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**A $\beta$ <sub>1-42</sub> inhibition of LTP is prevented by manipulation of a signalling pathway involving caspase-3, Akt and GSK-3 $\beta$**

Jihoon Jo<sup>1,5</sup>, Daniel J. Whitcomb<sup>1,5</sup>, Kimberly Moore Olsen<sup>2</sup>, Talitha L. Kerrigan<sup>1</sup>, Joyce Shih-Ching Lo<sup>2</sup>, Gilles Bru-Mercier<sup>1</sup>, Bryony Dickinson<sup>1</sup>, Sarah Scullion<sup>1,3</sup>, Morgan Sheng<sup>2</sup>, Graham Collingridge<sup>3,4</sup> and Kwangwook Cho<sup>1,3,CA</sup>

<sup>1</sup>Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology (LINE), School of Clinical Sciences, Faculty of Medicine and Dentistry, University of Bristol, Whitson Street, Bristol BS1 3NY, United Kingdom, <sup>2</sup>Department of Neuroscience, Genentech Inc, South San Francisco, CA, USA. <sup>3</sup>MRC Centre for Synaptic Plasticity, <sup>4</sup>School of Physiology & Pharmacology, University of Bristol, University Walk, Bristol BS8 1TD, United Kingdom

<sup>5</sup> These authors contributed equally to this work

<sup>CA</sup>Corresponding author: Correspondence should be addressed to K.C. (Kei.Cho@bristol.ac.uk).

**Amyloid- $\beta_{1-42}$  ( $A\beta$ ) is thought to be a major mediator of the cognitive deficits in Alzheimer's disease (AD). The ability of  $A\beta$  to inhibit hippocampal long-term potentiation (LTP) provides a cellular correlate of this action but the underlying molecular mechanism is only partially understood. We report a prominent role for a signaling pathway involving caspase-3, Akt1 and glycogen synthase kinase-3 $\beta$  in this effect in rats and mice.**

It is established that oligomeric forms of  $A\beta$  inhibit long-term potentiation (LTP)<sup>1, 2</sup> and enhance long-term depression (LTD)<sup>2-4</sup> but the mechanisms by which  $A\beta$  interferes with long-term synaptic plasticity remain to be established. To address this we investigated how  $A\beta$  could impair LTP in organotypic hippocampal slice cultures. A pairing protocol (200 pulses at 2 Hz,  $V_h$  0 mV) readily induced LTP in CA1 neurons in control slices ( $173 \pm 11$  %,  $n = 6$ , **Fig. 1a**). Pretreatment of  $A\beta$  (500 nM, 2 h) blocked the induction of LTP ( $104 \pm 10$  %,  $n = 6$ , **Fig. 1b**). We found that the forms of treated  $A\beta$  in this study were mostly in the range of 2-mer – 6-mer oligomeric species (details in supplementary methods).

Caspases are known to be involved in apoptosis, are associated with AD<sup>5-7</sup> and have been shown to be activated by  $A\beta$  exposure<sup>8</sup>. Recent evidence has implicated these proteases in hippocampal synaptic plasticity<sup>9</sup>. To determine whether caspases might be involved in  $A\beta$  regulation of LTP we biolistically transfected CA1 neurons with plasmids expressing well characterized caspase inhibitor protein constructs and made recordings 3 - 4 days later<sup>9</sup>. We first tested XIAP (X-chromosome-linked inhibitor of apoptosis protein), which is an inhibitor of caspase-3, -7 and -9<sup>9</sup>. This treatment completely prevented the inhibition of LTP by  $A\beta$ , which was evident in simultaneously recorded, neighbouring untransfected neurons (**Fig. 1c**). To confirm the role of caspases we further applied a pharmacological inhibitor and tested both organotypic and acute slice preparations (**Supplementary figure 1, 2**). Using organotypic slices, we found that Z-VAD-FMK, a pan-caspase inhibitor<sup>9</sup>, prevented the inhibition of LTP by  $A\beta$  (**Supplementary figure 1a**), whilst the compound had no effect on LTP *per se* (**Supplementary figure 1b**). Using acute hippocampal slices, prepared from 4 - 5 week old animals and extracellular field EPSP (fEPSP) recording,

we observed similar results (**Supplementary figure 1c, d**). In contrast, a scrambled peptide, Z-FA-FMK, was unable to prevent the inhibition of LTP by A $\beta$  (**Supplementary figure 2**).

