mM NaH_2PO_4 , 2mM CaCl_2 , 1mM MgSO_4 , 10mM D-
70 glucose, carbogenated with 95% O_2 / 5% CO_2). Hemispheres were separated with a
71 midsagittal cut and hippocampi were micro-dissected from each intact hemisphere.
72 Transverse hippocampal slices (400 μm) were cut using a McIlwain tissue chopper
73 (Mickle Laboratory Engineering) and allowed to recover in a submersion-type bath
74 filled with ACSF for at least 60 minutes. Before recording, hippocampal slices were
75 incubated in ACSF with pharmacological compounds at room temperature. Evoked
76 field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 region
77 using glass electrodes containing NaCl (3 M). Stimulating electrodes placed in the
78 subiculum and CA2 region (Schaffer collateral pathway). Stimuli were delivered
79 alternately to the two electrodes at a baseline rate of 0.066 Hz. Stimulus intensity
80 was set at 30-40% of saturating intensity. After 30 minutes of stable baseline, high-
81 frequency stimulation (2×100 pulses; 100 Hz, 30 s interval) was used for the LTP
82 induction protocol. LTP was gauged as the change of fEPSP slope relative to the
83 preconditioning baseline. Both control and experimental recordings were collected
84 using slices prepared from the same animal. Analysis of control LTP data across all
85 field recording experiments showed no significant difference between mean post-

86 conditioning fEPSPs in slices prepared from different rats (one-way ANOVA, $F(4,33)$
87 $= 2.147$, $p = 0.097$). Data were captured and analysed using LTP114j software. Data
88 are expressed relative to a normalized baseline. For analyses, the baseline was
89 defined as 5 points before tetanic stimulation and the post-conditioning time was set
90 at 75-80 minutes following recording commencement. The difference between
91 baseline and post-conditioning time-points was expressed as a percentage of
92 baseline \pm SEM, and was used to make comparisons between treatment groups.

93 2.2.2. Hippocampal slice culture and biolistic transfection

94 Organotypic slices were cultured based upon a method previously described by
95 Stoppini *et al.* (1991) [25]. Briefly, rats were decapitated, and their brains rapidly
96 removed and placed into ice-cold dissecting medium containing: 238 mM sucrose,
97 2.5 mM KCl, 26 mM NaHCO₃, 1 mM NaH₂PO₄, 5 mM MgCl₂, 11 mM D-glucose, and
98 1 mM CaCl₂. Hippocampi were extracted, and transverse hippocampal slices (350
99 μ m thickness) were cut and placed upon sterile, semi-porous membranes. These
100 were stored and maintained at the interface between air and culture medium
101 (containing: 78.8% Minimum Essential Medium, 20% heat-inactivated horse serum,
102 30 mM HEPES, 26 mM D-glucose, 5.8 mM NaHCO₃, 2 mM CaCl₂, 2 mM MgSO₄, 70
103 μ M ascorbic acid, 1 μ g/ml insulin, pH 7.3 and 320 – 330 mOsm/kg) inside a
104 humidified incubator at 35°C with a 5% CO₂-enriched atmosphere. Culture medium
105 was refreshed every 2 days, and slices were used for whole-cell recording at 6 – 8
106 days *in vitro* (DIV).

107 DNA-coated microcarriers for biolistic transfection of organotypic hippocampal slices
108 were prepared based on previously described methods [26]. At DIV 3–5, neurons

109 were transfected with plasmids expressing shRNA against rat tau protein (OriGene
110 Technologies). A mixture of four different tau shRNA constructs (1:1:1:1, in pGFP-V-
111 RS vector) was used for tau silencing. Phosphorylation-null (serine residues mutated
112 to alanine) human tau constructs of the AT8 [S199, S202, threonine 205 (T205)] and
113 PHF-1 (S396, S404) epitopes, in pCI-neo vectors, were kindly provided by Dr. A.
114 Takashima (Department of Aging Neurobiology, National Center for Geriatrics and
115 Gerontology, Obu, Japan). Individual mutations to the PHF-1 epitope (residues S396
116 or S404) were generated by site-directed mutagenesis (Agilent Technologies) of
117 2N4R human tau. Wild-type and triple mutant Akt1 (D108A/D119A/D462N) were
118 generated as previously described [18].

119 *2.2.3. Whole-cell patch clamp recording*

120 For whole-cell recordings from cultured organotypic slices, the recording chamber
121 was perfused with a buffer solution containing 119 mM NaCl, 2.5 mM KCl, 4 mM
122 CaCl₂, 4 mM MgCl₂, 26 mM NaHCO₃, 1 mM NaH₂PO₄, 11 mM glucose, 0.02 mM
123 picrotoxin, and 0.01 mM 2-chloroadenosine. The buffer solution was maintained at
124 29–30°C and saturated with 95% O₂/5% CO₂. Recording electrodes (5–6 MΩ)
125 containing CsMeSO₄ filling solution (130 mM CsMeSO₄, 8 mM NaCl, 4 mM Mg-ATP,
126 0.3 mM Na-GTP, 0.5 mM EGTA, 10 mM HEPES, and 6 mM QX-314, pH 7.2–7.3 and
127 270 –290 mOsm/kg) were used to voltage clamp CA1 pyramidal neurons. EPSCs
128 were recorded at a holding voltage of –70 mV, and only cells that had an initial R_s
129 (series resistance) < 20 MΩ that was maintained within 20% of that value from start
130 to finish were included in final data analysis. To induce LTP, depolarization of the cell
131 to 0 mV was paired with 200 pulses of 2 Hz stimulation to the Schaffer collateral

132 input. Analysis of control LTP data across all whole cell patch recording experiments
133 showed no variation in interleaved control LTP levels over time and in different slices
134 (one-way ANOVA, $F(4,11) = 1.775$, $p = 0.230$). The change in peak amplitude of the
135 EPSC, relative to baseline, was used to assess the effects of these stimulation
136 protocols on synaptic efficacy. For analysis purposes, the baseline was defined as 5
137 points before 2 Hz stimulation and the post-conditioning time was set as the last 5
138 points at the 40-minute time-point. The difference between baseline and post-
139 conditioning time-points was expressed as a percentage of baseline \pm SEM, and was
140 used to make comparisons between treatment groups.

