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Supplementary Materials for

BDNF Selectively Regulates $GABA_A$ Receptor Transcription by Activation of the JAK/STAT Pathway

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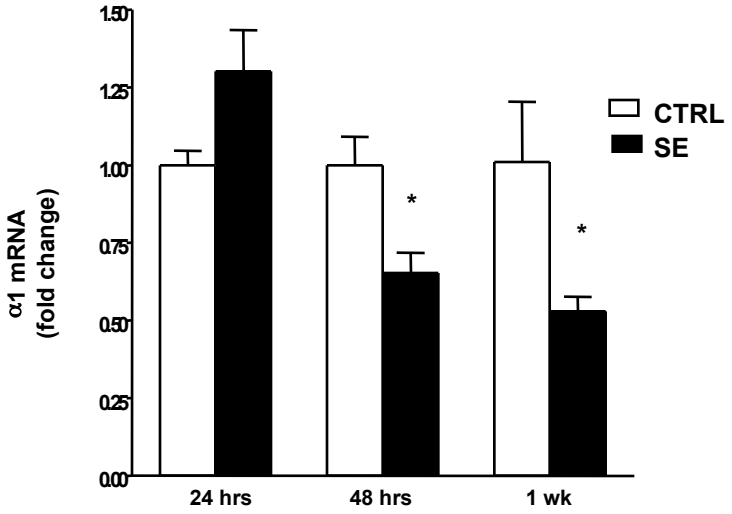
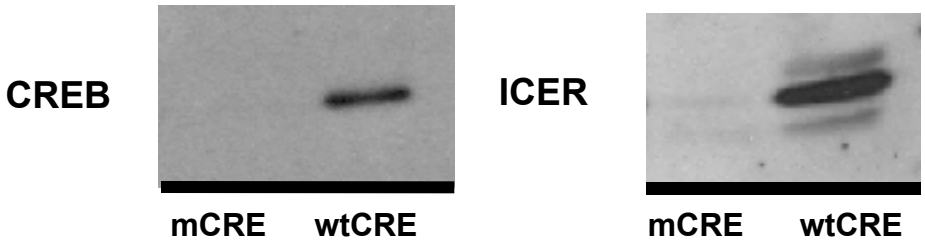
Fig. S6. BDNF increases binding of pSTAT3 to the endogenous ICER promoter as compared to isotype antibody control.

Supplemental Figure 1 (S1). ***GABAR α1 mRNA expression is decreased in DG after pilocarpine induced SE.***



Quantification of RT-PCR analysis of GABAR α1 mRNA expression in dentate gyrus (DG) after SE. RNA was extracted from microdissected DG of rats 24 hrs to 1 week after SE and from controls that were injected with a subconvulsive dose of pilocarpine. α1 mRNA expression was normalized to cyclophilin expression and expressed as fold change compared to controls (defined as 1). GABAR α1 mRNA expression changes are not detectable at 24 hrs (mean +/- SEM), then decrease by 34.8% at 48 hours (n=5 ctrl, n=6 SE; $p<0.05$) and 46.5% at 1 week (n=6 ctrl, n=7 SE, $p<0.05$).

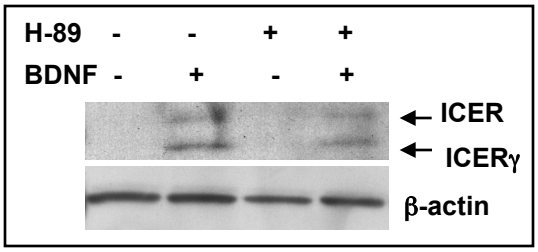
Supplemental Figure 2 (S2). ***Mutant CRE site does not bind CREB or ICER after DNA Pulldown.***

