Behavioural Brain Research 304 (2016) 51–59

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

# Adolescent social defeat alters *N*-methyl-D-aspartic acid receptor expression and impairs fear learning in adulthood

Andrew M. Novick[a,*], Mackenzie Mears[a], Gina L. Forster[a], Yanlin Lei[b], Shanaz M. Tejani-Butt[b], Michael J. Watt[a]

[a] *Center for Brain and Behavior Research, Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, 414 E. Clark St., Vermillion, SD 57069, USA*
[b] *Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, 600 S 43rd St., Philadelphia, PA 19104, USA*

## HIGHLIGHTS

- Adolescent defeat alters NMDA receptor expression in discrete brain areas.
- Adult fear conditioning and extinction are decreased following adolescent defeat.
- Altered glutamate signaling may underlie behavioral outcomes of adolescent stress.

## ARTICLE INFO

*Article history:*
Received 13 August 2015
Received in revised form 27 January 2016
Accepted 8 February 2016
Available online 10 February 2016

*Keywords:*
NMDA
Adolescence
Stress
Social defeat
Conditioned fear

## ABSTRACT

Repeated social defeat of adolescent male rats results in adult mesocortical dopamine hypofunction, impaired working memory, and increased contextual anxiety-like behavior. Given the role of glutamate in dopamine regulation, cognition, and fear and anxiety, we investigated potential changes to *N*-methyl-D-aspartic acid (NMDA) receptors following adolescent social defeat. As both NMDA receptors and mesocortical dopamine are implicated in the expression and extinction of conditioned fear, a separate cohort of rats was challenged with a classical fear conditioning paradigm to investigate whether fear learning is altered by adolescent defeat. Quantitative autoradiography was used to measure 3H-MK-801 binding to NMDA receptors in regions of the medial prefrontal cortex, caudate putamen, nucleus accumbens, amygdala and hippocampus. Assessment of fear learning was achieved using an auditory fear conditioning paradigm, with freezing toward the auditory tone used as a measure of conditioned fear. Compared to controls, adolescent social defeat decreased adult NMDA receptor expression in the infralimbic region of the prefrontal cortex and central amygdala, while increasing expression in the CA3 region of the hippocampus. Previously defeated rats also displayed decreased conditioned freezing during the recall and first extinction periods, which may be related to the observed decreases and increases in NMDA receptors within the central amygdala and CA3, respectively. The alteration in NMDA receptors seen following adolescent social defeat suggests that dysfunction of glutamatergic systems, combined with mesocortical dopamine deficits, likely plays a role in the some of the long-term behavioral consequences of social stressors in adolescence seen in both preclinical and clinical studies.



## 1. Introduction

Glutamate, as one of the primary excitatory amino acids, plays a ubiquitous role in brain function. Importantly, glutamate activity at *N*-methyl-D-aspartic acid (NMDA) receptors has been linked to changes in synaptic plasticity, learning and memory, and modulation of other neurotransmitter systems [1–3]. Given its influence on brain structure and function, alterations in NMDA receptor activity as a result of stressful experience have been linked to a variety of psychiatric disorders [3,4].

*Abbreviations:* NMDA, *N*-methyl-D-aspartic acid; mPFC, medial prefrontal cortex; CPu, caudate putamen; NAc, nucleus accumbens; CeA, central amygdala; BLA, basolateral amygdala; CA, cornu ammonis.

* Corresponding author. Current address: Butler Hospital, Department of Psychiatry and Human Behavior, Warren Alpert Medical School, Brown University, 345 Blackstone Blvd, Providence, RI 02906, USA.
*E-mail address:* andrew_novick@brown.edu (A.M. Novick).