141 **2.3. Western Blotting and Antibodies**

142 Hippocampal slices were collected and homogenised in lysis buffer (1% SDS,
143 292mM sucrose, 2.6mM EDTA, 1:10 Protease Inhibitor Cocktail (Sigma Aldrich),
144 1:100 Phosphatase Inhibitor Cocktail 3 (Sigma Aldrich)).

145 For immunoblotting, primary antibodies were used at the following dilutions: anti-
146 phospho-GSK-3 β Ser9 (rabbit polyclonal, Cell Signalling Technology, 1:500), anti-
147 GSK-3 β (H-76) (rabbit polyclonal, Santa Cruz Biotechnology, 1:500), anti-phospho-
148 Akt1 Ser473 (rabbit polyclonal, Cell Signalling, 1:1000), anti-phospho-Akt1 Thr308
149 (rabbit polyclonal, Cell Signalling, 1:1000), anti-Akt1 (rabbit polyclonal, Cell
150 Signalling, 1:1000), anti-PHF-1 (mouse monoclonal, kindly provided by Dr. P.
151 Davies, 1:1000), anti-phospho-Tau Ser396 (rabbit polyclonal, Life Technologies,
152 1:1000), anti-phospho-Tau Ser404 (rabbit polyclonal, Life Technologies, 1:1000),
153 anti-phospho-PHF-tau pSer202+Thr205 (AT8) (Mouse monoclonal, Thermo

154 Scientific, 1:1000), anti-Tau 5 (mouse monoclonal, Life Technologies, 1:1000), anti-
155 β -Actin (mouse monoclonal, Abcam, 1:10,000).

156 Membranes were incubated in horseradish peroxidase–conjugated secondary
157 antibodies at the following dilutions: anti-rabbit (goat polyclonal, Millipore, 1:5000),
158 anti-mouse (goat polyclonal, Millipore, 1:5000), followed by imaging with the G:Box
159 gel imaging system (Syngene) using the EZ-ECL detection system (Thermo
160 Scientific Inc.). Optical densities of immunoreactive bands were measured using
161 ImageJ software (NIH) and statistical analysis conducted with SigmaPlot (Systat
162 Software, Inc., USA). Example blots were cropped around regions of interest for
163 presentation in figures. For quantitative comparisons of treatment groups, optical
164 densities for proteins of interest were normalized to loading-control protein levels and
165 expressed relative to the average value from the control group.

166 **2.4. Akt1 kinase assay**

167 Akt1 activity was determined as described by the manufacturer's instructions (Cell
168 Signalling), with minor modifications. Acute hippocampal slices were washed with
169 TBS and homogenised in IP buffer (25 mM Tris pH 7.5, 150 mM NaCl, 1 % Triton X-
170 100, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 1 mM Na-Orthovanadate, 1X
171 phosphatase inhibitors and 1X Protease inhibitors). Lysates were centrifuged at
172 15,000 g for 15 minutes at 4°C and the supernatant transferred to fresh tubes.
173 Immunoprecipitation of Akt1 was performed using 500 μ g of lysate and incubated
174 with 2 μ g of Akt1 Ser473 antibody overnight at 4°C. Subsequently, 30 μ l of washed
175 Protein G agarose slurry was added and incubated at 4°C for 45 minutes with over-
176 end mixing. The beads were washed twice in IP buffer, followed by two washes in

177 NEB kinase buffer. The immobilised precipitated Akt1 was incubated in 40 μ l kinase
178 buffer containing 200 μ M ATP and 1 μ g of GSK3 β fusion protein at 30°C for 30
179 minutes. The reaction was stopped by addition of 20 μ l of 3X sample buffer. The
180 samples were vortexed and centrifuged prior to heating and protein separation by
181 SDS-PAGE.

182 **2.5. Statistical analysis**

183 Where practically possible, experiments were performed blind to experimental
184 condition. Results are presented as the mean \pm standard error of the mean (SEM).
185 Results were analysed using unpaired *t*-test for two independent groups or by one-
186 way ANOVAs with post-hoc Holm-Sidak analysis for multiple groups, as indicated in
187 the results text and figure legends. $p < 0.05$ was considered statistically significant. N
188 numbers represent samples from independent animals unless otherwise specified.

189 **3. Results**

190 *3.1. GCs activate a synapse weakening pathway to inhibit LTP*

191 In acutely prepared dorsal hippocampal slices, corticosterone (CORT; 1 μ M)
192 application for 2 hours (Ctrl: 146.12 \pm 2.9%; CORT: 114.33 \pm 5.6%, unpaired *t*-test
193 $t(13) = 5.2$, $p < 0.001$, Fig. 1a), or application of the synthetic glucocorticoid
194 dexamethasone (DEX; 200 nM, 2 hours, Ctrl: 164.35 \pm 4.5%; DEX: 133.92 \pm 3.9%,
195 unpaired *t*-test, $t(12) = 4.9$, $p < 0.001$, Fig. 1b), both inhibited LTP induction when
196 compared to untreated slices. No effects of CORT treatment on basal properties of
197 synaptic transmission were observed (Supplementary Fig. S1). Based on previous