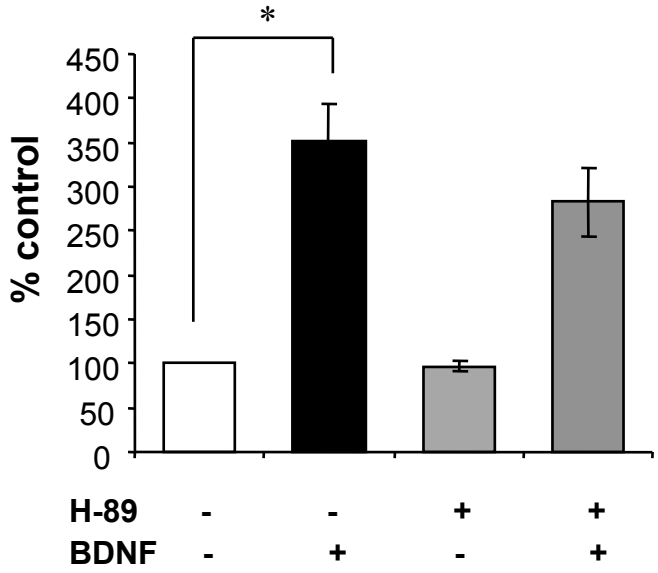


Nuclear extract was prepared from primary cultured neurons after 4 h forskolin treatment to induce ICER expression. 600 μg of nuclear extract was incubated with 1 mg of Dynabeads Streptavidin bound to either mutant or wildtype A1 CRE oligonucleotide duplexes. Eluted proteins were analysed by western blot with either anti-CREB or anti-CREM antibodies that detect ICER protein. A representative gel is shown.

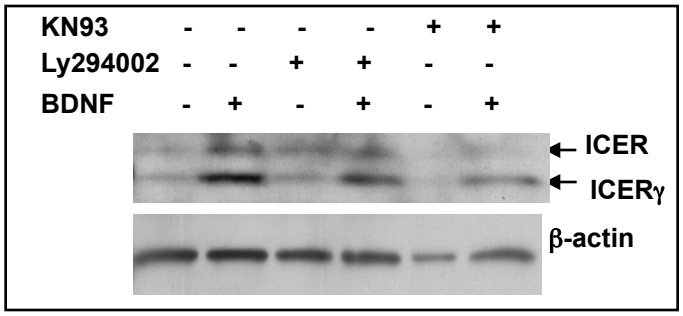
Supplemental Figure 3 (S3). ***BDNF-induced ICER is not dependent on PKA, CaM Kinase, or PI3 kinase activation.***

