Repeated social defeat decreased neurogenesis and impaired working memory in mice

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Abstract

Recent studies demonstrate that microglia activation and inflammatory cytokines are potent regulators of cognition and neuroplasticity. Moreover, repeated social defeat (RSD) in mice causes microglia activation and increased production inflammatory cytokines in the brain. The present examined the association between hippocampal cytokine expression, learning & memory, and hippocampal neurogenesis. We show that RSD increased production of hippocampal cytokines (i.e., IL-1b, IL-6, and TNFa) without reducing common neurogenic growth factors (e.g., VEGF, NGF, IGF1, and BDNF). Increased hippocampal cytokine expression was not associated with reduced proliferation or survival of hippocampal neural progenitor cells (NPCs) but was associated with reduced NPC differentiation into NeuN expressing neurons (BrdU+/NeuN+). Moreover, corresponding with increased hippocampal cytokines, impaired spatial memory recall was observed in the Morris water maze and Barnes maze that was independent of increased anxiety-like behaviors (i.e., thigmotaxis). Taken together, RSD-induced hippocampal cytokine expression was associated with impaired spatial memory recall and impaired NPC differentiation.

Introduction

The relationship between prolonged stress, inflammatory signaling, and changes in brain and behavior are of particular public mental health concern. For example, prolonged stressors are widely associated with increased inflammatory signaling [1-3], increased incidence of mental

health disorders [4], and accelerated cognitive decline [5, 6]. Experimental studies have demonstrated that microglia activation and inflammatory cytokine production are capable of potently regulating both neuroplasticity and cognition. For instance, inflammatory cytokines both centrally and peripherally induce microglia activation [7-9] and impair spatial learning and memory performance [10]. Moreover, microglia activation and cytokine production regulate neuronal plasticity [11] and neurogenesis [12-14] in vivo and ex vivo models. Furthermore, there are clinical links in human studies between inflammatory medical conditions and impaired cognitive performance [15-17]. Thus, associations between inflammatory signaling and cognitive impairment may be due to microglia activation that directly impairs neural plasticity.

Repeated social defeat (RSD) in mice recapitulates many of the immunological and behavioral consequences associated with prolonged stressors in humans [1, 18-21]. Moreover, we have previously demonstrated that RSD caused brain region specific trafficking of inflammatory myeloid cells to the hippocampus that was associated with increased microglia activation as determined by Iba1 immunoreactivity [22]. The purpose of the present study was to determine hippocampal cytokine expression, neurogenesis, and spatial memory performance in mice exposed to RSD.

Methods

Animals.

Male C57BL/6 (6-8 weeks old) and male CD-1 retired breeder mice were purchased from Charles River Breeding Laboratories (Wilmington, MA) and allowed to acclimate to their surroundings for 7-10 days before experiments. Mice were housed in 11.5”x 7.5”x 6” polypropylene cages. Rooms were maintained at 21°C under a 12 h light: 12 h dark cycle from

0600-1800 hrs with ad libitum access to water and rodent chow. All experiments took place between 0800 and 1100 hrs, unless otherwise noted. All procedures were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

Repeated Social Defeat (RSD).

RSD was performed as previously described [22]. In brief, an aggressive male intruder CD-1 mouse was introduced into cages of established male cohorts (3 per cage) of C57BL/6 mice for 2 hours between 17:00 and 19:00 for six consecutive nights. During each cycle, submissive behavior including upright posture, fleeing, and crouching were observed to ensure that the resident mice were exhibiting subordinate behavior. If the intruder did not initiate an attack within 5-10 minutes or was attacked by any of the resident mice, then a new intruder was introduced. At the end of the 2 h period, the intruder was removed and the residents were left undisturbed until the following day when the paradigm was repeated. Different intruders were used on consecutive nights. The health status of the mice was carefully examined throughout the paradigm. Mice that were injured or moribund were removed from the study. Consistent with previous studies using RSD (REF), less than 5% of mice met the early removal criteria. Control mice (CON) were left undisturbed in their home cages until sacrificed.

Behavior.

Similar to previous protocols, spatial memory [23] and working memory [24] were assayed in the Morris water maze. The apparatus consisted of a white 250 cm diameter tub filled with a mixture of water and white paint (at room temperature). A clear plastic platform (diameter 10 cm) was concealed 1 cm beneath the surface of the water, and was placed in a position 20 cm away from the edge of the tub. The area surrounding the apparatus consisted of two distinct

extra-maze geometric cues as well as additional spatial cues inherent to the room. The experimenter and recording equipment were concealed behind a curtain during testing. Behavior was recorded and analyzed by digital recording system (Noldus)

The spatial memory assay involved a 5 day acquisition phase and a probe trial on the 6th day. The acquisition phase consisted of four trials per day for 5 days, with the platform in the same position throughout the task. During the probe trial on the 6th day, the platform was removed and percent time in each quadrant was recorded. Maximum trial duration was 60 seconds. If the platform was not reached within 60 seconds, then the mouse was placed on the platform and a latency of 60 seconds was recorded. Mice were placed at one of four positions in a quasi-random order. Latency and total distance to reach the platform, and percent time in the outer annulus of the tub were recorded. During the probe trial on the sixth day, the platform was removed and total distance traveled and percent time in each quadrant was recorded for 60 seconds.

