

PKA Pathway Modulation in Autoimmune Encephalitis: Implications for Rescue of Synaptic Dysfunction

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Background

Autoimmune encephalitis (AIE) is a neuroinflammatory disorder that causes memory loss, cognitive impairment, behavioral changes, seizures, and movement disorders¹. The most common type of AIE is caused by autoantibodies that target and decrease the density of ionotropic glutamate N-methyl-D-aspartate receptors (NMDARs) that regulate responsiveness to synaptic transmission¹. Eliminating cell surface NMDARs through autoantibody-binding decreases the cells ability to integrate synaptic activity. The disrupted signaling pathways resulting from anti-NMDAR antibody-mediated receptor internalization remain unknown.

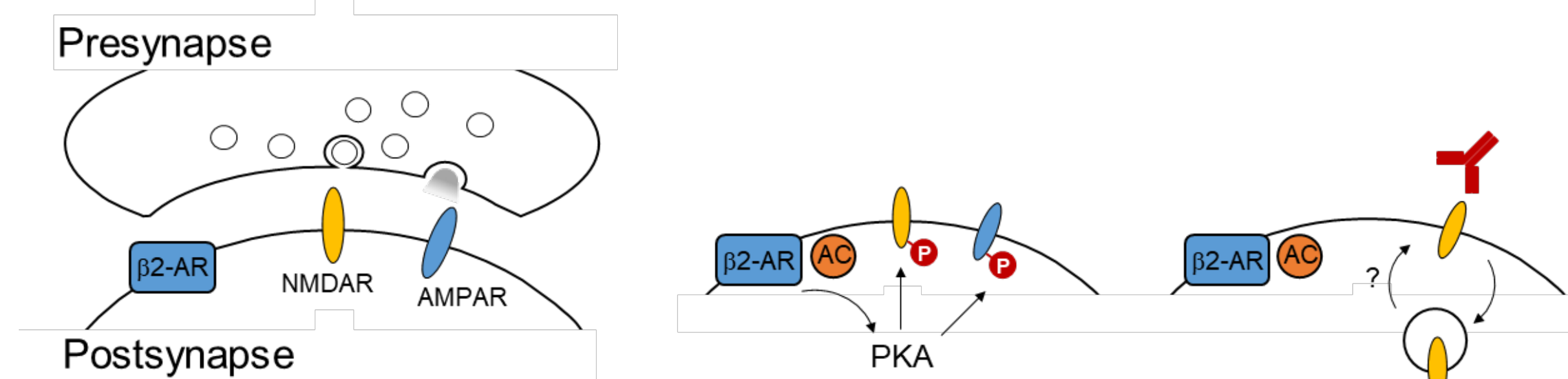
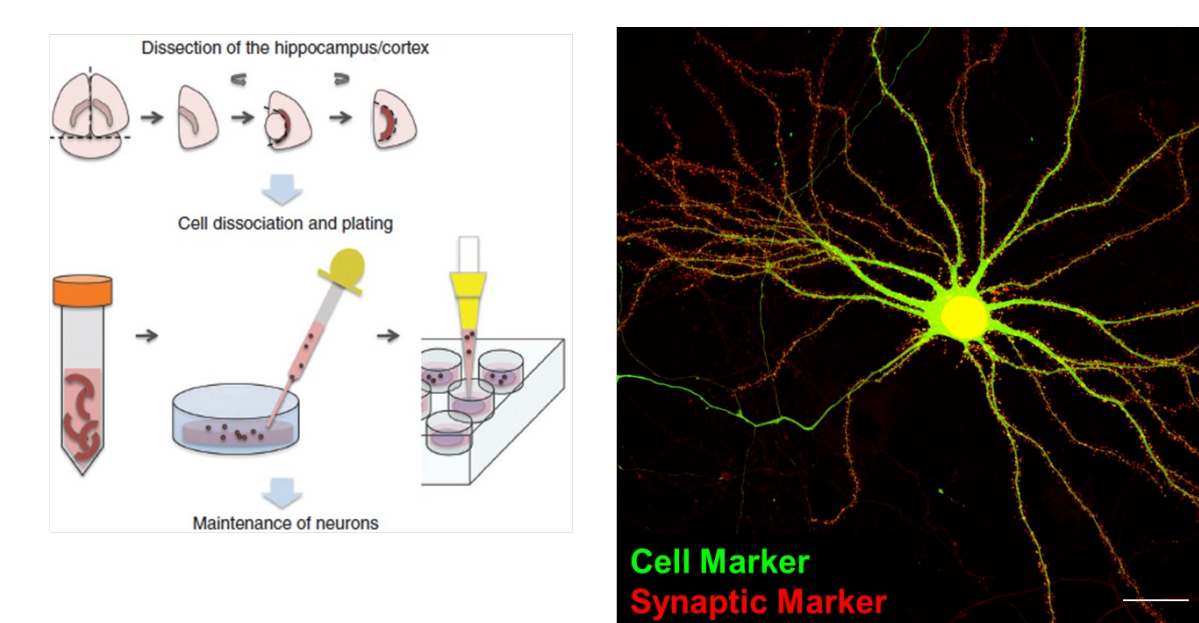


Figure 1: Schematic of GPCRs and glutamate receptors at synaptic sites implicated in learning and memory.