198 studies [18-21], we hypothesised that GCs activate a caspase-3-Akt1-GSK-3
199 signalling (CAG) cascade that induces aberrant expression of LTD and inhibits LTP.
200 We therefore tested whether GC exposure impairs LTP via activation of this CAG
201 cascade. Co-application of the GSK-3 inhibitor CT-99021 (1 μ M) prevented the LTP
202 impairment that was induced by CORT treatment alone (CORT: $114 \pm 5.3\%$;
203 CORT+CT-99021: $142.9 \pm 3.8\%$, unpaired t-test, $t(10) = 3.1$, $p = 0.0114$, Fig. 1c),
204 suggesting that GSK-3 activation is required for CORT-mediated inhibition of LTP.
205 Furthermore, LTP impairment induced by CORT was prevented by the caspase
206 inhibitor Z-DEVD-FMK (10 μ M) but not by the non-active control peptide Z-FA-FMK
207 (10 μ M), supporting a role for caspase-3 upstream of GSK-3 activation in the
208 aberrant modulation of synaptic plasticity (one-way ANOVA, $F(3,22) = 8.85$, $p =$
209 0.003 ; Holm-Sidak post hoc test for CORT+Z-DEVD-FMK ($149.7 \pm 6.4\%$, $p = 0.005$)
210 and CORT+Z-FA-FMK ($120.7 \pm 5.2\%$, $p = 0.793$) vs CORT ($118.5 \pm 5.8\%$), Fig. 1d).
211 Indeed, the activation of GSK-3 was robustly induced by CORT treatment and was
212 prevented by pre-treatment (30 min) and co-application of Z-DEVD-FMK (one-way
213 ANOVA, $F(3,36) = 14.41$, $p < 0.001$; Holm-Sidak post hoc test for CORT ($56.7 \pm$
214 9.6%) vs CORT+Z-DEVD-FMK ($89.1 \pm 6.4\%$, $p = 0.031$), Fig. 1e). Z-FA-FMK had no
215 effect on the CORT-induced activation of GSK-3 β (CORT ($56.7 \pm 9.6\%$) vs CORT+Z-
216 FA-FMK ($58.8 \pm 7.9\%$, $p = 0.858$, Fig. 1e) and neither Z-DEVD-FMK nor Z-FA-FMK
217 altered the total expression level of GSK-3 β (one-way ANOVA, $F(3,39) = 0.09$, $p =$
218 0.96 , Fig. 1e).

219 We also utilized a previously characterised XIAP Bir 1,2 protein, which inhibits
220 caspase-3 and 7 activation [18]. XIAP Bir 1,2 was biolistically transfected into
221 neurons of cultured hippocampal slices. In this cultured slice model, cells exposed to
222 CORT treatment (200 nM, 24 hours) exhibited impaired LTP when compared to

223 untreated cells (untreated: $160.1 \pm 14.0\%$; CORT: $93.7 \pm 15.2\%$, unpaired t-test $t(8)$
224 = 3.2, $p = 0.012$, Fig. 1f). This effect was dependent upon the presence of the GR,
225 such that CORT failed to block LTP in neurons transfected with GR shRNA when
226 compared to untransfected cells (untransfected: $85.7 \pm 12.5\%$; GR-shRNA
227 transfected: $157.3 \pm 24.9\%$, unpaired t-test $t(8) = 2.6$, $p = 0.033$, Fig. 1g). CORT
228 treatment did not inhibit LTP in XIAP Bir1,2 overexpressing cells when compared to
229 untransfected cells (untransfected: $103.6 \pm 20.8\%$; XIAP Bir1,2 transfected: $169.5 \pm$
230 20.2% , unpaired t-test $t(11) = 2.3$, $p = 0.044$, Fig. 1h), indicating a requirement for
231 caspase-3 / 7 activation for the inhibition of LTP by CORT. In line with our
232 hypothesis, these results suggest that CORT induces both GSK-3 and caspase
233 activation.

234 3.2. GCs inhibit LTP via Akt1 regulation

235 Since the mitochondrial permeability transition pore (mPTP) is important for the
236 activation of caspases [27], we tested whether mPTP regulates CORT-induced LTP
237 inhibition. The mPTP inhibitor TRO-19622 (25 μ M) prevented both the CORT-
238 mediated activation of GSK-3 β (one-way ANOVA, $F(2,15) = 11.74$, $p = 0.003$; Holm-
239 Sidak post hoc test for CORT+TRO-19622 ($94.9 \pm 6.9\%$, $p = 0.010$) vs CORT (69.7
240 $\pm 3.6\%$), Fig. 2a) and the inhibition of LTP (CORT: $114.3 \pm 5.8\%$; CORT+TRO-
241 19622: $144.1 \pm 2.2\%$, unpaired t-test $t(11) = 6.5$, $p = 0.001$, Fig. 2b). These results
242 imply that aberrant regulation of mPTP activates caspase and GSK-3, which
243 subsequently dysregulate LTP. Since caspase-3 can regulate GSK-3 through
244 cleavage of Akt1 [18,19], we hypothesised that the observed caspase mediated
245 upregulation of GSK-3 activity might be due to Akt1 cleavage and that this is a key

246 sequential step in the inhibition of LTP. Previous findings have shown that
247 phosphorylation of Akt1 modulates its cleavage in a site-specific manner [28]. We
248 therefore measured the phosphorylation of Akt1 at Thr308, a site correlated with
249 Akt1 cleavage and activity [29]. We found that Thr308 phosphorylation was
250 significantly reduced by CORT treatment (one-way ANOVA, $F(3,20) = 10.13$, $p <$
251 0.001 ; Holm-Sidak post hoc test for CORT ($42.2 \pm 12.3\%$, $p = 0.024$) vs control
252 ($100.0 \pm 13.0\%$), Fig. 2c) and that this reduction was prevented by pre-treating slices
253 with either Z-DEVD-FMK or the GR antagonist RU486 (500 nM), (CORT vs
254 CORT+Z-DEVD-FMK ($142.6 \pm 17.5\%$, $p < 0.001$), CORT vs CORT+RU486 ($114.7 \pm$
255 8.9% , $p = 0.005$, Fig. 2c). The total expression of Akt1 was unaffected by CORT, Z-
256 DEVD-FMK or RU486 (one-way ANOVA, $F(3,20) = 0.59$, $p = 0.632$, Fig. 2c). This
257 suggests that CORT inhibits Akt1 activity via caspase activation, and is a specific
258 consequence of GR activation. To further validate the effect of CORT on Akt1
259 activity, we used an *in vitro* kinase assay utilising immuno-precipitated Akt1 from
260 control and CORT treated hippocampal slices and a GSK-3 fusion protein as a
261 substrate to determine the activity of immobilised Akt1. We found that CORT
262 treatment reduced Akt1 activity as observed by a significant decrease in
263 phosphorylated GSK $\alpha\beta$ at serine residues 21 and 9 (pGSK $\alpha\beta$ Ser21/9) levels
264 compared to control (control: $100.0 \pm 8.44\%$; CORT: $73.9 \pm 3.67\%$, unpaired t-test
265 $t(4) = 7.1$, $p = 0.002$, Fig. 2d). We next aimed to determine whether caspase-3-
266 mediated Akt1 cleavage is important for the inhibition of LTP by CORT. To do this,
267 we biolistically transfected mutant Akt1 protein (Akt1 triple mutant, resistant to
268 cleavage by caspase-3[18]) into CA1 hippocampal neurons. CORT treatment did not
269 impair LTP in cells transfected with the Akt1 triple mutant when compared to
270 untransfected cells (untransfected: $104.9 \pm 7.2\%$; Akt1 triple mutant transfected;

