Dysregulation of Stress Signaling and Synaptic Proteome in Depression

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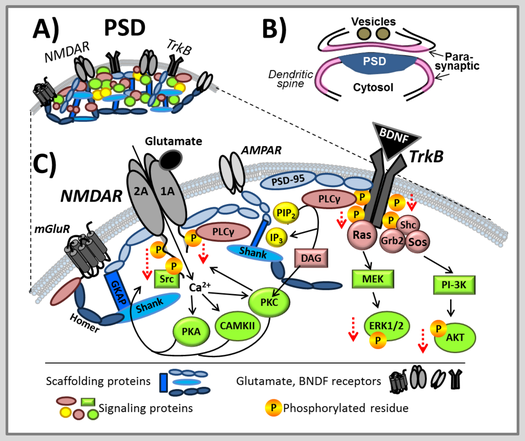
Introduction

- Synaptic dysfunction is implicated in the pathophysiology of depression.[1] Postsynaptic density (PSD) proteins can regulate synaptic function through interactions with NMDA (NR) and TrkB receptors.
- Depression is more prevalent in women[2] and sex differences exist in studies of synaptic dysfunction.[3] We reported structural deficits and decreased PSD-95 and spinophilin in the anterior hippocampus in behaviorally depressed adult female monkeys.[4,5]



- **PURPOSE:** To test the hypothesis that altered synaptic integrity in behaviorally depressed monkeys is mediated by dysregulated NR and TrkB signaling, and disrupted PSD protein composition.

Methods

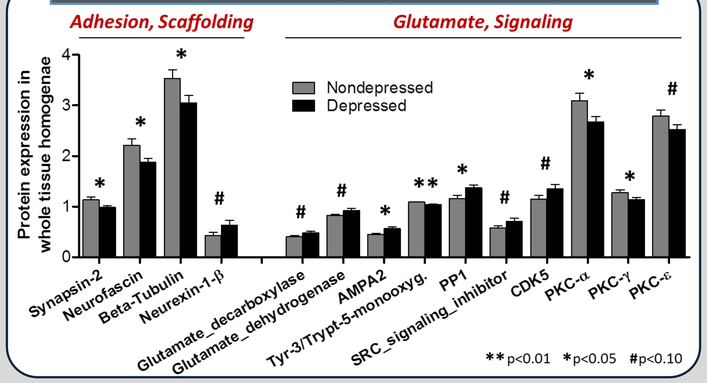
- Spontaneously occurring depressive behavior was observed 2x/wk for 4 yrs in 36 socially-housed, adult female monkeys. N=8 depressed monkeys matched with 8 nondepressed body weight, cortisol, estradiol and social status.[4,5]

Depressive behavior (left) was defined as a slumped/ collapsed body posture and open eyes accompanied by a lack of responsiveness to environmental stimuli.[4,5]

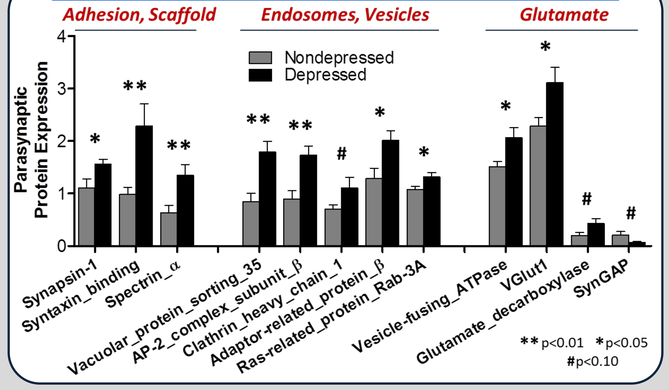
- Nuclear, cytosolic, vesicular, parasynaptic, and PSD compartments were biochemically fractionated from the anterior CA1 and mixed with L-Lysine $^{13}C_6$ labeled mouse brain (stable isotope labeling of amino acids in mammals, SILAM).[6] Proteins were separated with SDS-PAGE and trypsin-digested.
- Liquid chromatography-selected reaction monitoring mass spectrometry (LC-SRM/MS) was used for quantification of **>200 target synaptic proteins**.[6]
- Postmortem anterior entorhinal cortex was incubated with NDMA or BDNF, NR complexes were immunoprecipitated, and signaling partners of NR or TrkB were assessed by immunoblotting.

