Supplement for:

Role of adult hippocampal neurogenesis in persistent pain

A. Vania Apkarian[1,*], Amelia A. Mutso[1], Maria V. Centeno[1], Lixin Kan[2], Melody Wu[3], Marjorie Levinstein[3], Ghazal Banisadr[4], Kevin T. Gobeske[2], Richard J. Miller[4], Jelena Radulovic[5], René Hen[3], John A. Kessler[2]

Departments of [1]Physiology, [2]Neurology, [4]Molecular Pharmacology and Biological Chemistry, [5]Psychiatry and Behavioral Sciences, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA

[3]Department of Pharmacology, Columbia University, New York Psychiatric Institute, NY 10032, USA

6 supplemental figures

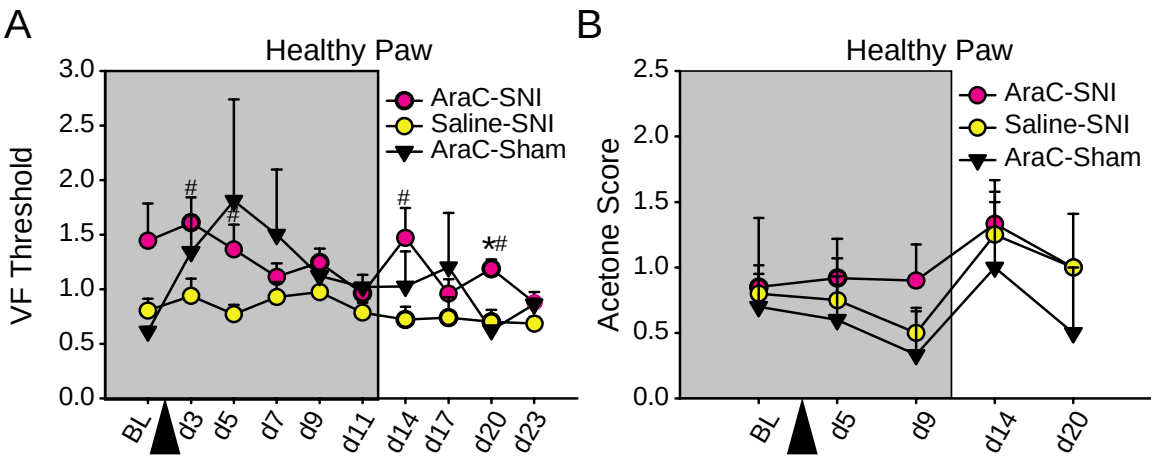

Figure S1. *Arac-SNI, Arac-Sham and Saline-SNI mice do not differ from each other in touch and heat sensitivity tested on the healthy paw. Post-hoc analysis indicates transient touch sensitivity differences at specific timepoints between Arac and Saline animals .*

(A) Von Frey 50% withdrawal thresholds (grams) of Saline-SNI, AraC-SNI, and AraC-Sham animals in the healthy paw. There were no significant ANOVA differences for group, time, or group by time interaction. Post-hoc analysis shows specific time group differences. Saline-SNI and AraC-SNI have transient differences in healthy paw mechanical threshold at days 3 ($p=0.04$), 5 ($p=0.04$), 14 ($p=0.04$), and 20 ($p=0.03$), with AraC-SNI having higher thresholds at each timepoint. AraC-SNI and AraC-Sham also differ significantly in healthy paw mechanical thresholds at day 20 ($p=0.02$).

(B) Cold allodynia scores. No differences in threshold are observed in the healthy paw for group, time, or group by time interaction. Error bars are SEM, Post-hoc *$p<0.05$ AraC-SNI vs. AraC-Sham, #$p<0.05$ Saline-SNI vs. AraC-SNI.

Triangle is time of SNI or Sham injury incurred to the opposite paw.