271 144.8 ± 13.5%, unpaired t-test $t(9) = 2.5$, $p = 0.036$, Fig. 2e) but inhibited LTP in cells
272 transfected with wild-type Akt1 (untransfected: 117.4 ± 11.7.%; Akt1 wild-type: 107.4
273 ± 16.3%, unpaired t-test $t(10) = 0.5$, $p = 0.628$, Fig. 2f).

274 3.3. GCs induce pTau specifically at the PHF-1 epitope

275 Together, these results suggest that CORT-mediated LTP inhibition relies upon the
276 cleavage of Akt1 by caspase-3 and this might be a critical step to further
277 downstream signal cascades which ultimately execute functional synapse
278 weakening. What remains to be shown, however, are the potential downstream
279 substrates that mediate synaptic weakening and dysregulate LTP. GSK-3 is a well-
280 characterised kinase of the tau protein, governing the phosphorylation of tau (pTau)
281 at multiple sites [30]. Interestingly, pTau at Ser396, one of the GSK-3 target sites
282 closely associated with pathogenesis in AD [30], was recently revealed as necessary
283 for LTD [21]. Given previous evidence showing increased pTau following stress [31],
284 we postulated that CORT-induced activation of GSK-3, via upstream caspase-3-Akt1
285 regulation, might culminate in greater pTau and thus aberrant modulation of synaptic
286 plasticity. Therefore, we tested whether caspase, mPTP and GSK-3 activation are
287 involved in CORT-mediated changes to pTau. CORT treatment significantly
288 increased immunoreactivity with the PHF-1 antibody, which detects pTau at amino
289 acids Ser396 and Ser404 (S396/404) (one-way ANOVA, $F(3,20) = 4.83$, $p < 0.001$;
290 Holm-Sidak post hoc test for control (100.0 ± 16.0%) vs CORT (135.9 ± 7.9%, $p =$
291 0.001, Fig. 3a), and this effect of CORT occurred in a caspase dependent manner
292 (CORT vs CORT+Z-DEVD-FMK (90.4 ± 4.4%, $p < 0.001$, Fig. 3a). We also tested
293 AT8, an antibody that detects alternate GSK-3 phosphorylation residues Ser-202
294 and Thr-205, and found no change in phosphorylation at these sites (one-way
295 ANOVA, $F(3,20) = 0.43$, $p = 0.734$, Fig. 3b). The CORT-mediated increase in PHF-1

296 immunoreactivity was also prevented by co-treatment with an inhibitor of the mPTP
297 ((one-way ANOVA, $F(2,15) = 9.96$, $p < 0.001$; Holm-Sidak post hoc test for control
298 ($100.0 \pm 4.2\%$) vs CORT ($139.7 \pm 5.9\%$, $p = 0.003$), CORT vs CORT+TRO-19622
299 ($88.2 \pm 10.0\%$, $p < 0.001$, Fig. 3c) or an inhibitor of GSK-3 ($139.8 \pm 8.0\%$) vs
300 CORT+CT-99021 ($104.1 \pm 10.4\%$, $p = 0.008$, Fig. 3e). AT8 immunoreactivity was
301 again unchanged in both cases (one-way ANOVA, $F(2,15) = 0.58$, $p = 0.573$, Fig.
302 3D; one-way ANOVA, $F(2,24) = 0.38$, $p = 0.69$, Fig. 3f). These data show that CORT-
303 triggered signalling cascades drive an increase in pTau, specifically at residues of
304 the PHF-1 epitope.

305 *3.4. CORT-induced inhibition of LTP is mediated by specific pTau at Ser396*

306 Based on previous findings that pTau is required for LTD induction as well as A β -
307 mediated LTP inhibition [21, 22], we were interested in determining whether CORT-
308 induced LTP inhibition is due to aberrant synapse weakening mediated through
309 pTau. To test this, endogenous tau was first knocked down by rat tau small hairpin-
310 forming interference RNA (shRNA) in cultured hippocampal slices [21], allowing us to
311 determine whether tau itself is critical to CORT-mediated LTP inhibition. Indeed,
312 robust LTP was induced in rat tau shRNA transfected neurons, but not in
313 untransfected neurons, that were treated with CORT (untransfected: $105.6 \pm 17.9\%$;
314 tau shRNA transfected: $162.9 \pm 19.5\%$, unpaired t-test $t(12) = 2.4$, $p = 0.032$, Fig.
315 4a). In addition, neurons where endogenous tau was replaced with S396/404 residue
316 phospho-null human tau (S396/404A) readily exhibited LTP following treatment with
317 CORT when compared to untransfected cells (untransfected: $100.1 \pm 12.1\%$,
318 S396/404A transfected: $145.5 \pm 12.1\%$, unpaired t-test $t(11) = 2.2$, $p = 0.040$, Fig.

319 4b), indicating that tau and its phosphorylation are important for the effect of CORT
320 on LTP. We next wanted to determine whether there was a selective requirement for
321 phosphorylation at either of these sites within this mechanism. We therefore
322 generated and transfected neurons with single phospho-null tau mutants in
323 conjunction with rat tau shRNA. We found that, following CORT treatment, LTP was
324 present in neurons expressing the S396A mutant (untransfected: $84.0 \pm 9.9\%$; S396A
325 transfected: $177.0 \pm 30.4\%$, unpaired t-test $t(11) = 2.7$, $p = 0.020$, Fig. 4c) but not in
326 neurons expressing the S404A form (untransfected: $101.9 \pm 14.2\%$; S404A
327 transfected: $108.8 \pm 17.6\%$, unpaired t-test $t(8) = 0.3$, $p = 0.770$, Fig. 4d). In contrast,
328 neurons where endogenous tau was replaced with Ser199/202/Thr205 residue
329 phospho-null human tau (S/T199/202/205A) were still sensitive to CORT-mediated
330 inhibition of LTP (untransfected: $85.4 \pm 13.0\%$; S/T199/202/205A transfected: 117.4
331 $\pm 11.7\%$, unpaired t-test $t(9) = 1.8$, $p = 0.100$, Fig. 4e). Together, these data suggest
332 that pTau at the Ser396 residue is specifically required for CORT-induced inhibition
333 of LTP.