Working memory was assayed in an adaptation of the Morris water maze [24]. All conditions were the same as described above, unless otherwise noted. There were three massed trials per day with an inter-trial interval of 30 s and maximum trial duration of 120 s. Each trial lasted until the platform was reached or until the maximum trial duration elapsed. If the platform was not reached within 120 s, then the mouse was placed on the platform and a score of 120 s was recorded. In a pseudorandom and experimentally balanced manner, mice were placed in one of four quadrants approximately 3 cm from the edge of the tub. The location of the platform was changed every day, but remained in the same place during trials on the same day. Average velocity, latency to platform, distance to platform, and duration spent in the outer annulus were recorded.

Bromodeoxyuridine (BrdU) Assay.

During the last three nights of RSD (i.e., cycles 4, 5, & 6), mice were injected with 50 mg/kg BrdU at 16:00 (1 hr before social defeat). Brains were collected for BrdU immunohistochemistry 17 hrs, 10 days, or 28 days after the last injection of BrdU.

Immunohistochemistry.

Brains were collected from mice after transcardiac perfusion with sterile PBS followed by 4% formaldehyde. Brains were post-fixed in 4% formaldehyde for 24 hrs followed by 30% sucrose for 48 hrs. Fixed brains were then frozen with isopentane (-78º C) and sectioned at 25 µm (for immnofluorescent double labeling) or 40 µm (for single enzymatic labeling of $BrdU^+$ cells) throughout the entire hippocampus using a Microm HM550 cryostat. Brain regions were identified by reference markers in accordance with the stereotaxic mouse brain atlas 9 (REF).

For quantification of $BrdU^+$ cells from brains collected 17hrs post injection (i.e., 14 hrs after the final cycle of RSD), every 6^{th} section (40 µm thick) was collected for analysis. Total number of $BrdU^+$ cells in the hippocampus was estimated by multiplying the number of cells counted in each section by six.

For phenotypic analysis of $BrdU^+$ cells at 10 and 28 days post injection, double labeling was performed with rat anti-BrdU (AbD Serotec, mAb 1:1000) and rabbit anti-NeuN (1:1000) or rabbit anti-GFAP (Dako, Rabbit1:1000). Sections were washed, denatured in 2N HCL at 37° C for 30 min, and blocked with 3% normal goat serum. Sections were then incubated in primary antibodies at 4° C for 24 hr and then secondary antibodies at 4° overnight. The secondary antibodies used were goat anti-rat 488 and goat anti-rabbit 594 (Alexafluor, 1:500). Sections were cover-slipped with Fluoromount (Beckman Coulter, Inc., Fullerton, CA) and stored at -20°C. Fluorescent sections from brains collected 28 days post BrdU injection were visualized

using confocal laser scanning microscope. Sections collected 14 hrs or 10 days post injection were visualized using wide field fluorescent microscope.

RNA isolation and real time PCR.

Brain tissue samples were collected from the hippocampus and cortex both immediately after and 14 hrs after the final cycle of RSD. RNA was isolated from homogenized brain regions using Tri-reagent/Isopropanol precipitation, RNA was reverse transcribed to cDNA using an RT-RETROscript kit (Ambion, Austin, TX), and RNA concentration was determined by spectrophotometry. Quantitative PCR was performed using the Applied Biosystems (Foster, CA) Assay-on-Demand Gene Expression protocol as previously described. In brief, experimental cDNA was amplified by real-time PCR where a target cDNA (e.g., IL-6, BDNF, VEGF) and a reference cDNA (glyceraldehyde-3-phosphate dehydrogenase; GAPDH) were amplified simultaneously using an oligonucleotide probe with a 5' fluorescent reporter dye (6-FAM) and a 3' quencher dye (NFQ or TAMRA). Fluorescence was determined on an ABI PRISM 7300-sequence detection system (Applied Biosystems, Foster, CA). Data were analyzed using the comparative threshold cycle (Ct) method and results are expressed as fold difference from GAPDH normalized to controls.

Experimental design.

In the first experiment, hippocampus was collected immediately after the sixth cycle of RSD (i.e., at 19:00) and was snap frozen and used for real time q-PCR analysis (see methods). In a second experiment, two separate cohorts of mice were subjected to RSD or CON conditions and subsequently used for behavioral analysis. In the first cohort, behavior in the working memory Morris water maze (see Methods) was assessed following CON or RSD conditions. In the second cohort, behavior in the Morris water maze (see Methods) was assessed following

CON or RSD conditions. In a third experiment, three separate cohorts of mice were subjected to RSD or CON conditions. All three cohorts were injected with BrdU during the last three cycles of RSD (see methods). Brains were subsequently collected for immunohistochemistry. Tissue was collected from the first cohort 14 hours after the final cycle of social defeat; tissue from cohorts two and three were collected 10 and 28 days post-RSD.

Statistical analysis.

To ensure a normal distribution, data were subjected to Shapiro-Wilk test using Statistical Analysis Systems (SAS) statistical software (Cary, NC). Observations greater than 3 interquartile ranges from the first and third quartile were considered outliers and were excluded in the subsequent analysis. To determine significant main effects and interactions between main factors, data were analyzed using one- or two-way ANOVA using the General Linear Model procedures of SAS. When there was a main effect of experimental treatment, differences between group-means were evaluated by an F-protected t-test using the Least-Significant Difference procedure of SAS. All data are expressed as treatment means ± standard error of the mean (SEM).