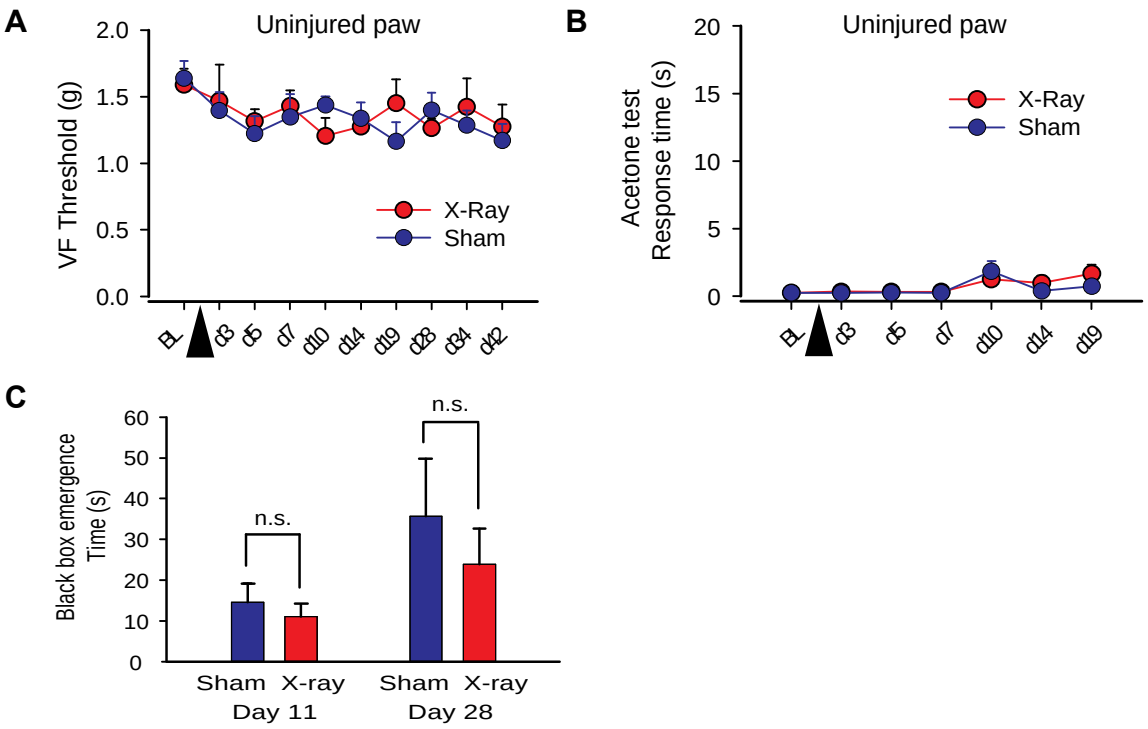

Figure S2. *Touch and cold sensitivity for uninjured paw, and black box emergence behavior, between X-ray and sham brain radiated WT mice, all of which received a SNI neuropathic injury to the contralateral hind-paw.*

(A) Uninjured paw tactile thresholds were not different between the two groups, and also were not different from baseline values.

(B) Uninjured paw cold sensitivity (acetone test response time) were not different between the two groups, and also were not different from baseline values.

Black triangle time when SNI was induced to the opposite paw.

(C) Time for emergence from black box showed a time effect ($F_{1,18}$=5.62, p<0.03) but no group effect and no group by time interaction effect. At day 11 and day 28 there were no differences between groups (sham vs. x-ray, n.s.). Thus both groups exhibit task repetition based learning but do not differentiate in anxiety levels.

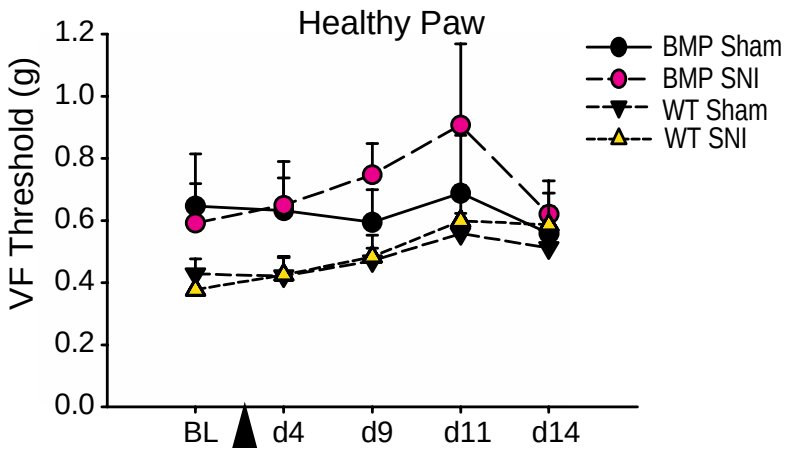

Figure S3. *BMP and WT animals, receiving sham or SNI injury, do not differ in mechanical sensitivity for the non-injured paw.*

There are no significant differences in Von Frey 50% withdrawal thresholds (grams) between the groups for the healthy paw, at baseline and following SNI or Sham injury of the contralateral hind-paw. Error bars are SEM.
Triangle is SNI or Sham injury incurred to the opposite paw.