334 **4. Discussion**

335 Our data are consistent with a serial synaptic weakening mechanism in which the
336 activation of caspase-3 leads to cleavage of Akt1, removing the tonic inhibition of
337 GSK-3, which subsequently increases pTau and causes inhibition of LTP. Caspase-
338 3, GSK-3 and pTau are all critically involved in LTD, and aberrant LTD expression by
339 these molecules has been implicated in the pathophysiology of synapse weakening
340 and neurodegeneration [18,19,21,32-35]. Interestingly, LTP induction can inhibit LTD
341 signalling cascades [17] and, conversely, aberrant activation of LTD and/or synapse

342 weakening signalling cascades inhibits LTP expression [18,19]. This suggests that
343 LTP and LTD signalling molecules are tightly regulated and are maintained in
344 balance at basal states. A given stimuli (i.e., GCs), therefore, could tip this balance in
345 favor of LTP or LTD by acting as a catalyst for signalling mechanisms associated
346 with one or the other form of plasticity. Previous studies have demonstrated that the
347 inhibition of aberrantly expressed LTD and synapse weakening signalling molecules
348 restores LTP expression ordinarily inhibited in an A β neurotoxicity model [19,35].
349 Therefore, a compelling explanation for the data presented in this study is that GC-
350 activated synapse weakening signalling results in the inhibition of LTP.

351 Given the numerous studies showing GC-facilitated LTD [11,12,14], there was
352 significant scope for GCs activating key LTD/synapse weakening molecules. Among
353 many of the LTD/synapse weakening molecules, we were specifically interested in
354 GSK-3, considered pivotal in LTD expression and known to be involved in certain
355 types of neurodegeneration [17,36]. Underlying our focus on GSK-3 were the
356 previous observations broadly linking GCs with upregulated GSK-3 activity [31,37],
357 findings which are consistent with the results presented here. A next important step
358 will be to determine the mechanisms by which GCs can modulate GSK-3 activity.
359 GCs activate caspase-3 in cultured rat hippocampal neurons [38] and activation of
360 GR specifically is known to regulate this proapoptotic pathway [39]. Indeed, our
361 TRO-19622 experiment data suggest that GCs regulate the proapoptotic mPTP and
362 cytochrome *c*, and this may serve as a key step in the activation of GSK-3. We have
363 previously suggested that caspase-3-mediated cleavage of Akt-1 is critical for the
364 activation of GSK-3 by A β and aberrant expression of LTD in the hippocampus [18].
365 Furthermore, the constitutive inhibition of GSK-3 is primarily governed by the PI3K-
366 Akt signaling pathway [40], which is subject to GC regulation [15,16]. It has also

367 been suggested that GCs inhibit the canonical wingless (WNT) pathway through
368 Dickkopf 1 (DKK1) [41], which can regulate GSK-3 activity [42]. Therefore, it is clear
369 that signalling pathways converging on GSK-3 are indeed liable to modulation by
370 GCs, and such events could serve as the means by which synapse weakening is
371 induced by GCs.

372 In the view of activation of GSK-3 by GCs, we hypothesised that the downstream
373 signalling of GSK-3 would promote LTD and/or synapse weakening molecules.
374 Given that GSK-3 is a typical tau kinase and our previous study suggested that tau is
375 essential for LTD expression [20], this hypothesis was tested by knockdown of tau.
376 As we recently found that tau phosphorylation by GSK-3 is a critical molecular step
377 in LTD induction but has no role in LTP [21], we also showed that a specific
378 phospho-null mutation of tau blocked GC-mediated inhibition of LTP. Growing
379 evidence now links GC exposure with tau phosphorylation [31,38,43-46], though the
380 significance of this had remained relatively under explored. Our study now strongly
381 suggests that GC-mediated GSK-3 activation regulates aberrant tau phosphorylation
382 and this causes inhibition of LTP. The real consequences of this could then be two-
383 fold; firstly, the dysregulation of synaptic function as a result of the synapse
384 weakening pathway activation. Secondly, GC-induced tau phosphorylation *per se*
385 could seed tauopathies. It remains to be determined, however, whether there is a
386 specific role of membrane-bound-GRs or cytosolic GRs in activating these signalling
387 pathways that mediate the inhibition of LTP by CORT.

388 Of particular interest is our novel observation that specific phosphorylation of tau at
389 Ser396 is central to GC-induced synapse weakening. Aside from the established role
390 of this phosphorylation signal with physiological LTD [20,21], it is intriguing to note
391 other studies linking the phosphorylation of tau at this residue with pathological

392 conditions. In particular, GCs have been shown to induce the accumulation of tau
393 specifically phosphorylated at Ser396 within the synaptic compartment [47].
394 Furthermore, tau phosphorylated at Ser396 is accumulated in the synaptic
395 compartment of Alzheimer's disease and α -synucleinopathy brains and is considered
396 to be one of the earliest cellular events associated with these pathologies [48,49].
397 Therefore, the GC-activated caspase-3-GSK-3-pTau signalling pathway may be a
398 potential molecular mechanism of stress mediated synapse weakening that primes
399 neurodegenerative and precedes cognitive decline onset. Control of such aberrant
400 synapse weakening signals may therefore be a useful symptom modification strategy
401 for improvement of cognitive impairments, such as in dementia.

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408 **Author Contributions**

409 The study was conceived and designed by K.C. Electrophysiological studies were
410 conducted by J.H.Y., P.R., C.M.P., and biochemical assays were conducted by C.B.,
411 G.W., T. P. and Y.S.L. The manuscript was written by D.J.W., P.R., J.H.Y., C.B. and
412 K.C.