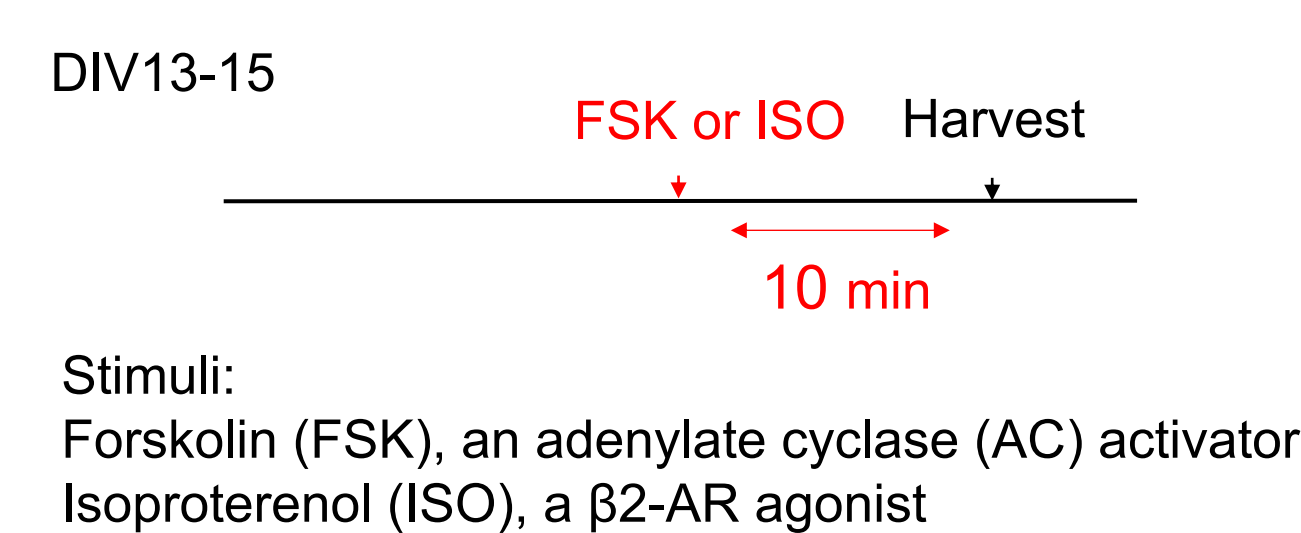
Mechanisms to restore NMDAR function in AIE are lacking. β_2 -adrenergic receptors (β_2 -ARs) are a type of G-protein coupled receptor (GPCR) that have been implicated in learning and memory. When stimulated, β_2 -ARs initiate a signaling cascade that activates protein kinase A (PKA)². This study aimed to elucidate the relationship between PKA signaling pathways and internalization of NMDARs as part of a larger effort to expand the knowledge for novel treatment modalities for AIE.