Results

RSD elevated inflammatory cytokine mRNA and increased growth factor mRNA in the HPC

In a recent report, RSD increased hippocampal IL-1β mRNA expression [22], and hippocampal cytokine expression has been linked to contrast with this, RSD did not reduce mRNA expression of the pro-neurogenic growth factors: brain derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and insulin-like growth factor-1 (IGF1). In fact, RSD increased mRNA expression of NGF ($F_{(1,9)} = 11.16$, p

< 0.05) and VEGF ($F_{(1,9)}$ = 11.62, p < 0.01). Taken together, increased mRNA cytokine expression following RSD was not paralleled by reductions in pro-neurogenic growth factors.

Table 1

Hippocampus

0 Hrs Post RSD

Gene	CON	RSD
IL-1β	1.08 ± 0.18	*6.78 ± 0.40
TNF-α	1.03± 0.10	*3.34 ± 0.71
IL-6	1.02 ± 0.09	*1.65 ± 0.25
Arg 1	1.08 ± 0.17	1.06 ± 0.11
BDNF	1.10 ± 0.11	1.02 ± 0.07
VEGF	1.02 ± 0.08	*1.41 ± 0.06
NGF	1.02 ± 0.11	*1.55 ± 0.05
IGF1	1.04 ± 0.02	0.92 ± 0.02

Table 1. RSD increased Hippocampal cytokine expression. Mice were exposed to RSD or CON conditions and sacrificed immediately after the final cycle. Relative mRNA fold expression is shown numerically ± SEM. Means with asterisk (*) are significantly different from CON (p < .05)

RSD impaired search strategy in the working memory Morris water maze

Previous reports implicate cytokines as a regulator of hippocampal-dependent memory functions. For example, in two different reports, peripheral LPS injection impaired spatial working memory [24] and hippocampal IL-1 impaired spatial memory recall [25]. Therefore it is possible that RSD-induced hippocampal cytokine expression would cause similar effects on hippocampus dependent memory functions. To test this, mice were exposed to CON or RSD conditions and then working memory was assessed as previously reported [24], in an adaptation of the Morris was maze. Mice were tasked with learning the location of a hidden platform that changed location across each test day. Fig. 1A shows that mice exposed to RSD had increased latency to reach the platform across test days ($F(1,95) = 4.38$, $p < 0.05$). This main effect of experimental condition was characterized by a tendency for increased latency on test days 1-3 ($p \leq 0.1$ for all). Increased latency was not associated with altered swim speed (Fig. 1B). Congruently, Fig. 1C shows that RSD also tended to increase the distance traveled across test days ($F(1,95) = 3.39$, $p < 0.1$), and this was characterized by a tendency for increased distance traveled on test days 1-3 ($p < 0.10$ for all). It is important to note that an anxiety-like search strategy was observed in mice exposed to RSD

(Fig. 1D). RSD increased time spent in the periphery of the maze (i.e., thigmotaxis) across test days ($F(1,95) = 10.63$, $p = 0.0001$), that was characterized by increased thigmotaxis on days 1-3 ($p < 0.05$). This thigmotactic search strategy is apparent in the representative search path in Fig. 1E. Taken together, RSD caused an anxiety-like search strategy that was associated with increased latency and increased distance to reach the hidden platform in the working memory Morris water maze, and these behavioral deficits were only observed for the first three days of testing.