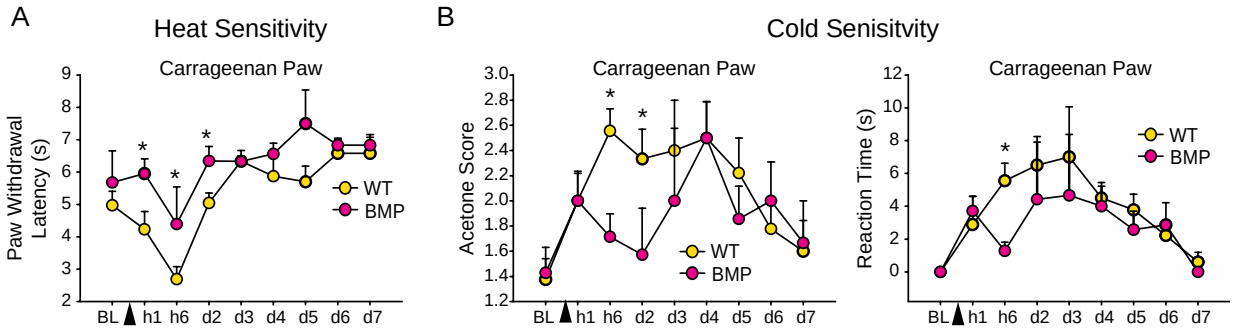

Figure S4. *BMP mice exhibit reduced heat and cold hyperalgesia at the injured hind-paw following inflammatory injury, as compared to WT littermate mice.*

(A) Paw withdrawal latency to heat stimulus. BMP animals have significantly higher heat thresholds in the carrageenan-injected paw compared to WT animals, group effect

($F_{1,22}$=15.73, p<0.001). Post-doc differences are seen at time points hour 1 through day 2 (p<0.01), but not at baseline. This indicates diminished heat hyperalgesia in BMP animals.

(B) Cold allodynia score and reaction times. BMP animals differ in cold sensitivity, with acetone scores being significantly lower (less pain) in BMP animals than WT at hour 6 (Mann-Whitney U-test, U=21, Z=3.37, p<0.001) and day 2 (U=38, Z=2.28, p=0.03), and lower reaction times to cold stimulation at hour 6 compared to WT animals (t(14)=3.27, p<0.01). This indicates less cold allodynia in BMP animals than WT animals.

Error bars are SEM, *p<0.05.

Triangle is time of carrageenan injection.

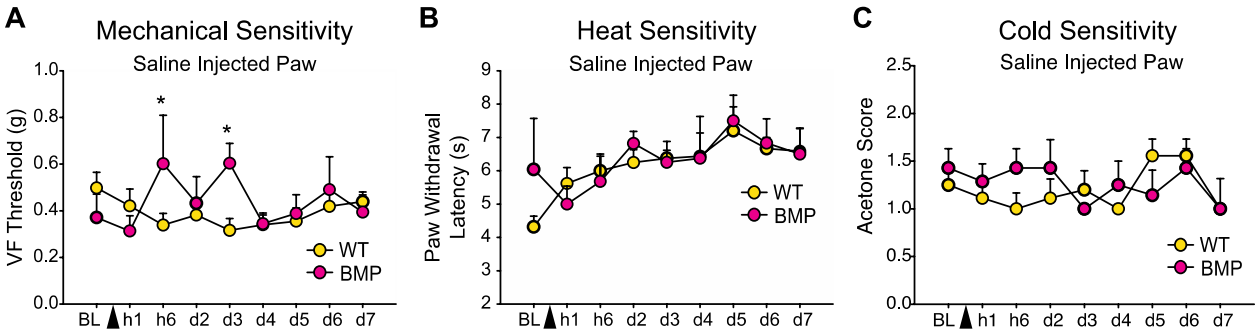

Figure S5. *BMP animals exhibit no group or group by time differences from WT littermates for mechanical, heat, and cold stimuli applied to the saline-injected paw.*

(A) Von Frey 50% withdrawal thresholds (grams) of BMP and WT littermates in the saline-injected paw. Transient post-hoc significant increases in BMP mechanical thresholds are seen in the saline-injected paw at hour 6 (t(23)=2.51, p=0.02) and day 3 (t(23)=4.15, p<0.01).