Results

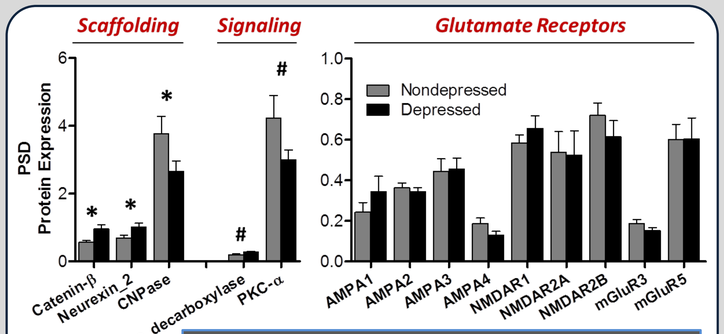
1. Expression of key synaptic proteins in whole tissue was altered in behaviorally depressed monkeys.



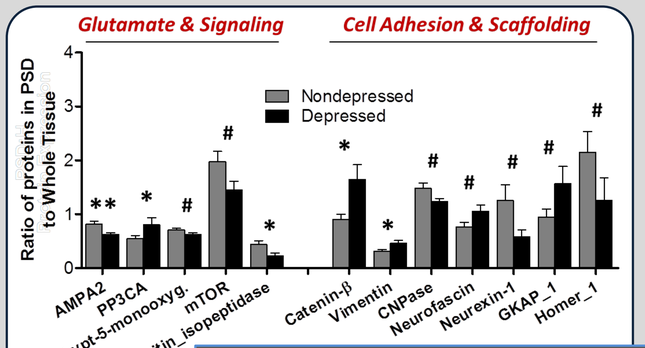
2. Expression of many synaptic proteins in the parasynaptic fraction was greater in behaviorally depressed compared to nondepressed monkeys.



3. PSD protein composition differed minimally between behaviorallly depressed and non-depressed monkeys. *p<0.05; #p<0.10.