So far, our results suggested that caspases play a role in the A $\beta$ -inhibition of LTP. To further characterize specifically which caspases were involved in this mechanism, we selectively inhibited members of the caspase family and tested the inhibition of LTP by A $\beta$ . LTP was similarly observed in neurons transfected with BIR1,2 (an XIAP construct containing the N-terminal two baculoviral IAP repeat (BIR) domains), which inhibits caspase-3 and 7<sup>9</sup>, whereas again LTP was absent in simultaneously recorded, untransfected neurons (**Fig. 1d**). In contrast, neither BIR3 (an N-terminally truncated form of XIAP, containing the third BIR domain), which inhibits caspase-9<sup>9</sup> nor CrmA (cytokine response modifier A, a cowpox virus serpin), which inhibits caspase-1, -5, and -8<sup>9</sup>, was able to prevent the inhibition of LTP by A $\beta$  (**Fig. 1e, f**). The ability of XIAP and BIR1,2 to rescue A $\beta$  inhibition of LTP was not due to an independent effect on LTP since none of the constructs examined directly affected LTP (control: 173  $\pm$  11 %; XIAP: 164  $\pm$  18 %; BIR1,2: 166  $\pm$  15 %;  $p > 0.05$  compared with control, data not shown). These results implicate caspase-3 and/or caspase-7 in the A $\beta$  inhibition of LTP.

To further substantiate the roles of caspases in A $\beta$  inhibition of LTP and to gain additional insights into the isoform involved, we used slices from caspase-3 knockout mice. Theta burst stimulation (TBS) induced LTP in wild type mice (caspase-3<sup>+/+</sup>) that was blocked by A $\beta$  (**Fig. 2a**). However, A $\beta$  had no effect on LTP in caspase-3 knockout mice (caspase-3<sup>-/-</sup>) ( $p > 0.05$ , compared with no A $\beta$  treatment, **Fig. 2b**).

These results implicate caspase-3 in A $\beta$  inhibition of LTP but do not establish how this occurs. Considering that caspase-3 can cleave Akt to inhibit its function, a mechanism prominent in synaptic plasticity<sup>9</sup>, and since Akt activity inhibits apoptosis, we tested whether Akt cleavage by caspase-3 plays a role in A $\beta$  inhibition of LTP. For this, we transfected a triple mutant Akt1 protein (D108/119A/D462N) that is resistant to cleavage by caspase-3 *in vitro*<sup>9</sup>. This treatment completely prevented the inhibition of LTP by A $\beta$ , which was evident in simultaneously recorded,

neighbouring untransfected neurons (**Fig. 2c**). In contrast to mutant Akt1, expression of wild type Akt1 was not able to prevent the A $\beta$ -induced inhibition of LTP (**Fig. 2d**). Once again, these treatments had no effect on LTP *per se* (control cells:  $174 \pm 9$  %; Akt-1 mutant:  $179 \pm 24$  %; Akt-1 wildtype:  $185 \pm 17$  %;  $p > 0.05$  compared with control cells; data not shown). Our results therefore indicate that cleavage of Akt1 by caspase-3 is required for A $\beta$ -induced inhibition of LTP.

Another protein that has been widely implicated in AD is glycogen synthase kinase-3 $\beta$  (GSK-3)<sup>10</sup>, the activity of which is normally suppressed by Akt. We speculated that caspase-3 could inhibit LTP by the cleavage of Akt, resulting in activation of GSK-3 $\beta$ <sup>9, 11</sup>. Consistent with this mechanism, using immuno-blotting assays, we found that A $\beta$  activated both caspase-3 (**Fig. 3a**) and GSK-3 $\beta$  (**Fig. 3b**). To test this hypothesis more directly, we used the specific GSK-3 inhibitor CT-99021 (1  $\mu$ M)<sup>12</sup> and found that pre-treatment with this compound completely prevented A $\beta$  from inhibiting LTP, assessed using whole-cell patch clamp recording in cultured slices (**Fig. 3c**). CT-99021 itself had no effect on LTP induction (**Fig. 3d**). Using fEPSP recording in acute slice from 4 - 5 weeks old rats, we also confirmed that CT-99021 prevented the A $\beta$ -inhibition of LTP (**Fig. 3e**).

Since CT-99021 is membrane permeant, we were able to apply the compound after LTP had been inhibited by A $\beta$ , in order to see whether the deficit could be reversed. For this, we used a two-input experimental design and induced LTP using a tetanus delivered to each input separated by 1 h. Under control conditions LTP was readily induced in both inputs (**Fig. 3f**). In contrast, A $\beta$  resulted in complete block of LTP in both inputs (**Fig. 3g**). Interestingly, treatment with CT-99021 enabled LTP to be induced normally (monitored using input 2) subsequent to when A $\beta$  had fully prevented the generation of LTP (monitored using input 1) in the same population of neurons (**Fig. 3h**).