Figure 1

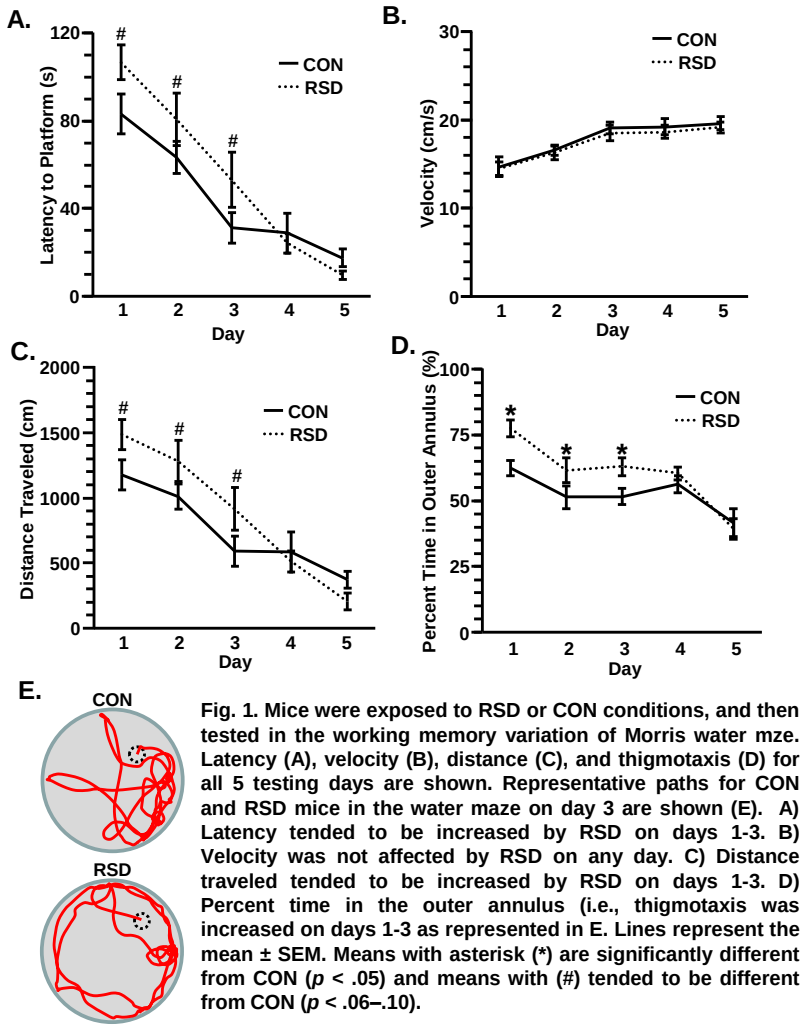

Fig. 1. Mice were exposed to RSD or CON conditions, and then tested in the working memory variation of Morris water mze. Latency (A), velocity (B), distance (C), and thigmotaxis (D) for all 5 testing days are shown. Representative paths for CON and RSD mice in the water maze on day 3 are shown (E). A) Latency tended to be increased by RSD on days 1-3. B) Velocity was not affected by RSD on any day. C) Distance traveled tended to be increased by RSD on days 1-3. D) Percent time in the outer annulus (i.e., thigmotaxis was increased on days 1-3 as represented in E. Lines represent the mean ± SEM. Means with asterisk (*) are significantly different from CON ($p < .05$) and means with (#) tended to be different from CON ($p < .06–.10$).

RSD impaired recall but not acquisition in the Morris water maze

Previous reports reveal that Mice were allowed to acquire the location of the hidden platform (Fig. 2A) for the first five days of testing and on the sixth day were subjected to a probe trial, where the hidden platform was removed and percent time spent in the target quadrant was measured. No differences in latency or distance to reach the platform were observed during the acquisition period (Fig. 2B-C). However, during the probe trial, mice exposed to RSD spent

significantly less time in the target quadrant ($p<0.05$), and this was not associated with differences in total distance traveled during the probe trial (Fig. 2D). Collectively, RSD impaired spatial memory recall in the Morris water maze probe trial, without affecting spatial memory acquisition during the five days of acquisition. Furthermore, reduced time spent in the target quadrant during the probe trial was not attributable to confounding factors such as anxiety-like search strategies or reduced swim speed.

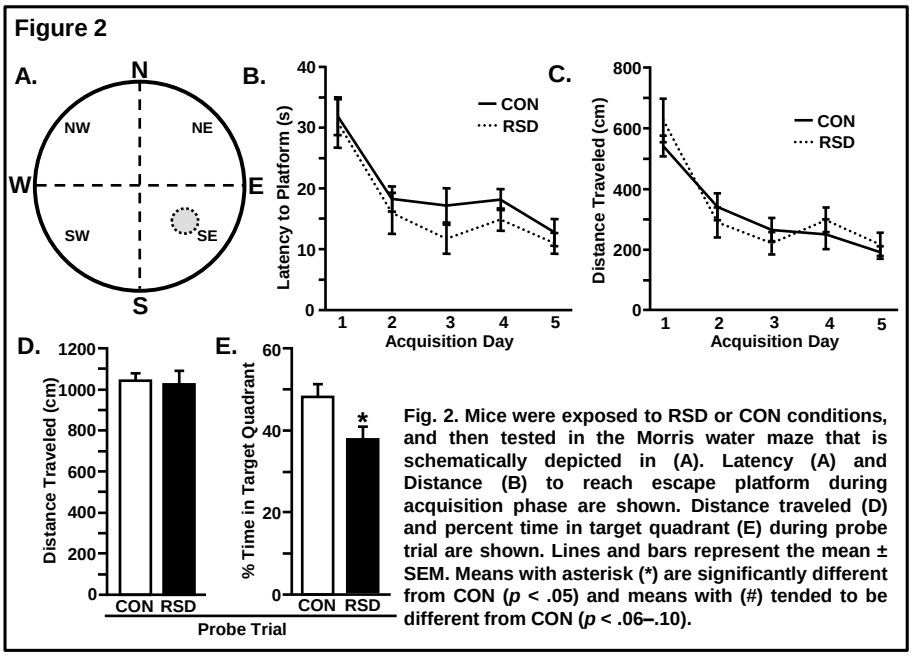

Fig. 2. Mice were exposed to RSD or CON conditions, and then tested in the Morris water maze that is schematically depicted in (A). Latency (A) and Distance (B) to reach escape platform during acquisition phase are shown. Distance traveled (D) and percent time in target quadrant (E) during probe trial are shown. Lines and bars represent the mean ± SEM. Means with asterisk (*) are significantly different from CON ($p < .05$) and means with (#) tended to be different from CON ($p < .06$–$.10$).

Impaired spatial memory recall in the Barnes maze was not associated with anxiety, impaired encoding, or deficient memory consolidation

Because RSD-induced anxiety-like behavior persists for at least 8 days [26], it is possible that decreased time spent in the target quadrant is attributable to reduced encoding efficiency. Moreover, reduced time spent in the target quadrant during the probe trial could be attributable to reduced persistence related to increased anxiety. Therefore, spatial memory was assessed in the Barnes maze, which has been shown to circumvent anxiety-related deficits observed in other learning and memory assays. Furthermore, mice were allowed to acquire the location of the escape hole in the Barnes maze for four days prior to being exposed to RSD or CON conditions.