(B) No differences were seen in paw withdrawal latency to heat stimuli between BMP and WT littermates.

(C) No differences were seen in cold sensitivity between BMP and WT animals in the saline-injected paw.

Error bars are SEM, *p<0.05. Triangle is time of saline injection.

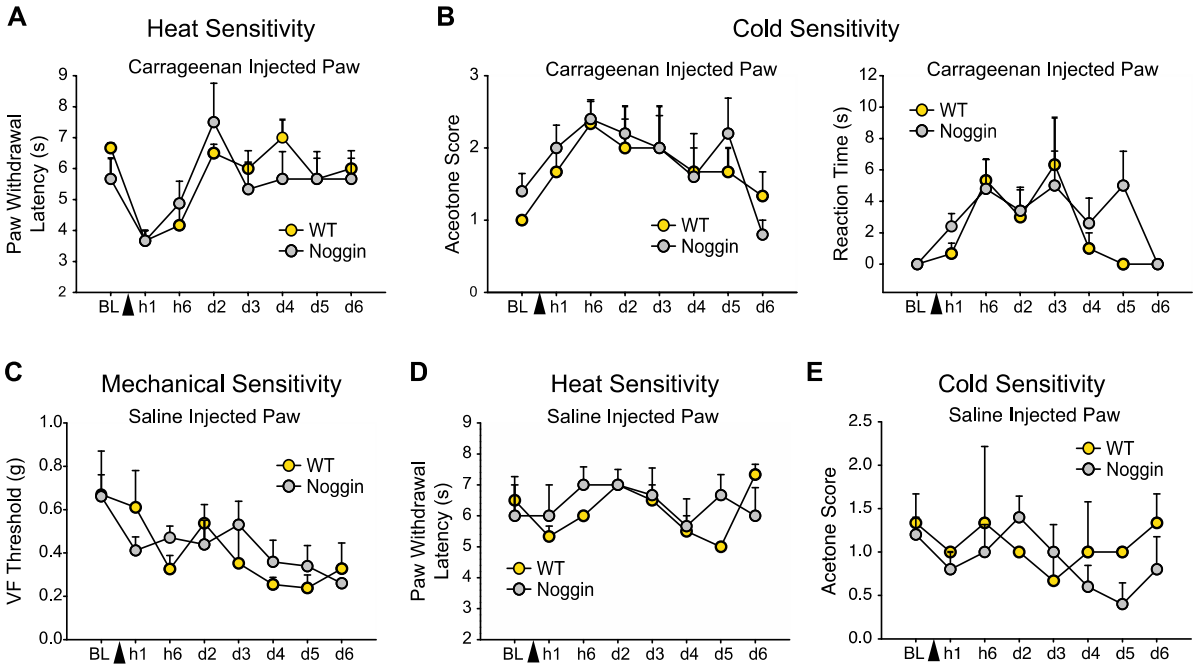

Figure S6. *Noggin animals exhibit no group, time, or group by time differences from WT littermates for heat, and cold stimuli applied to the carrageenan-injected paw (A, B), and also no group, time, or group by time differences for mechanical, heat, or cold stimuli applied to the saline-injected paw (C, D, E).*

(A) Paw withdrawal latency to heat stimuli applied to the carrageenan-injected paw, in Noggin and WT mice.

(B) Cold allodynia scores and reaction times to acetone applied to the carrageenan-injected paw, in Noggin and WT mice.

(C) Von Frey 50% withdrawal thresholds (grams) of Noggin and WT littermates for stimuli applied to the saline-injected paw.

(D) Paw withdrawal latency to heat stimuli applied to the saline-injected paw, in Noggin and WT mice.
(E) Cold allodynia score to acetone applied to the saline-injected paw, in Noggin and WT mice.
Error bars are SEM. Triangles are carrageenan or saline injection times.