A number of different signaling molecules, including a variety of protein kinases and cyclooxygenases, have been identified as playing a role in the A $\beta$  inhibition of LTP<sup>13-15</sup>. We have potentially identified a new pathway involved in this process, which may be highly relevant to the neuropathology of AD given the evidence implicating both

caspases and GSK-3 $\beta$  in this disorder. Our data are most consistent with a serial mechanism in which activation of caspase-3 leads to cleavage of Akt that then removes a tonic inhibition of GSK-3 $\beta$ . Further studies will be required to establish the upstream activators and downstream effectors of this process and how this relates to the other signaling processes involved in A $\beta$  inhibition of LTP. Importantly, our finding that a GSK-3 $\beta$  inhibitor can reverse the LTP deficit caused by A $\beta$  treatment raises the possibility that this pathway may be a useful therapeutic target for combating cognitive decline in AD.

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#### AUTHORS CONTRIBUTIONS

The study was conceived by K.C., the experiments were designed by K.C. M.S. and G.L.C., carried out by J.J., D.J.W., K.O., T.L.K, J.S.L, G.B., B.D, S.S. and the manuscript was written by G.L.C., M.S. and K.C.

## Figure Legend

### Figure 1: Identification of a role for caspases in A $\beta$ inhibition of LTP

(a) A pairing protocol induced LTP in control cultured slices (n = 6), (b) but not following 2 h A $\beta$  pretreatment (n = 6). (c-f) CA1 neurons were transfected with XIAP, BIR 1,2, BIR 3 or CrmA (co-transfected with GFP) in A $\beta$ -treated slices. (c) LTP was induced in XIAP transfected (open symbol, n = 6) but not in simultaneously recorded non-transfected cells (closed symbol, n = 6). (d) LTP was induced in BIR 1,2 transfected cells (open symbol, n = 6) but not in non-transfected cells (closed symbol, n = 6). (e) LTP was inhibited in BIR 3 transfected cells (n = 6), (f) and CrmA transfected cells (n = 6). Error bars represent s.e.m.

### Figure 2: Caspase-3 and cleavage of Akt is involved in the A $\beta$ inhibition of LTP

(a) LTP can be induced in acute hippocampal slices from caspase-3<sup>+/+</sup> mice (closed symbol, n = 12), but is inhibited following A $\beta$  treatment (open symbol, n = 11). (b) LTP can be induced in acute hippocampal slices from caspase-3 knockout mice (caspase-3<sup>-/-</sup>) (closed symbol, n = 12), even following A $\beta$  treatment (open symbol, n = 11). (c) LTP was induced in triple mutant Akt1 transfected cells but not in neighbouring non-transfected cells in the same slices receiving A $\beta$  pretreatment (n = 6). (d) LTP could not be induced in wild type Akt1 transfected cells following A $\beta$  treatment (n = 6).

### Figure 3: A $\beta$ inhibition of LTP is reversed by blockade of GSK-3

(a) Active caspase-3 was enhanced by 30 min and 2 h A $\beta$  treatment. Bar chart indicates pooled data (n = 4, four independent experiments from four animals). (b) Increase in GSK-3 $\beta$  activity (reduction in phosphorylation of serine 9) after 30 min and 2 h of A $\beta$  treatment. Bar chart indicates pooled data (n = 4, four independent experiments from four animals). (c) The prevention of A $\beta$ -inhibition of LTP by pre-incubation of cultured slices with CT-99021 (1  $\mu$ M) (n = 6). (d) CT-99021 alone had no effect on LTP (n = 6). (e) CT-99021 prevented A $\beta$ -inhibition of LTP in acute slices (CT-99021 with A $\beta$ : triangle, n = 6; A $\beta$  alone: circle, n = 5). (f) LTP induction in two independent pathways following first tetanus (input 1) and second tetanus (input 2; 60 min after first tetanus) in control slices (n = 5). (g) LTP was completely inhibited in both inputs following A $\beta$  treatment (n = 5) for 2 h prior to the first tetanus.

**(h)** Pooled data showing that CT-99021 (applied from 30 min prior to the second tetanus) rescued A $\beta$ -inhibition of LTP (n = 5). \* indicates  $p < 0.05$ . \*\* indicates  $p < 0.01$ .