Methods

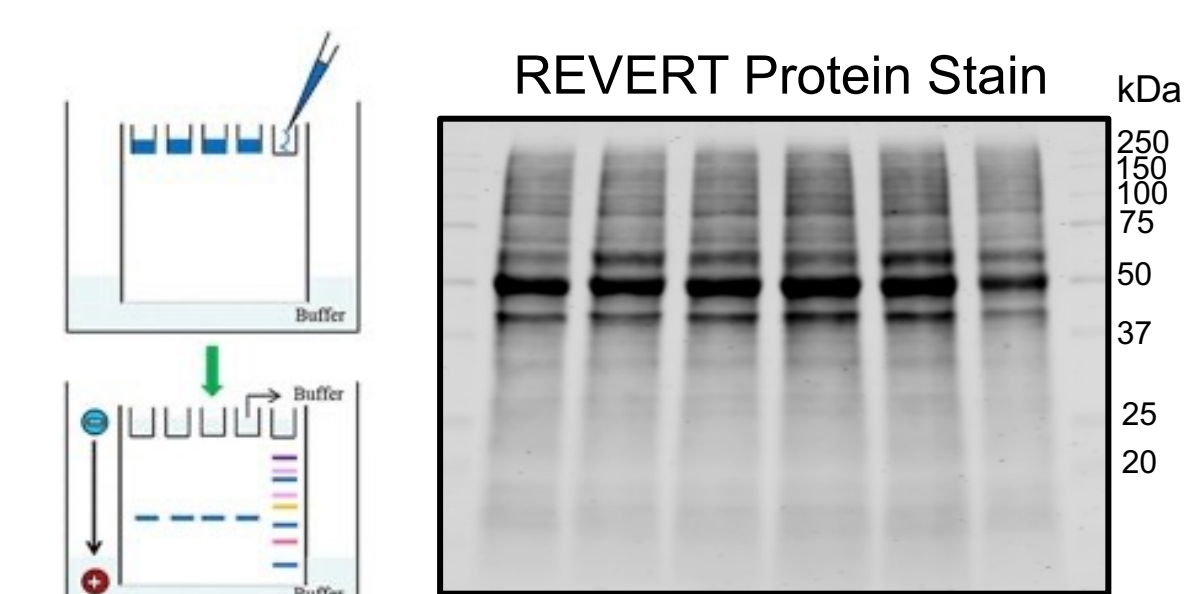
1. Rat Primary Neuron Culture



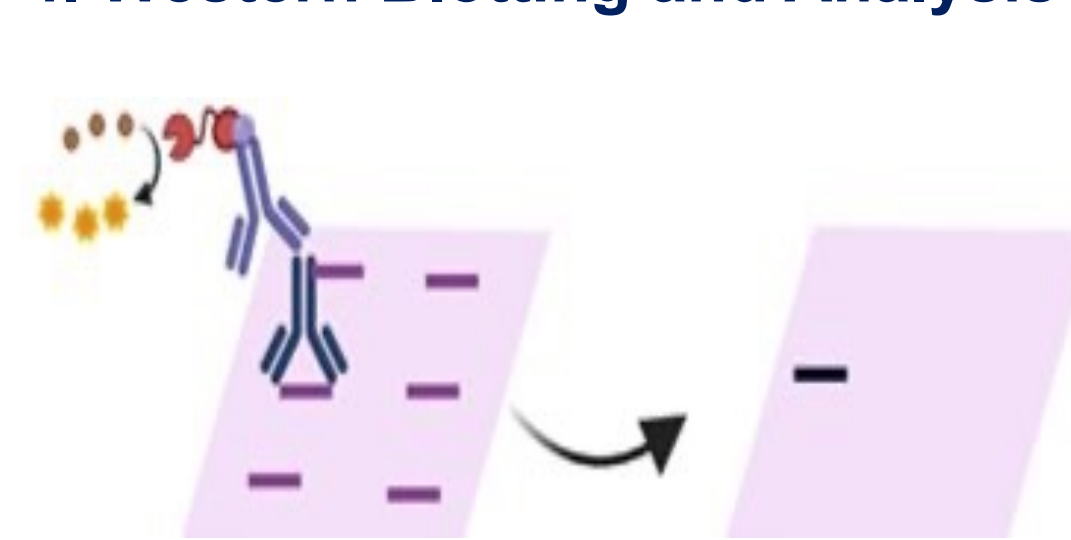
2. Pharmacology of the PKA Pathway



3. SDS-PAGE and Protein Stain



4. Western Blotting and Analysis



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Phosphorylation of PKA substrates increases with Forskolin Treatment