Figure 3

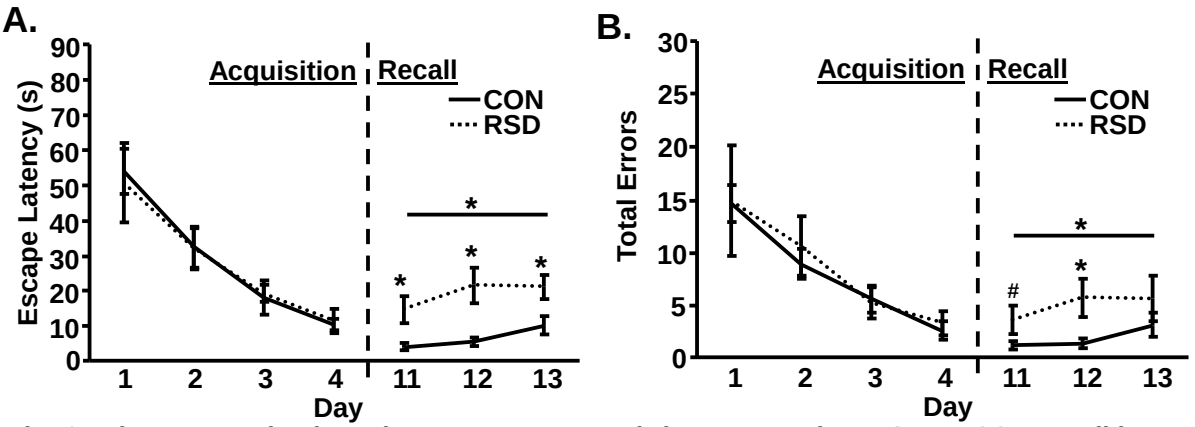

Fig. 3. Mice were trained on the Barnes maze and then exposed to RSD or CON conditions. Latency (A) and Errors (B) to reach escape hole during acquisition and testing phase are shown. Lines represent the mean ± SEM. Means with asterisk (*) are significantly different from CON ($p < .05$) and means with (#) tended to be different from CON ($p < .06–.10$).

Thus, any potential confounds related to impaired encoding efficiency or reduced persistence were avoided. Accordingly, there were no differences in the number of errors, latency, or distance traveled between experimental groups on any acquisition day (data not shown). Following RSD, mice were tested on the Barnes maze for three days. RSD significantly increased latency to escape ($p<0.05$; Fig. 3A), increased number of errors ($p<0.05$; Fig. 3B), and distance traveled (data not shown) to reach the platform across test days. It is important to note that since mice were allowed to acquire the location of the escape hole prior to exposure to stress, impaired memory encoding during acquisition cannot explain the observed deficits. Moreover, since the Barnes maze consists of a circular arrangement of dummy holes around the edge of the maze, it is unlikely that anxiety-like search strategies can explain the observed impairments. Taken together, these data collected in the Barnes maze confirm that RSD caused impaired spatial memory recall independent of anxiety-like search strategies or impaired memory encoding. Despite this, it was yet unclear if RSD was causing a transient impairment in recall efficiency or if RSD caused impairments in spatial memory consolidation.

If spatial memory consolidation was impaired, then deficits in spatial memory recall would be persistent. To test this, mice were allowed to acquire the location of the escape platform for four days and were subsequently exposed to either CON or RSD conditions. Accordingly, there were no differences in the number of errors, latency, or distance traveled between experimental groups on any acquisition day (Fig. 4 A&B). Twenty eight days after RSD, CON and RSD mice were tested on the Barnes maze for three days. RSD had no effect on errors, latency or distance traveled on any of the three testing days. It is important to note that all mice retained a strong spatial memory of the location of the escape hole, as their performance during testing was substantially improved over day 1 of acquisition. Collectively, these combined data imply that RSD caused transient deficits in spatial memory recall in the Barnes maze that were not associated with anxiety-like search strategies, impaired encoding, or deficient consolidation.

Figure 4

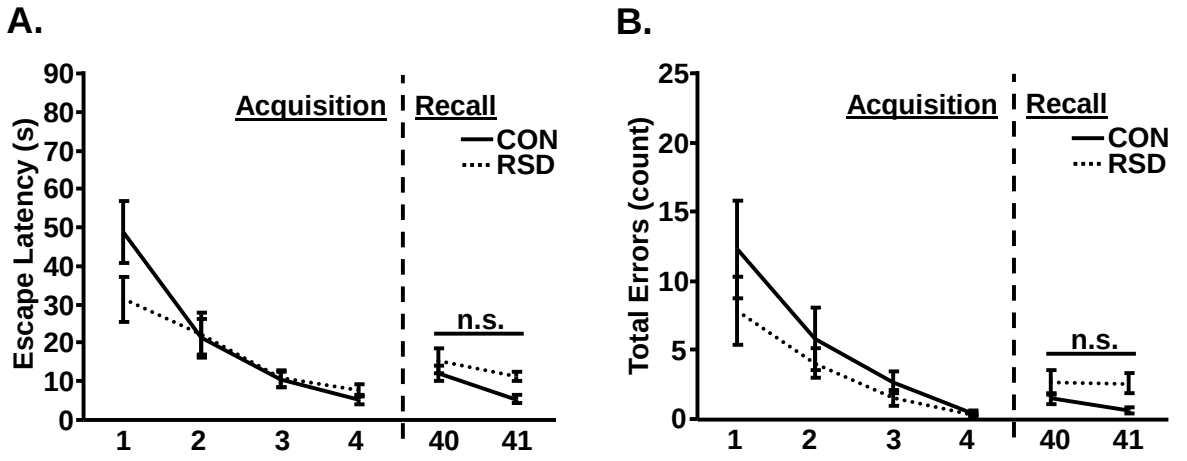

Fig. 4. Mice were trained on the Barnes maze and then exposed to RSD or CON conditions. 28 days after RSD, mice were tested on the Barnes maze. Latency (A) and Errors (B) to reach escape hole during acquisition and testing phase are shown. Lines represent the mean ± SEM. Means with asterisk (*) are significantly different from CON ($p < .05$) and means with (#) tended to be different from CON ($p < .06–.10$).