http://dx.doi.org/10.1016/j.bbr.2016.02.013
0166-4328/

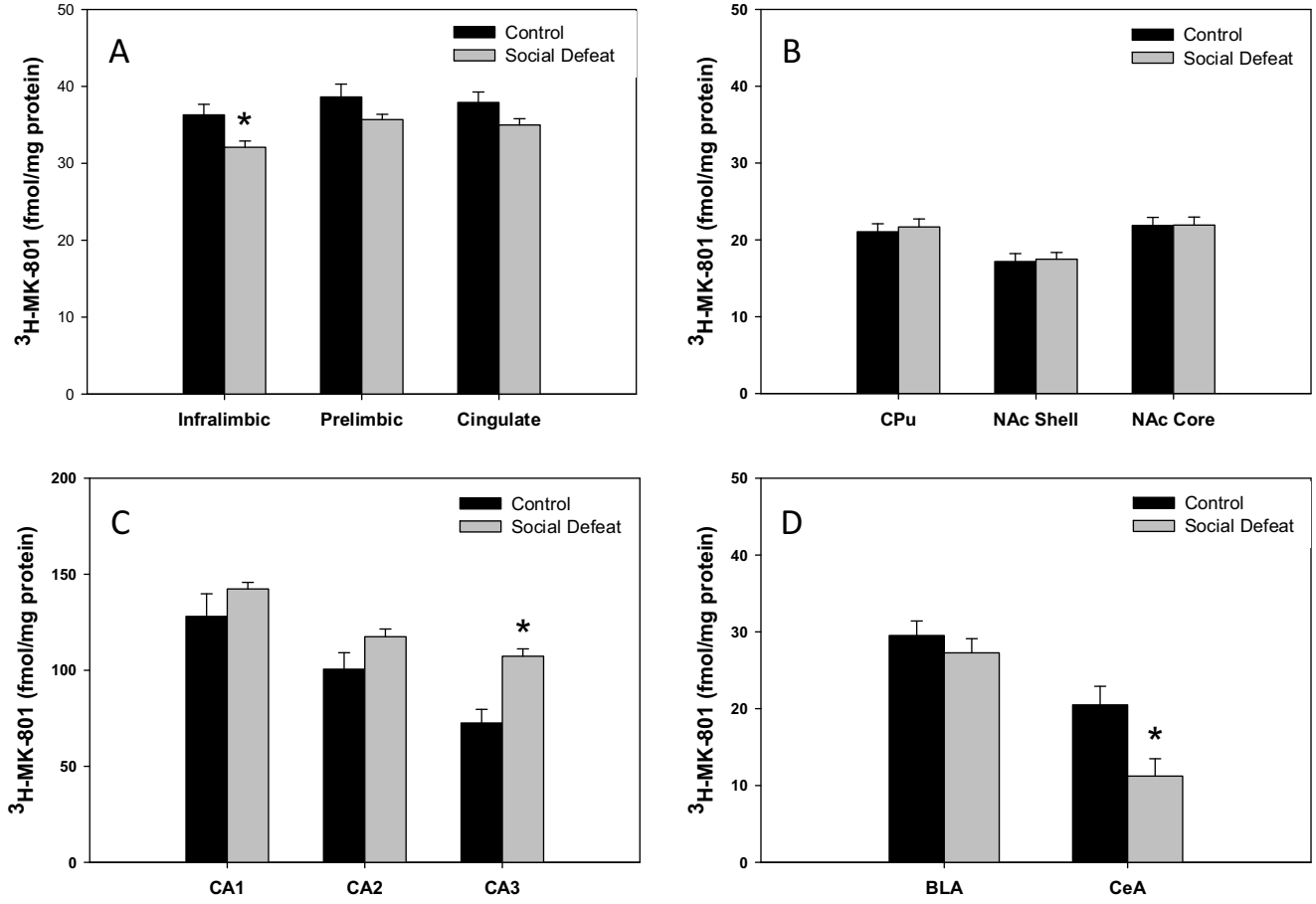


**Fig. 1.** Specific binding of [3H]-MK801 to NMDA receptors in brain regions of adult rats that underwent adolescent social defeat *versus* controls. (A) Medial prefrontal cortex (mPFC), (B) caudate putamen (CPu) and nucleus accumbens (NAc), (C) hippocampal CA, and (D) basolateral amygdala (BLA) and central nucleus of the amygdala (CeA). Data are expressed as the mean ± S.E.M. determinations made in quadruplicate sections from each brain. *N* = 6–9/group. *Significant difference between treatment groups ($p < 0.05$).

**Table 1**
Freezing duration (s) across each 10 min time block within each testing session. Values represent mean ± S.E.M. #Difference among time blocks (one way repeated ANOVA within stress treatment, SNK $p < 0.005$), *Difference between control and defeat within the 11–20 min (tone) time block (two way repeated ANOVA stress x session, SNK $p < 0.002$). *N* = 12/group.

| Session | 0–10 min | 11–20 min (tone) | 21–30 min | ANOVA (one way repeated measures within stress treatment) |
|---|---|---|---|---|
| Conditioning | | | | |
| Control | 3.86 + 0.73 | 357.43 + 25.85# | 367.11 + 41.19# | $F(2,22) = 68.03$, $p < 0.001$ |
| Defeat | 2.85 + 0.71 | 360.41 + 24.25# | 372.10 + 46.89# | $F(2,21) = 60.8$, $p < 0.001$ |
| Recall | | | | |
| Control | 71.44 + 34.34 | 522.61 + 15.89# | 67.18 + 15.83 | $F(2,20) = 114.6$, $p < 0.001$ |
| Defeat | 17.76 + 7.67 | 387.82 + 38.78#* | 73.61 + 26.74 | $F(2,21) = 73.9$, $p < 0.001$ |
| Extinction 1 | | | | |
| Control | 12.63 + 2.37 | 300.68 + 45.08# | 29.64 + 6.67 | $F(2,22) = 40.3$, $p < 0.001$ |
| Defeat | 19.73 + 7.09 | 172.78 + 39.27#* | 17.15 + 7.53 | $F(2,21) = 14.8$, $p < 0.001$ |
| Extinction 2 | | | | |
| Control | 5.51 + 0.53 | 170.96 + 29.80# | 16.52 + 2.75 | $F(2,21) = 29.7$, $p < 0.001$ |
| Defeat | 3.12 + 0.66 | 104.89 + 26.48# | 11.02 + 2.51 | $F(2,20) = 10.5$, $p < 0.001$ |
| Extinction 3 | | | | |
| Control | 8.44 + 1.19 | 90.29 + 10.87# | 25.98 + 4.99 | $F(2,17) = 38.6$, $p < 0.001$ |
| Defeat | 5.61 + 0.83 | 56.46 + 11.57# | 18.98 + 3.51 | $F(2,21) = 11.9$, $p < 0.001$ |
| Extinction 4 | | | | |
| Control | 5.38 + 0.91 | 44.32 + 4.31# | 12.22 + 3.19 | $F(2,21) = 36.5$, $p < 0.001$ |
| Defeat | 3.47 + 0.76 | 33.05 + 7.58# | 12.77 + 3.68 | $F(2,21) = 13.6$, $p < 0.001$ |

Psychiatric disorders are particularly common in individuals who have experienced severe social stressors during adolescence [5], but the role of NMDA receptors in outcomes of adolescent social stress has yet to be fully delineated. It is known that NMDA receptor expression undergoes changes throughout the brain during adolescence [6], potentially enhancing the vulnerability of adolescents to stress-induced NMDA receptor dysfunction. In support of this, rats exposed to social instability stress during adolescence have been