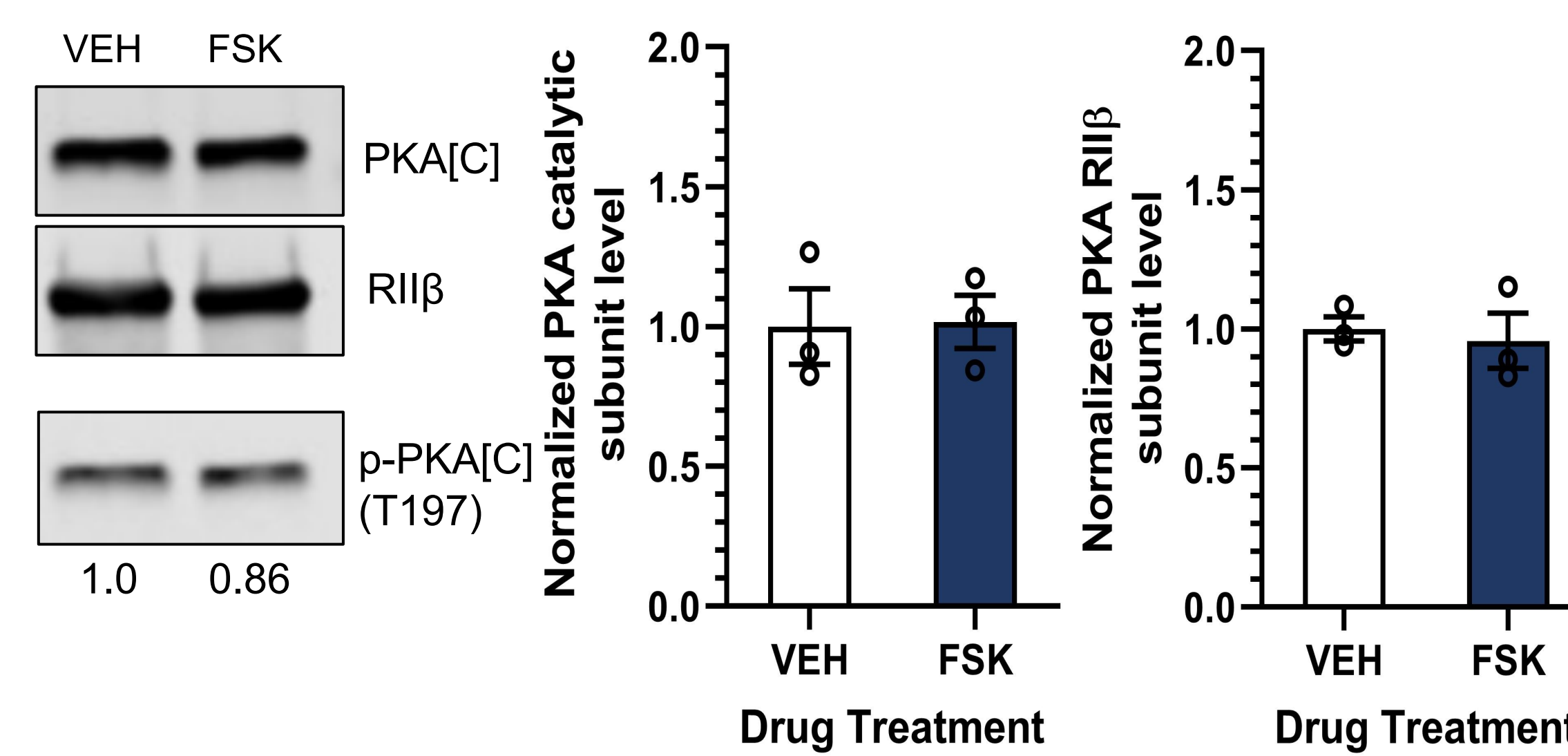


Figure 2: Western blot analysis of DIV13 primary neurons treated with 10 μ M of FSK for 10 minutes. Phosphorylation and expression changes were analyzed through densitometry for PKA[C], RII β , and phospho-PKA[C] (T197). Histograms represent mean \pm SEM. N=6 per group.

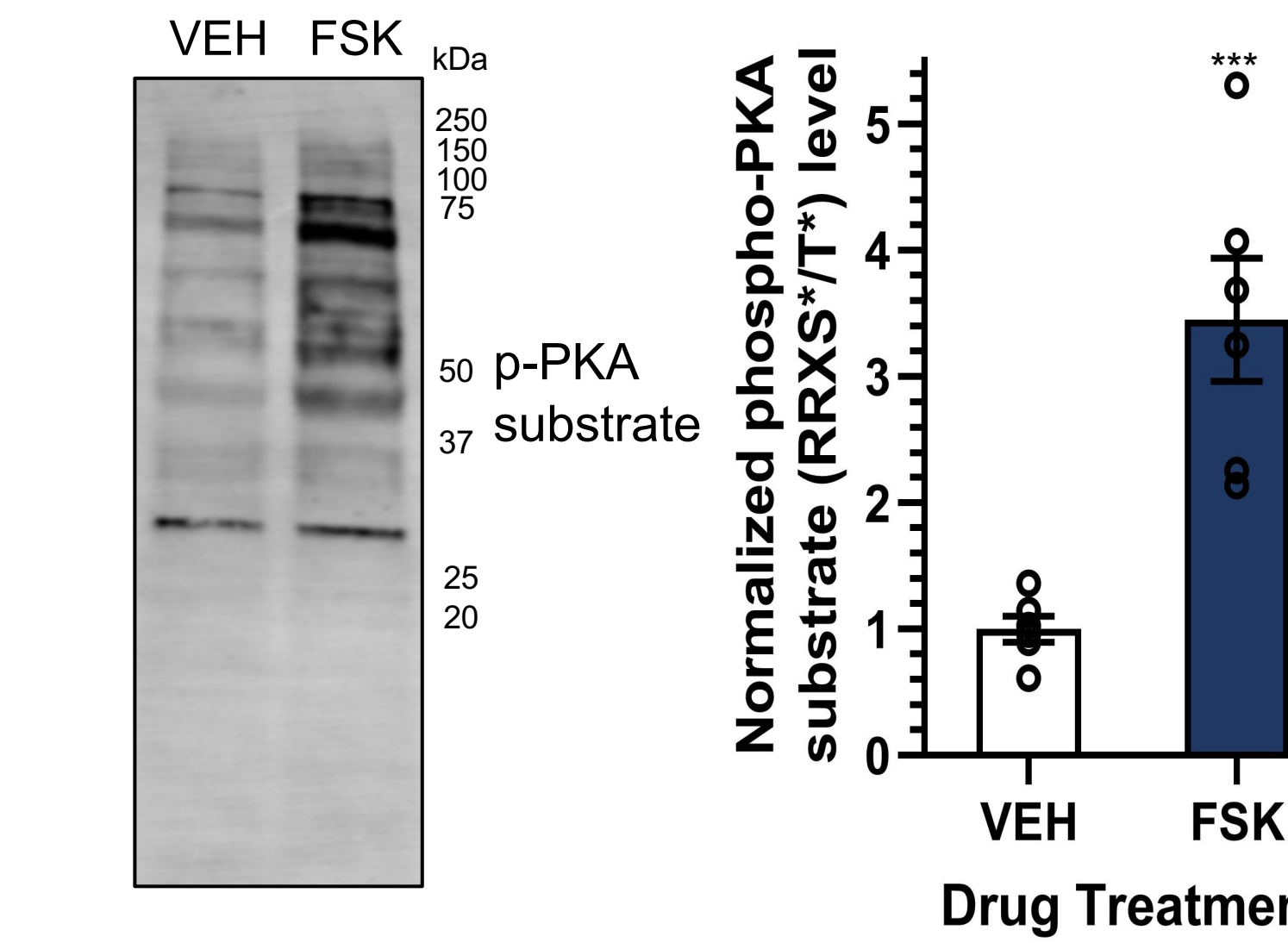


Figure 3: Western blot analysis for phospho-PKA substrate of DIV13 primary neurons treated with 10 μ M of FSK for 10 minutes. Histograms represent mean \pm SEM. *** $p < 0.001$. N=6 per group.