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Fig. 1

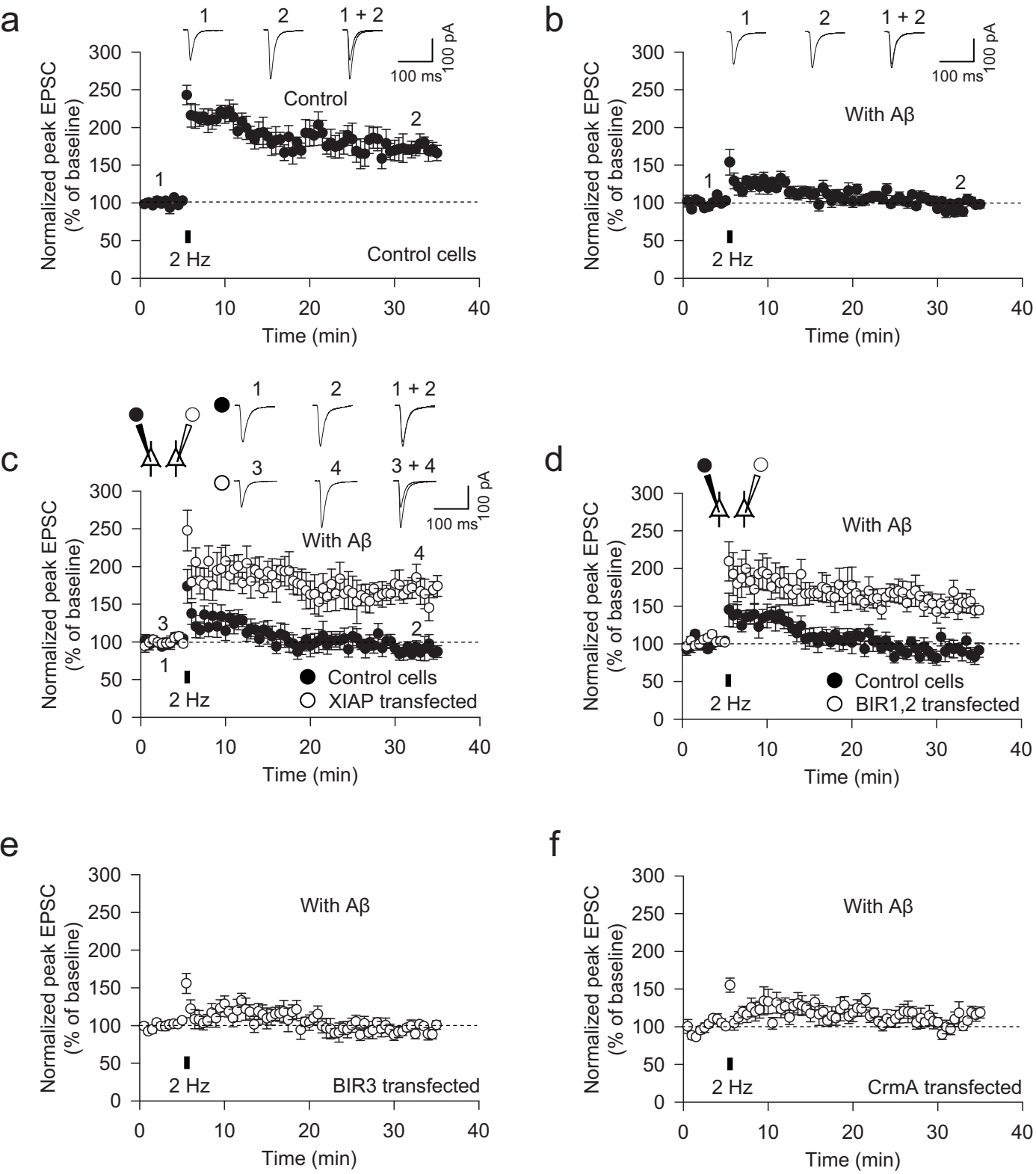
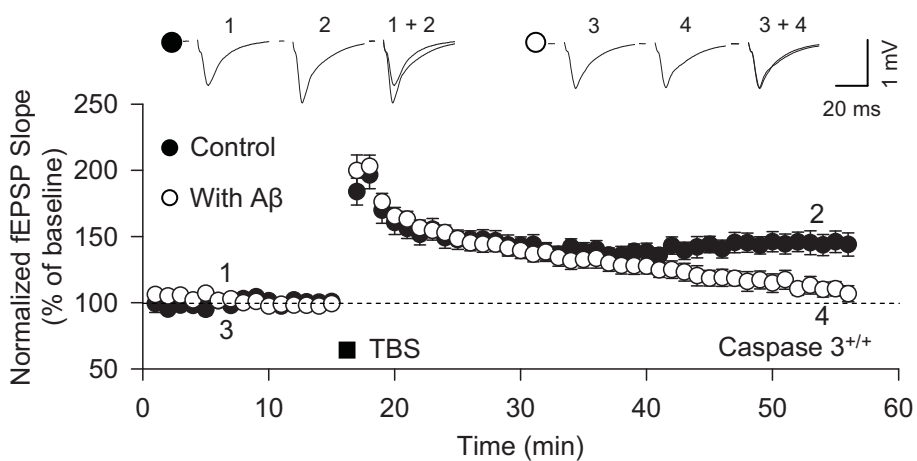
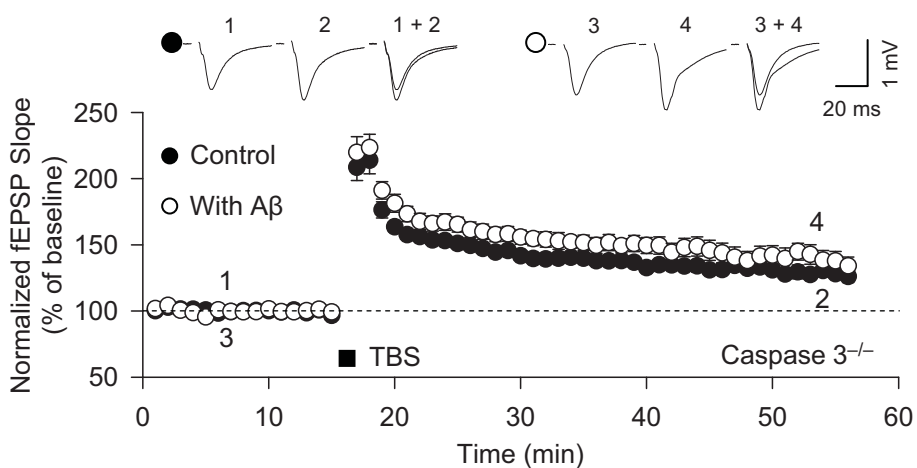