RSD did not affect NPC proliferation/survival in the hippocampus

There is evidence that cognitive impairments following prolonged psychological stress are related to deficits in hippocampal neurogenesis and to brain cytokine expression. For example, chronic isolation stress reduced neurogenesis and impaired several measures of learning and memory in an IL-1 receptor dependent manner [27]. Therefore, the effect of RSD on neurogenesis was determined. To mark proliferating neural progenitor cells (NPCs) in the dentate gyrus of the hippocampus, BrdU was injected during the last three cycles of RSD, and BrdU+ cells in the dentate gyrus were determined by IHC 14 hrs after the last cycle. Fig. 5A shows represetnaive images of BrdU labeling in the dentate gyrus. Fig. 5B shows quantification of $BrdU^+$ cells in CON and RSD dentate gyrus and that the number of BrdU+ cells in RSD mice was not different from CON. Despite this, it is unclear if reduced BrdU+ cells in the dentate actually represent a functional neurogeneic output. For example, it is possible that reduced proliferation does not correspond with reduced production of neurons in the hippocampus due to differential regulation of survival and differentiation of proliferating cells. Some reports suggest

Figure 5

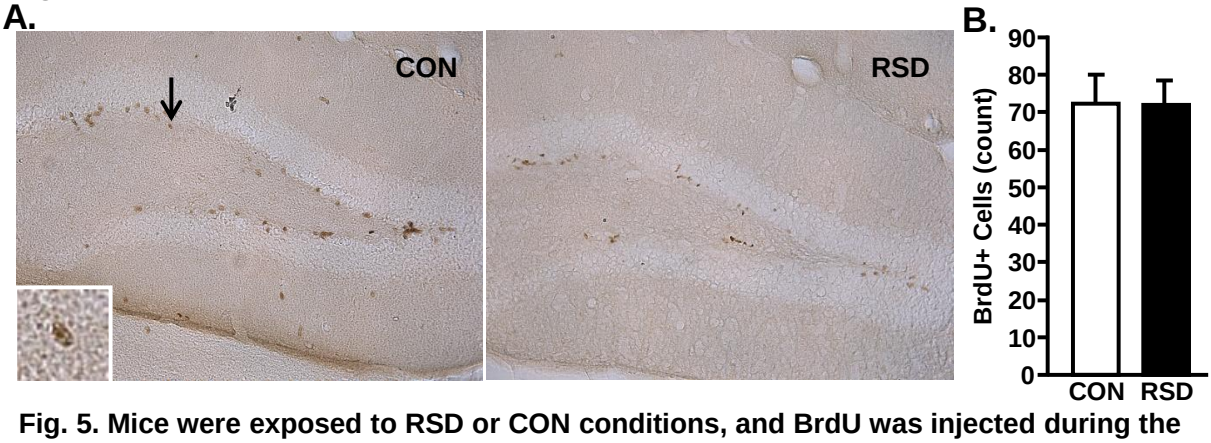

Fig. 5. Mice were exposed to RSD or CON conditions, and BrdU was injected during the last three cycles; mice were sacrificed 14 hrs later. Representative images of BrdU labeling in the hippocampus are shown (A) that were blindly quantified (B). Bars represent the mean ± SEM. Means with asterisk (*) are significantly different from CON ($p < .05$) and means with (#) tended to be different from CON ($p < .06–.10$).

that cytokines, such as IL-1β, are capable of redirecting NPC fate away from neuronal lineage and toward an astrocytic phenotype [14]. To begin to assess the eventual phenotypic fate of the hippocampal NPCs after dividing during RSD, mice were exposed to either CON or RSD conditions and were injected with BrdU during the last three cycles of RSD. At 10 days post-RSD, the expression of the immature neuronal marker doublecortin (DCX) was determined on the BrdU+ cells in the dentate via fluorescent immunohistochemisry (Fig. 6). At 10 days post-RSD, BrdU+ cell counts were still not different (data not shown). Despite this, the proportion of BrdU+ cells that colocalized with DCX was lower in mice exposed RSD (Fig. 6B; $t(10) = 2.54$, $p < 0.05$). Fig. 6A insets show a BrdU+/DCX+ cell (left) and BrdU+/DCX- cell (right). Taken together, RSD reduced the number of BrdU+ cells that in the hippocampus 14 hrs after stress, and these reductions persisted for at least ten days. Moreover, the proportion of BrdU+ cells expressing an early neuronal maturation marker DCX was lower in mice exposed RSD, potentially representing impaired neuronal maturation of NPCs.