Goals

1. To define PKA signaling pathways in primary neurons using readouts of intracellular targets of the PKA pathway.
2. To characterize the pharmacological activation of the PKA pathway using Forskolin.
3. To examine the impact of activation of β_2 -adrenergic receptors on PKA signaling using Isoproterenol.

Conclusions

Through quantitative analysis and comparison of vehicle-treated control samples and those treated with AC and β -AR activators, we have established that these pharmacological interventions effectively enhance PKA activity in primary neurons. Application of Forskolin for 10 minutes in primary neurons led to a statistically significant increase in the phospho-PKA substrate motif level. Dose-dependent increases in phospho-PKA substrate levels were also observed in primary neurons following the application of Isoproterenol. The observed increase in PKA activity suggests that the AC and β -AR pathways play crucial roles in regulating PKA signaling in our experimental model.

Dose-dependent increase of PKA activation by Isoproterenol

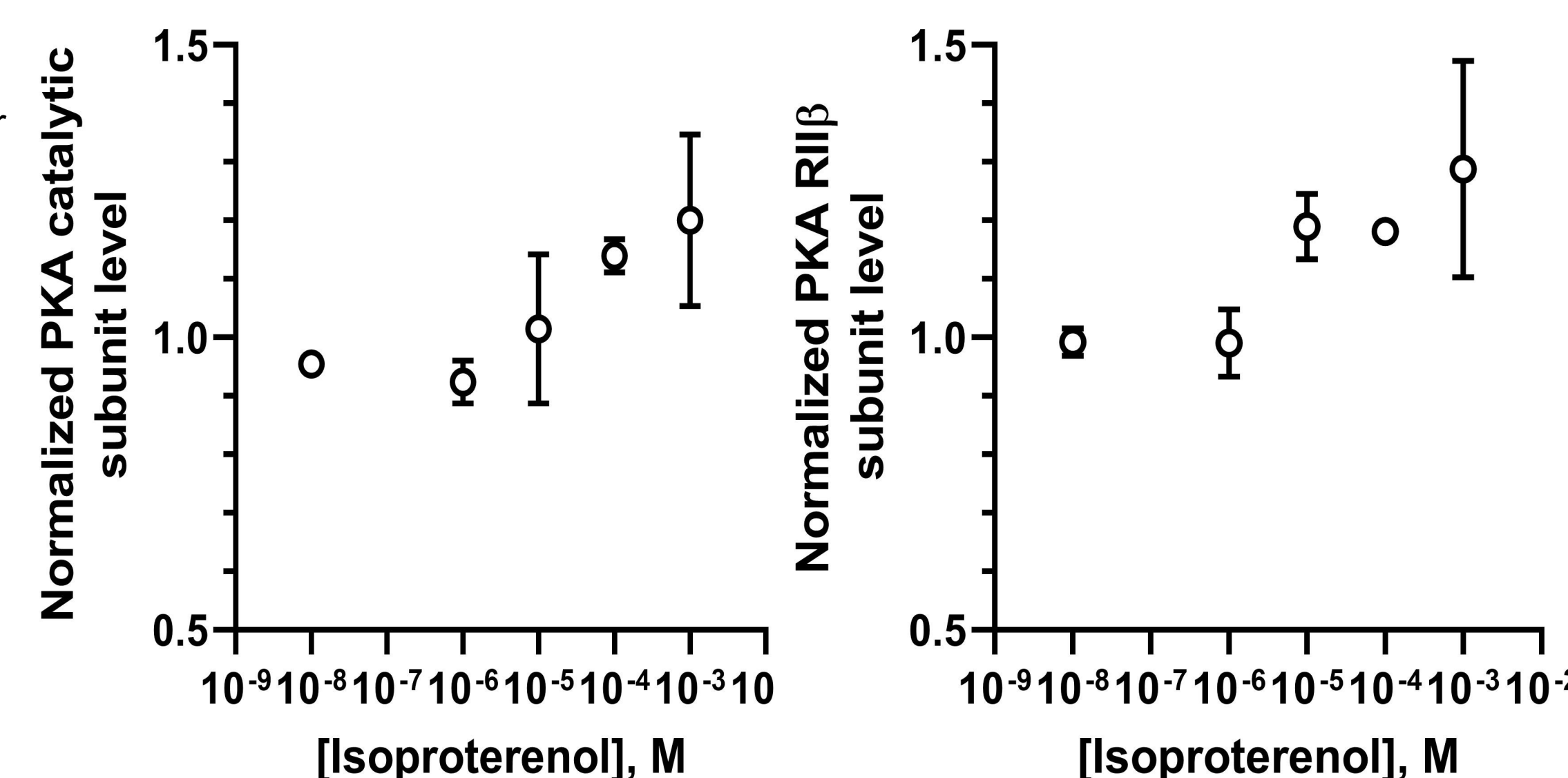
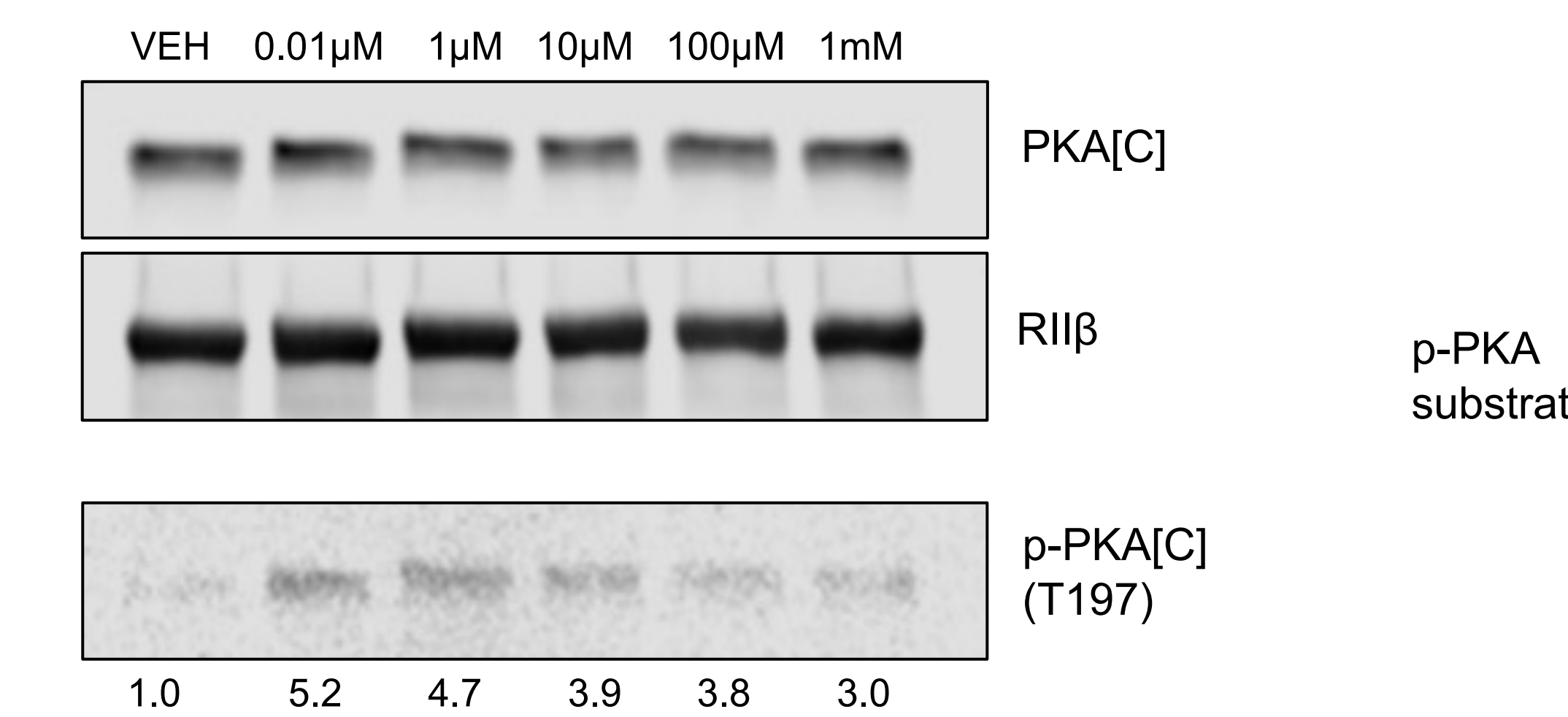


Figure 4: Western blot analysis of DIV13 primary neurons treated with varying doses of ISO for 10 minutes. Phosphorylation and expression changes were analyzed through densitometry for PKA[C], RII β , and phospho-PKA[C] (Thr197). Plots represent mean \pm SEM. N=2 per group.