Fig. 2

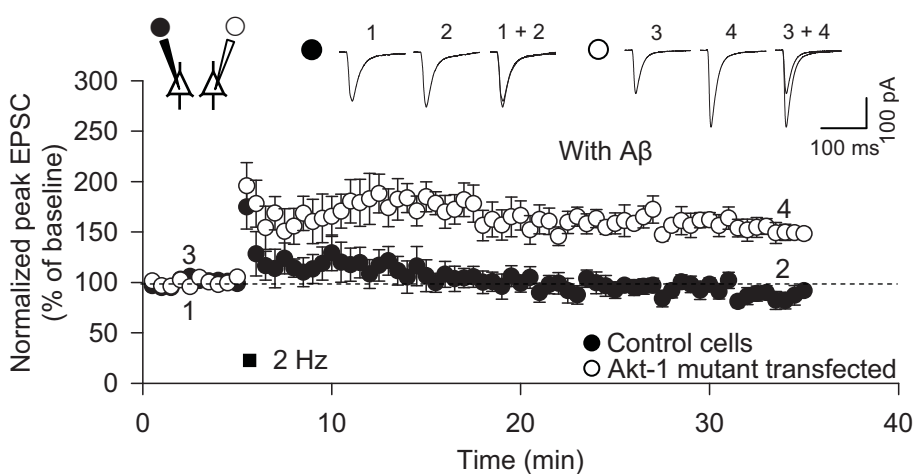
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b



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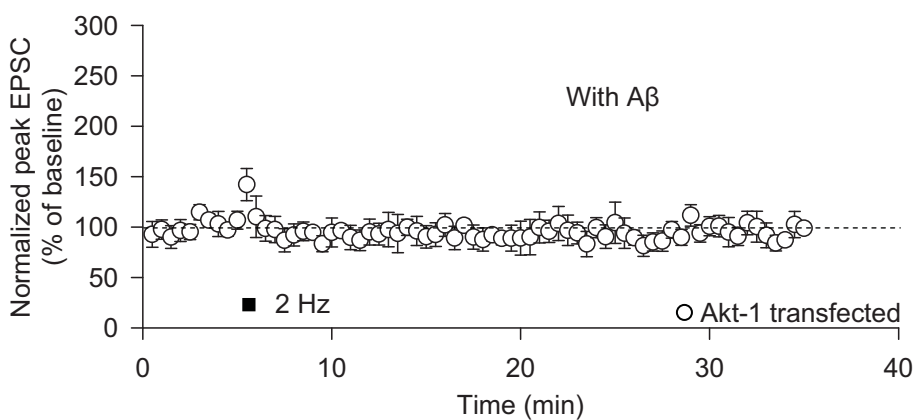


Fig. 3