Figure 6

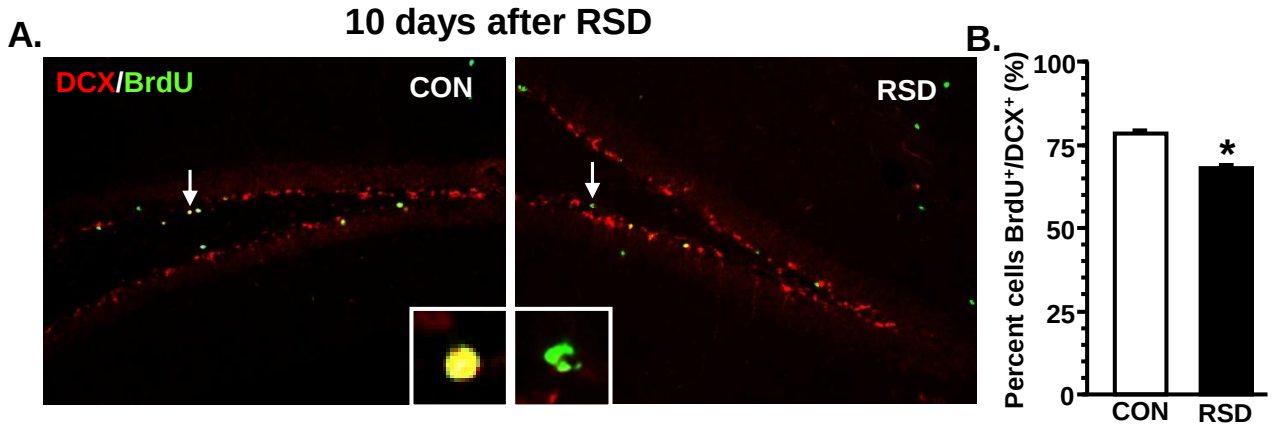

Fig. 6. Mice were exposed to RSD or CON conditions, and BrdU was injected during the last three cycles; mice were sacrificed 10 days later. Representative images of BrdU DCX double labeling in the hippocampus are shown (A) that were blindly quantified (B). Bars represent the mean ± SEM. Means with asterisk (*) are significantly different from CON ($p < .05$) and means with (#) tended to be different from CON ($p < .06–.10$).

RSD impaired neuronal differentiation of hippocampal NPCs

Cytokines have been implicated as regulators of NPC proliferation and maturation. For example in vitro NPCs express IL-1 receptor 1 (IL-1R1) and are redirected toward an astrocytic fate in the presence of IL-1 [14]. In light of evidence that RSD increases IL-1β mRNA (Table 1) and that fewer BrdU+ cells colocalize with a neuronal maturation marker (Fig. 6B), it is possible