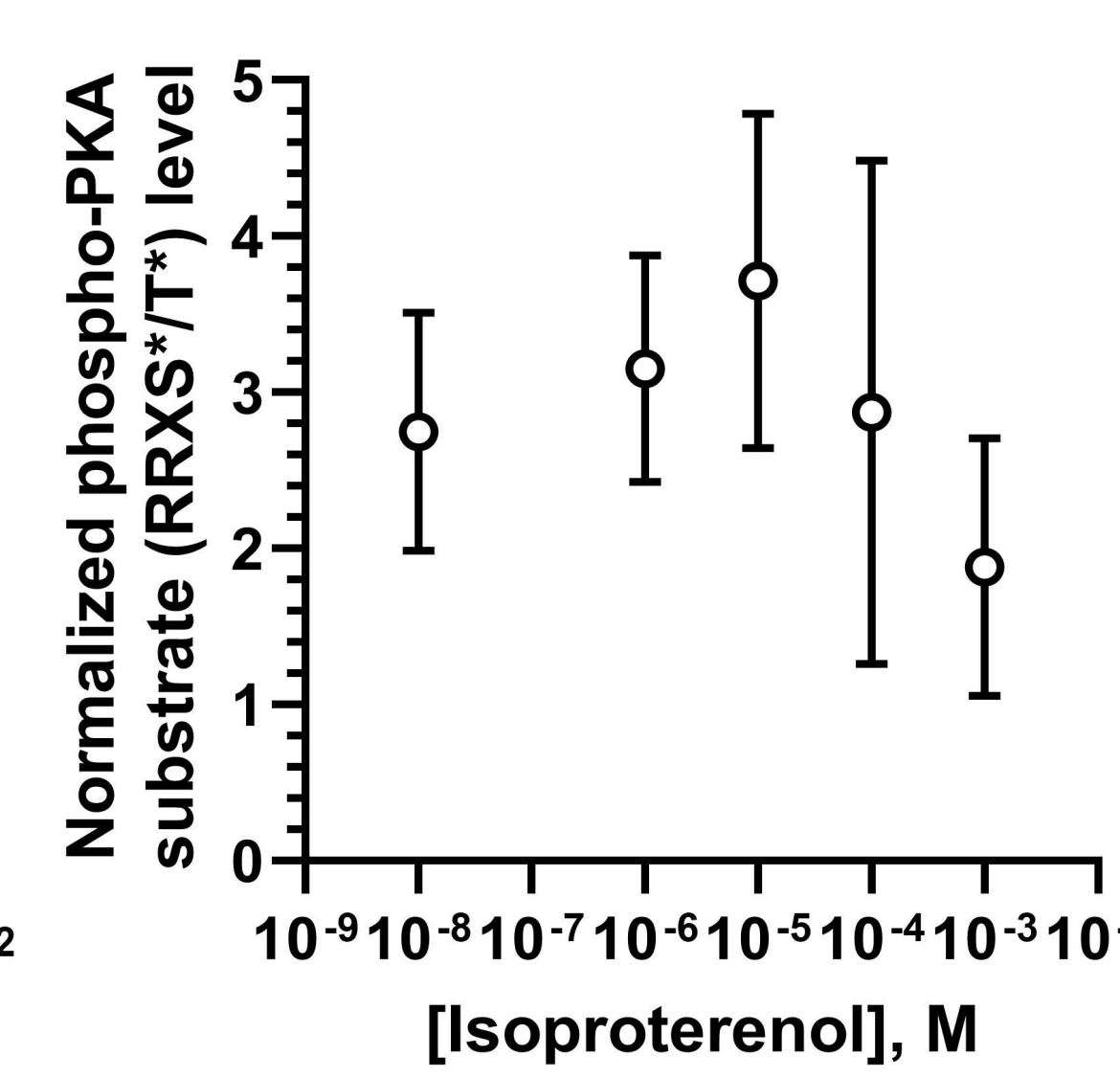
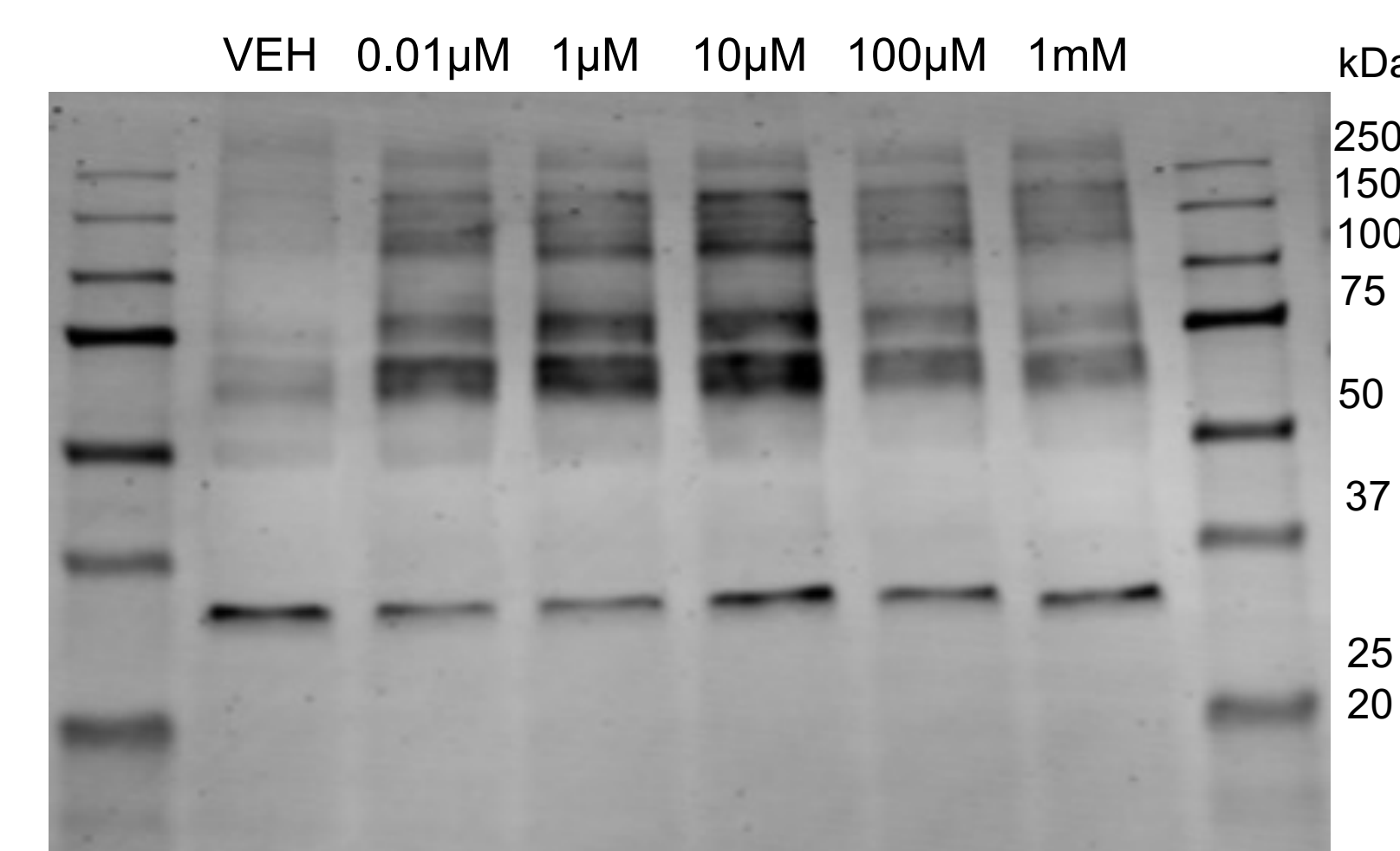
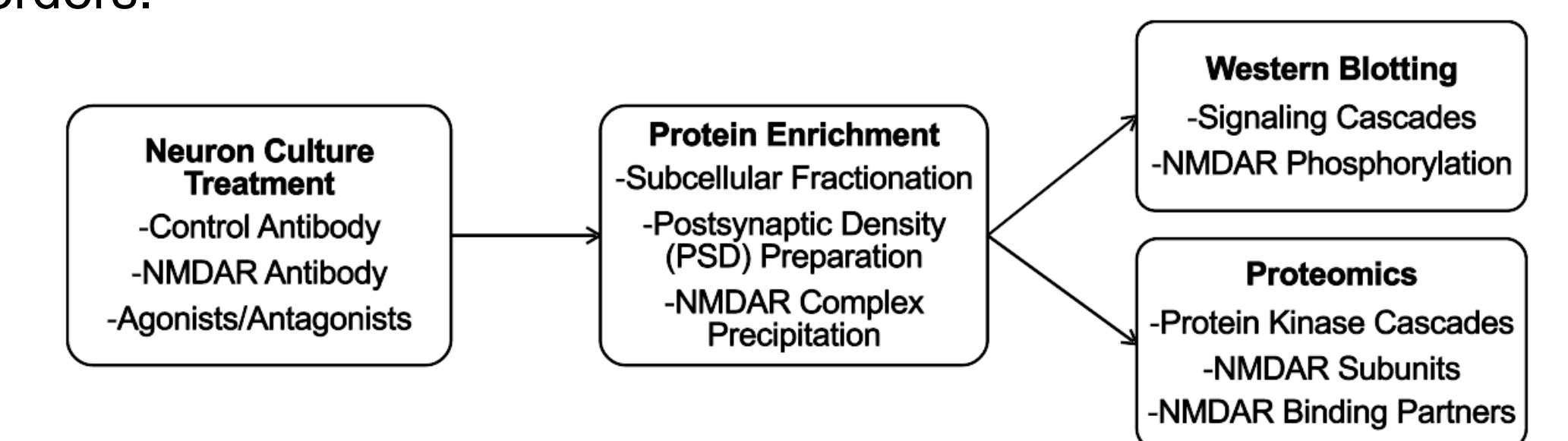


Figure 5: Western blot analysis for phospho-PKA substrate of DIV13 primary neurons treated with varying doses of ISO for 10 minutes. Plots represent mean \pm SEM. N=2 per group.

Future Directions

In future investigations, we plan to expand our research by incorporating anti-NMDAR antibody treatments that mimic disease-related NMDAR dysregulation prior to pharmacological activation of PKA pathway. We also plan to expand our western blot analyses to include a wider range of PKA machinery targets (*i.e.* PKA subunits, A-kinase anchoring proteins), as well as phosphorylation of PKA motifs on AMPA receptors (pS845 GluA1) and CREB (pS133 CREB). These readouts will be compared among groups to confirm differences in PKA signaling when GluN1 human monoclonal antibodies are applied. By elucidating the molecular mechanisms underlying these interactions, we hope to identify novel avenues for intervention and contribute to the development of targeted treatments for AIE and related disorders.



References

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3. Beaudoin, G., Lee, SH., Singh, D. et al. Culturing pyramidal neurons from the early postnatal mouse hippocampus and cortex. *Nat Protoc* 7, 1741–1754 (2012). doi:10.1038/nprot.2012.099
4. Methods images adapted from bioRad.