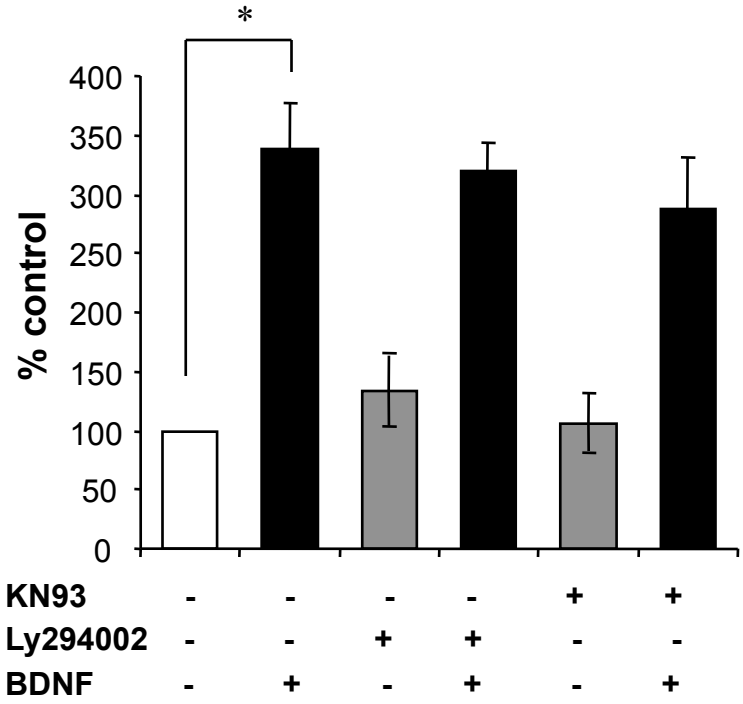
A





B

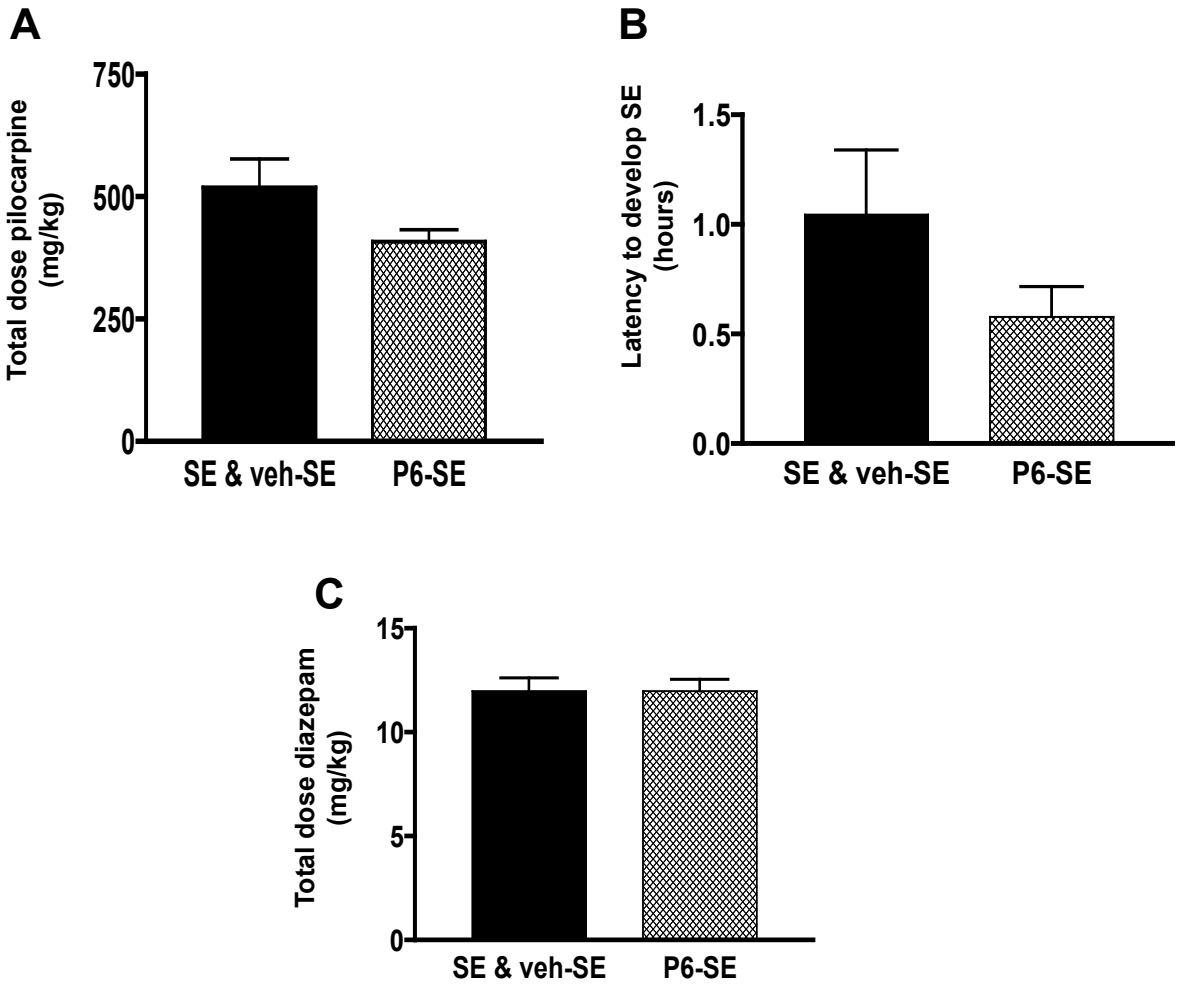




Supplemental Figure 3 (S3). ***BDNF-induced ICER is not dependent on PKA, CaM Kinase, or PI3 kinase activation.***

Cultures were pretreated with A) the PKA inhibitor H-89 (10 μM), B) the CaM kinase inhibitor KN93 (10 μM), PI3 kinase inhibitor LY294002 (20 μM) or vehicle (DMSO) for 1 h. Cultures were then treated with either vehicle (H_2O) or BDNF (50ng/ml) for 4h. Total cellular proteins were extracted. Western blot was performed using polyclonal CREM antibodies. Proteins were visualized using ECL following incubation with an anti-rabbit HRP-conjugated antibody. Representative Western blots are shown in A and B. Normalized data (ICER/β-actin) is presented in the bar graphs and expressed as percent change with respect to vehicle-treated cultures (defined as 100%). β-actin abundance did not vary with BDNF treatment and was used as an internal control. Significant changes are as indicated (* $p<0.05$; mean ± SEM; n=3).