413 **References**

- 414 1. de Kloet, E.R., Joels, M. & Holsboer, F. Stress and the brain: from adaptation
415 to disease. *Nat. Rev. Neurosci.* **6**, 463-75 (2005).
- 416 2. Lupien, S.J. et al. Cortisol levels during human aging predict hippocampal
417 atrophy and memory deficits. *Nat. Neurosci.* **1**, 69-73 (1998).
- 418 3. Starkman, M.N., Gebarski, S.S., Berent, S. & Schteingart, D.E. Hippocampal
419 formation volume, memory dysfunction, and cortisol levels in patients with
420 Cushing's syndrome. *Biol. Psychiatry.* **32**, 756-65 (1992).
- 421 4. Sapolsky, R.M., Romero, L.M. & Munck, A.U. How do glucocorticoids
422 influence stress responses? Integrating permissive, suppressive, stimulatory,
423 and preparative actions. *Endocr. Rev.* **21**, 55-89 (2000).
- 424 5. Cho, K. Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive
425 deficits. *Nat. Neurosci.* **4**, 567-8 (2001).
- 426 6. Newcomer, J.W., Craft, S., Hershey, T., Askins, K. & Bardgett, M.E.
427 Glucocorticoid-induced impairment in declarative memory performance in
428 adult humans. *J. Neurosci.* **14**, 2047-53 (1994).
- 429 7. Kirschbaum, C., Wolf, O.T., May, M., Wippich, W. & Hellhammer, D.H. Stress-
430 and treatment-induced elevations of cortisol levels associated with impaired
431 declarative memory in healthy adults. *Life Sci.* **58**, 1475-83 (1996).
- 432 8. Conrad, C.D., Galea, L.A., Kuroda, Y. & McEwen, B.S. Chronic stress impairs
433 rat spatial memory on the Y maze, and this effect is blocked by tianeptine
434 pretreatment. *Behav. Neurosci.* **110**, 1321-34 (1996).

- 435 9. Sousa, N., Lukoyanov, N.V., Madeira, M.D., Almeida, O.F. & Paula-Barbosa,
436 M.M. Reorganization of the morphology of hippocampal neurites and
437 synapses after stress-induced damage correlates with behavioral
438 improvement. *Neuroscience*. **97**, 253-66 (2000).
- 439 10. de Quervain, D.J., Aerni, A., Schelling, G. & Roozendaal, B. Glucocorticoids
440 and the regulation of memory in health and disease. *Front. Neuroendocrinol.*
441 **30**, 358-70 (2009).
- 442 11. Kim, J.J., Foy, M.R. & Thompson, R.F. Behavioral stress modifies
443 hippocampal plasticity through N-methyl-D-aspartate receptor activation. *Proc.*
444 *Natl. Acad. Sci. U S A*. **93**, 4750-3 (1996).
- 445 12. Xu, L., Anwyl, R. & Rowan, M.J. Behavioural stress facilitates the induction of
446 long-term depression in the hippocampus. *Nature*. **387**, 497-500 (1997).
- 447 13. Nabavi, S. et al. Engineering a memory with LTD and LTP. *Nature*. **511**, 348-
448 52 (2014).
- 449 14. Yang, C.H., Huang, C.C. & Hsu, K.S. Behavioral stress modifies hippocampal
450 synaptic plasticity through corticosterone-induced sustained extracellular
451 signal-regulated kinase/mitogen-activated protein kinase activation. *J.*
452 *Neurosci*. **24**, 11029-34 (2004).
- 453 15. Jeanneteau, F., Garabedian, M.J. & Chao, M.V. Activation of Trk neurotrophin
454 receptors by glucocorticoids provides a neuroprotective effect. *Proc. Natl.*
455 *Acad. Sci. U S A*. **105**, 4862-7 (2008).

- 456 16. Yang, S., Roselli, F., Patchev, A.V., Yu, S. & Almeida, O.F. Non-receptor-
457 tyrosine kinases integrate fast glucocorticoid signaling in hippocampal
458 neurons. *J. Biol. Chem.* **288**, 23725-39 (2013).
- 459 17. Peineau, S. et al. LTP inhibits LTD in the hippocampus via regulation of
460 GSK3beta. *Neuron.* **53**, 703-17 (2007).
- 461 18. Li, Z. et al. Caspase-3 activation via mitochondria is required for long-term
462 depression and AMPA receptor internalization. *Cell.* **141**, 859-71 (2010).
- 463 19. Jo, J. et al. Abeta(1-42) inhibition of LTP is mediated by a signaling pathway
464 involving caspase-3, Akt1 and GSK-3beta. *Nat. Neurosci.* **14**, 545-7 (2011).
- 465 20. Kimura, T. et al. Microtubule-associated protein tau is essential for long-term
466 depression in the hippocampus. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **369**,
467 20130144 (2014).
- 468 21. Regan, P. et al. Tau phosphorylation at serine 396 residue is required for
469 hippocampal LTD. *J. Neurosci.* **35**, 4804-12 (2015).
- 470 22. Shipton OA, et al. Tau protein is required for amyloid {beta}-induced impair-
471 ment of hippocampal long-term potentiation. *J Neurosci* **31**, 1688-92 (2011).
- 472 23. Ittner, L.M. & Gotz, J. Amyloid-beta and tau--a toxic pas de deux in
473 Alzheimer's disease. *Nat. Rev. Neurosci.* **12**, 65-72 (2011).
- 474 24. Sotiropoulos, I. & Sousa, N. Tau as the Converging Protein between Chronic
475 Stress and Alzheimer's Disease Synaptic Pathology. *Neurodegener. Dis.* **16**,
476 22-5 (2016).