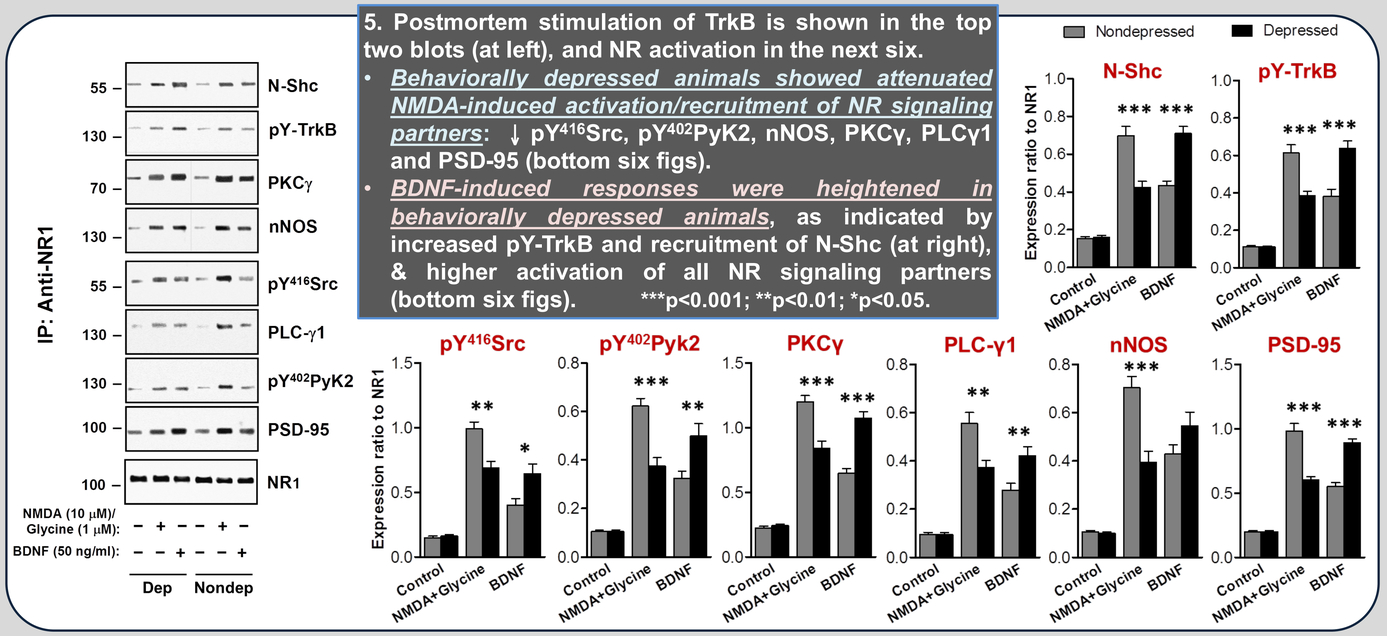


4. Targeting of proteins to the PSD may be altered in behaviorally depressed monkeys. **p<0.01; *p<0.05; #p<0.10.

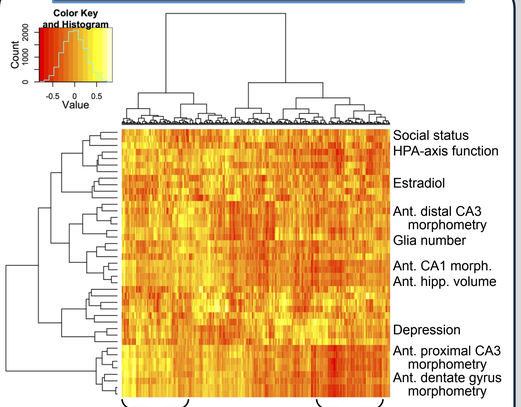


5. Postmortem stimulation of TrkB is shown in the top two blots (at left), and NR activation in the next six.

- ***Behaviorally depressed animals showed attenuated NMDA-induced activation/recruitment of NR signaling partners***: ↓ pY[416]Src, pY[402]PyK2, nNOS, PKCγ, PLCγ1 and PSD-95 (bottom six figs).
- ***BDNF-induced responses were heightened in behaviorally depressed animals***, as indicated by increased pY-TrkB and recruitment of N-Shc (at right), & higher activation of all NR signaling partners (bottom six figs). ***p<0.001; **p<0.01; *p<0.05.



6. PSD proteomic data correlates with behavioral, physiological and hippocampal morphometric data.



Presynaptic: SYN2, SV2A
Trafficking: AP2A1, ARF1
G-protein signaling: GNAQ, GNB1
IRS1 signaling: YWHAZ, YWHAQ, YWHAE
Scaffolding, cell adhesion: SEPT4, SPTBN1, CASK, CTNNB1
Glutamate: GRIA2
Protein degradation: UBR4
Mitochondrial, glucose: CKB, TPI1, PRDX5, HK1, AK1

Signaling kinases, phosphatases: PKCα, PKCγ, mTOR, PPP3R1, DLGAP2
Glutamate: GRIK2, GRIA4, GRIN1
Dendritic spines: PPP1R9B
Scaffolding, cytoskeletal, adhesion: HOMER1, DCTN1, NCAM1
Membrane trafficking: VPS35, RAb14
Growth factor signaling: FLOT2
Calcium channels: CACNB1
HSP Signaling: STIP1, HSP90AB1

Conclusions

- Synaptic protein composition in behaviorally depressed female monkeys is altered in ways that may affect the structure, strength and activity of synapses, Specifically, the observed alterations are likely to precipitate changes in NR and TrkB signaling.
- NR function is attenuated in behaviorally depressed females, pointing to PLC-γ1, PKCγ, Src, nNOS, PSD-95 and Pyk2 as loci of dysregulation.
- BDNF signaling is heightened in depressed females, and accompanied by an increased association between TrkB and NR.[7]
- In the same well-characterized and matched sample of behaviorally depressed female monkeys, hippocampal proteomic data correlates with behavioral, physiological and hippocampal morphometric data.

References & Acknowledgments

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