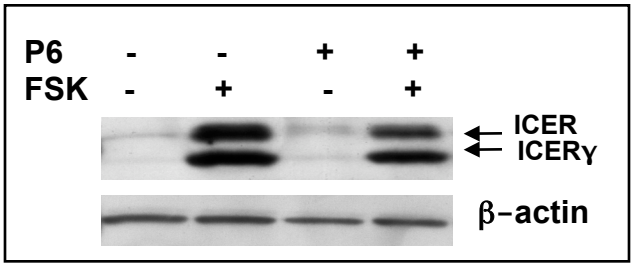
Supplemental Figure 4 (S4). ***Inhibition of JAK/STAT pathway via slow infusion of P6 beginning 48 hours before SE does not affect SE.***

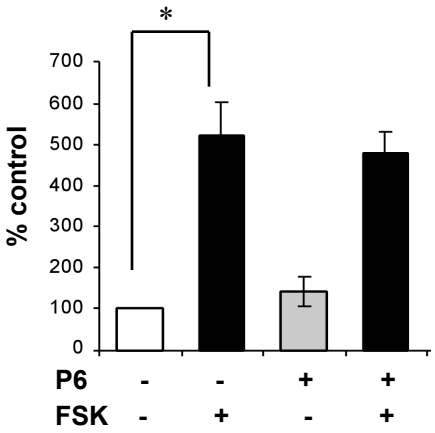


Supplemental Figure 4 (S4). ***Inhibition of JAK/STAT pathway via slow infusion of P6 beginning 48 hours before SE does not affect SE.***

The total dose of pilocarpine (mg/kg) required to induce SE, latency (hours) to develop SE, and total dose of diazepam (mg/kg) required to stop SE were not significantly different ($p>0.05$) between SE-only (SE, n=8) and vehicle pump-implanted (veh-SE, n=6), results were therefore pooled. A) The total dose of pilocarpine (in mg/kg) required to induce SE was not different between SE & veh-SE and P6-SE rats (n= 14 SE & veh-SE, n=8 P6-SE; $p>0.05$). B) The latency (in hours) between pilocarpine injection and development of SE was not different between the groups. ($p>0.05$). C) The total dose of diazepam (mg/kg) required to stop SE was not different between groups ($p>0.05$). All Statistics were performed using the Mann-Whitney test.

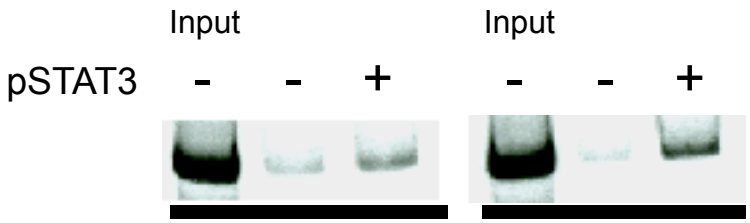
Supplemental Figure 5 (S5). ***No effect of P6 on PKA induced ICER synthesis.***





Cultures were pretreated with a pan JAK inhibitor P6 (500 nM) or vehicle (DMSO) for 1 hr and then treated with either vehicle (Me_2SO) or FSK (20 μM) for an additional 4 hrs. Total cellular proteins were extracted and Western blot was performed using polyclonal CREM antibodies (left panel). Proteins were visualized using ECL. Amounts of β-actin protein did not vary with FSK treatment and were used as an internal control. Bar graph of data quantitation (right panel) shows no effect of P6 on the marked induction of ICER after FSK treatment.

Supplemental Figure 6 (S6). ***BDNF increases binding of pSTAT3 to the endogenous ICER promoter as compared to isotype antibody control.***



An increase in pSTAT3 binding to the endogenous ICER promoter is observed in hippocampal cells following 2 hours of BDNF treatment as measured by chromatin immunoprecipitation. Control isotype antibody is chicken-polyhistidine tag (Chemicon) which gives a similar non-specific signal as detected with IgG (see Methods). “+” indicates presence of pSTAT3 in the precipitation reaction. “-” indicates presence of isotype antibody for reactions that do not contain Input DNA.