- 477 25. Stoppini, L., Buchs, P.A. & Muller, D. A simple method for organotypic
478 cultures of nervous tissue. *J. Neurosci. Methods.* **37**, 173-82 (1991).
- 479 26. Lo, D.C., McAllister, A.K. & Katz, L.C. Neuronal transfection in brain slices
480 using particle-mediated gene transfer. *Neuron.* **13**, 1263-8 (1994).
- 481 27. Garrido, C. et al. Mechanisms of cytochrome c release from mitochondria.
482 *Cell Death Differ.* **13**, 1423-33 (2006).
- 483 28. Liao, Y. & Hung, M.C. Physiological regulation of Akt activity and stability. *Am*
484 *J. Transl. Res.* **2**, 19-42 (2010).
- 485 29. Alessi DR, et al. Mechanism of activation of protein kinase B by insulin and
486 IGF-1. *EMBO J* **15**, 6541-6551 (1996).
- 487 30. Mandelkow EM, et al. Glycogen synthase kinase-3 and the Alzheimer-like
488 state of microtubule-associated protein tau. *FEBS Lett* **314**, 315-21. (1992).
- 489 31. Sotiropoulos, I. et al. Stress acts cumulatively to precipitate Alzheimer's
490 disease-like tau pathology and cognitive deficits. *J. Neurosci.* **31**, 7840-7
491 (2011).
- 492 32. Regan, P., Whitcomb, D.J. & Cho, K. Physiological and Pathophysiological
493 Implications of Synaptic Tau. *The Neuroscientist.* 1-15. (2016).
- 494 33. Regan, P., Hogg, E., Whitcomb, D.J. & Cho, K. Long-term depression: a new
495 conceptual understanding of Alzheimer's disease. *Eur. J. Neurodegener. Dis.*
496 **2**, 79-89 (2014).
- 497 34. Carroll, J.C. et al. Chronic stress exacerbates tau pathology,
498 neurodegeneration, and cognitive performance through a corticotropin-

- 499 releasing factor receptor-dependent mechanism in a transgenic mouse model
500 of tauopathy. *J. Neurosci.* **31**, 14436-49 (2011).
- 501 35. Kailainathan et al. Activation of a synapse weakening pathway by human Val
502 66 but not Met66 pro-brain-derived neurotrophic factor (proBDNF). *Pharmacol*
503 *Res* **104**, 97-107 (2015).
- 504 36. Lei, P., Ayton, S., Bush, A.I. & Adlard, P.A. GSK-3 in Neurodegenerative
505 Diseases. *Int. J. Alzheimers Dis.* 2011, 189246 (2011).
- 506 37. Sotiropoulos, I. et al. Glucocorticoids trigger Alzheimer disease-like
507 pathobiochemistry in rat neuronal cells expressing human tau. *J. Neurochem.*
508 **107**, 385-97 (2008).
- 509 38. Liu, B. et al. Neuroprotective effects of icariin on corticosterone-induced
510 apoptosis in primary cultured rat hippocampal neurons. *Brain Res.* **1375**, 59-
511 67 (2011).
- 512 39. Crochemore, C. et al. Direct targeting of hippocampal neurons for apoptosis
513 by glucocorticoids is reversible by mineralocorticoid receptor activation. *Mol.*
514 *Psychiatry.* **10**, 790-8 (2005).
- 515 40. Doble, B.W. & Woodgett, J.R. GSK-3: tricks of the trade for a multi-tasking
516 kinase. *J. Cell Sci.* **116**, 1175-86 (2003).
- 517 41. Matrisciano, F. et al. Induction of the Wnt antagonist Dickkopf-1 is involved in
518 stress-induced hippocampal damage. *PLoS One.* 6, e16447 (2011).
- 519 42. Metcalfe, C. & Bienz, M. Inhibition of GSK3 by Wnt signalling--two contrasting
520 models. *J. Cell Sci.* **124**, 3537-44 (2011).

- 521 43. Cuadrado-Tejedor, M. et al. Chronic mild stress in mice promotes cognitive
522 impairment and CDK5-dependent tau hyperphosphorylation. *Behav. Brain*
523 *Res.* **220**, 338-43 (2011).
- 524 44. Liu, Y. et al. Tau phosphorylation and mu-calpain activation mediate the
525 dexamethasone-induced inhibition on the insulin-stimulated Akt
526 phosphorylation. *PLoS One.* **7**, e35783 (2012).
- 527 45. Zhang, L.F. et al. Increased hippocampal tau phosphorylation and axonal
528 mitochondrial transport in a mouse model of chronic stress. *Int. J*
529 *Neuropsychopharmacol.* **15**, 337-48 (2012).
- 530 46. Joshi, Y.B., Chu, J. & Pratico, D. Stress hormone leads to memory deficits
531 and altered tau phosphorylation in a model of Alzheimer's disease. *J*
532 *Alzheimers Dis.* **31**, 167-76 (2012).
- 533 47. Pinheiro, S. et al. Tau Mislocation in Glucocorticoid-Triggered Hippocampal
534 Pathology. *Mol. Neurobiol.* **53**, 4745-53 (2016).
- 535 48. Muntane, G., Dalfo, E., Martinez, A. & Ferrer, I. Phosphorylation of tau and
536 alpha-synuclein in synaptic-enriched fractions of the frontal cortex in
537 Alzheimer's disease, and in Parkinson's disease and related alpha-
538 synucleinopathies. *Neuroscience.* **152**, 913-23 (2008).
- 539 49. Mondragon-Rodriguez, S., Perry, G., Luna-Munoz, J., Acevedo-Aquino, M.C.
540 & Williams, S. Phosphorylation of tau protein at sites Ser(396-404) is one of
541 the earliest events in Alzheimer's disease and Down syndrome. *Neuropathol.*
542 *Appl. Neurobiol.* **40**, 121-35 (2014).

543 **Figure Legends**

544 **Figure 1: Glucocorticoids impair LTP via caspase-mediated GSK-3 activation**

545 **(a, b, c and d)** Field recording in rat hippocampus CA1. *Top*: Example traces of
546 fEPSPs from indicated timepoints. *Bottom*: Mean fEPSP slope shown as percentage
547 of the established baseline. **(a)** LTP is inhibited by CORT treatment ($n = 8$). **(b)** Bath
548 application of DEX inhibits LTP ($n = 6$). **(c)** Treatment with CT-99021 prevents LTP
549 impairment induced by CORT ($n = 6$). **(d)** Z-DEVD-FMK blocks impairment of LTP by
550 CORT ($n = 6$), but scrambled peptide Z-FA-FMK does not ($n = 6$). Bath application of
551 CORT and CT-99021, and delivery of tetanus (100Hz, 100 pulses) indicated by black
552 rectangles. **(e)** Z-DEVD-FMK rescues the reduction of GSK-3 inhibitory
553 phosphorylation caused by CORT treatment ($n = 10$), but Z-FA-FMK does not ($n =$
554 10). *Left*: Cropped example blots showing immunoreactive bands from different
555 treatment groups. *Top Right*: Quantification of changes in immunoreactivity of pS9
556 GSK-3 β normalised against total GSK-3 β . *Bottom Right*: Levels of total GSK-3 β
557 normalised against β -actin loading control. **(f, g and h)** Whole cell patch clamp
558 recording in CA1 region of cultured hippocampal slices. *Top*: Example traces of
559 EPSCs from indicated timepoints. *Bottom*: Mean peak EPSC shown as percentage
560 of the established baseline. **(f)** CORT treatment blocks LTP induction ($n = 5$, control
561 $n = 5$). **(g)** GR shRNA prevents CORT-induced LTP impairment (GR shRNA
562 transfected $n = 5$, untransfected $n = 5$). **(h)** XIAP Bir1,2 prevents CORT-induced LTP
563 impairment (XIAP Bir1,2 transfected $n = 7$, untransfected $n = 6$). All bars and circles
564 represent the mean \pm SEM. Statistical significance was determined by one-way
565 ANOVA and Holm-Sidak post hoc analysis indicated as * $p < 0.05$, ** $p < 0.01$, *** $p <$
566 0.001.

567 **Figure 2: Caspase-mediated Akt1 cleavage is required for the inhibition of LTP**
568 **by CORT**

569 (a) TRO-19622 prevents the reduction of pS9GSK-3 β caused by CORT treatment (n
570 = 6). *Left*: Cropped example blots showing immunoreactive bands from different
571 treatment groups. *Top Right*: Quantification of changes in immunoreactivity of pS9
572 GSK-3 β normalised against total GSK-3 β . *Bottom Right*: Levels of total GSK-3 β
573 normalised against β -actin loading control. (b) TRO-19622 inhibits CORT-induced
574 LTP impairment ($n = 6$). (c) *Left*: Cropped example blots showing immunoreactive
575 bands from different treatment groups. *Middle*: Quantification of pAkt1(Thr308)
576 immunoreactivity normalised to total Akt1. *Right*: Total Akt1 normalised to β -actin
577 loading control. The reduction in Thr308 phosphorylation of Akt1 following CORT
578 treatment ($n = 6$) was rescued by Z-DEVD-FMK ($n = 6$) and RU486 ($n = 6$). (d)
579 CORT treatment reduces Akt1 activity as determined by a significant decrease in
580 pGSK $\alpha\beta$ Ser21/9 levels compared to control ($n = 3$). (e and f) Whole cell patch
581 clamp recording in CA1 region of cultured hippocampal slices. *Top*: Example traces
582 of EPSCs from indicated timepoints. *Bottom*: Mean peak EPSC shown as
583 percentage of the established baseline. (e) CORT does not impair LTP in slices
584 transfected with Akt1 triple mutant (Akt1 triple mutant transfected $n = 6$,
585 untransfected $n = 5$), (f) whereas Akt1 wild-type has no effect on CORT-induced LTP
586 impairment (Akt1 wild-type $n = 7$, untransfected $n = 6$). 2 Hz stimulation indicated by
587 black rectangles. All bars and circles represent the mean \pm SEM. Statistical
588 significance was determined by one-way ANOVA and Holm-Sidak post hoc analysis
589 and unpaired t-test indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

590 **Figure 3: Caspase, mPTP and GSK-3 activation regulate CORT-mediated**
591 **changes to pTau**

592 Western blots showing changes in phosphorylation of tau. *Left:* Cropped example
593 blots showing immunoreactive bands from different treatment groups. *Right:*
594 Quantification of pTau antibody immunofluorescence normalised to total tau (Tau5).
595 **(a and b)** pTau measured by PHF-1 is increased by CORT and rescued by Z-DEVD-
596 FMK, but not scrambled peptide Z-FA-FMK ($n = 6$). Immunoreactivity of AT8 is
597 unchanged by any treatment ($n = 6$). **(c and d)** TRO-19622 also prevents the
598 increase in PHF-1 immunoreactivity caused by CORT ($n = 6$), without affecting levels
599 of AT8 ($n = 6$). **(e and f)** Additionally, CT-99021 blocks the increase in PHF-1
600 immunoreactivity caused by CORT ($n = 9$), while AT8 immunoreactivity is not
601 affected by CORT or CT-99021 ($n = 9$). All bars represent the mean \pm SEM.
602 Statistical significance was determined by one-way ANOVA and Holm-Sidak post
603 hoc analysis indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

604 **Figure 4: pTau at Ser396 is specifically required for CORT-induced inhibition of**
605 **LTP**

606 Whole cell patch clamp recording in CA1 region of cultured hippocampal slices. *Top:*
607 Example traces of EPSCs from indicated timepoints. *Bottom:* Mean peak EPSC
608 shown as percentage of the established baseline. **(a)** LTP can still be induced after
609 CORT treatment in neurons transfected with tau shRNA (tau shRNA $n = 8$,
610 untransfected $n = 6$). **(b)** Neurons expressing the tau S396/404A mutant exhibited
611 LTP after treatment with CORT (tau S396/404A transfected $n = 8$, untransfected $n =$
612 5). **(c)** LTP can be induced after CORT treatment in neurons transfected with the

613 single residue S396A tau mutant (tau S39A transfected $n = 7$, untransfected $n = 6$).
614 **(d)** CORT impairs LTP in neurons expressing tau S404A (tau S404A transfected $n =$
615 5, untransfected $n = 5$). **(e)** The tau S/T 199/202/205A mutant was unable to prevent
616 inhibition of LTP by CORT (tau S/T 199/202/205A transfected $n = 6$, untransfected n
617 = 5). 2Hz stimulation indicated by black rectangles. All bars and circles represent the
618 mean \pm SEM. Statistical significance was determined by unpaired t-test.