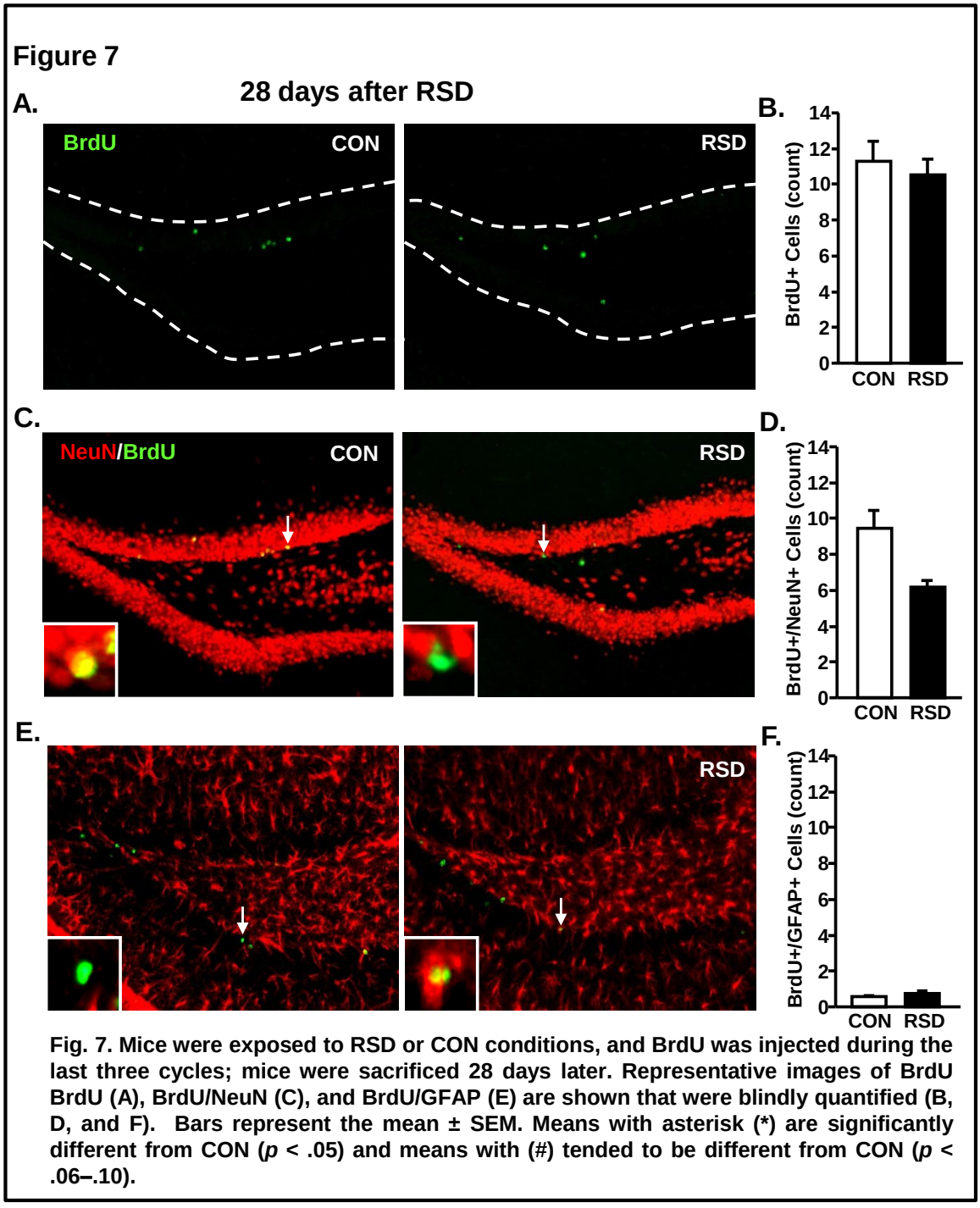

Fig. 7. Mice were exposed to RSD or CON conditions, and BrdU was injected during the last three cycles; mice were sacrificed 28 days later. Representative images of BrdU BrdU (A), BrdU/NeuN (C), and BrdU/GFAP (E) are shown that were blindly quantified (B, D, and F). Bars represent the mean ± SEM. Means with asterisk (*) are significantly different from CON ($p < .05$) and means with (#) tended to be different from CON ($p < .06–.10$).

that RSD may have reduced the neurogenic capacity of hippocampal NPCs. To test this, similar to as above, mice were exposed to CON or RSD conditions and were injected with BrdU during the last three cycles of RSD. However, brains were collected 28 days after the last BrdU injection, allowing for enough time for the dividing and BrdU-labeled NPCs to fully express mature phenotypic markers, such as NeuN (mature neurons) or GFAP (astrocytes). BrdU+ cells from sections collected 28 days after RSD were colabeled for NeuN (Fig. 5C) and GFAP (Fig. 6E). Notable, the number of BrdU+ cells at this time point was not different between experimental conditions (Fig. 6A). Importantly, the number of BrdU+ cells that colocalized with the mature neuronal nuclear marker NeuN was significantly reduced in mice exposed RSD ($t(9) = 2.95$ $p < 0.05$). There were no differences in the number of BrdU+ cells that colocalized with GFAP, but it should be noted that double positive cells were very few in both groups. Taken out to a late time point, fewer surviving BrdU+ cells matured into a granule neuronal phenotype in mice exposed RSD.

Discussion

The results of this study show that RSD increased hippocampal cytokine expression and that this was associated with transient deficits in spatial memory recall and long term deficits in hippocampal neuronal differentiation. We show that RSD increased hippocampal IL-1b, TNFa, and IL-6 without reducing the common neurogenic growth factors BDNF, NGF, IGF, or VEGF. Moreover, increased hippocampal cytokine expression was associated with transient impairments in spatial memory recall in the Barnes and Morris water mazes. The deficits in memory recall were transient as they were no longer detectable 28 days after RSD. Further studies showed that impaired spatial memory recall was not associated with reduced BrdU+ cells in the dentate gyrus

of the hippocampus; however, fate mapping of proliferating BrdU+ cells revealed that RSD significantly reduced the number of BrdU+ that matured into NeuN expressing mature neurons 28 days after injections. Due to the temporal aspects of this neurogenic impairment, it is unlikely that long-term impairments in neuronal differentiation were causing the transient impairments in spatial memory recall. Despite this, the data presented here demonstrate that RSD significantly influences neuroinflammatory signaling, brain plasticity, and spatial learning and memory.

It is of particular interest that RSD impaired NPC differentiation, as this demonstrates a novel pathway in which chronic or prolonged stress exposure can additively impair the production and integration of new adult born neurons into dentate circuitry. While RSD did not reduce the total number of BrdU+ cells that proliferated and persisted to the 28 days time, RSD did reduce the number that had matured and presumably integrated into dentate circuitry. While we cannot reasonably ascribe any cognitive deficits (observed here) to impaired NPC differentiation, it is quite likely that the cumulative effect of chronic stress-induced impairments in neuronal differentiation would certainly be associated with a phenotype. For example, clinical studies have shown that depressed patients have reduced hippocampal volume [28]; this could be caused by chronic reductions in NPC differentiation. It is also important to note that increased IL-1b expression was associated with impaired neuronal differentiation of hippocampal progenitors and that recent in vitro reports show that IL-1b is capable of causing this phenomenon in a NPC-autonomous fashion [14]. Taken together, data reported here demonstrate that RSD caused increased hippocampal cytokine expression, impaired spatial memory recall, and reduced neuronal cell fate of NPCs in the dentate gyrus.

References

1. Powell, N.D., et al., *Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via beta-adrenergic induction of myelopoiesis.* Proc Natl Acad Sci U S A, 2013. **110**(41): p. 16574-16579.
2. Cohen, S., et al., *Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk.* Proc Natl Acad Sci U S A, 2012. **109**(16): p. 5995-9.
3. Miller, G.E., et al., *A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling.* Biol Psychiatry, 2008. **64**(4): p. 266-72.
4. Haroon, E., C.L. Raison, and A.H. Miller, *Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior.* Neuropsychopharmacology, 2012. **37**(1): p. 137-62.
5. Vitaliano, P.P., et al., *Psychophysiological mediators of caregiver stress and differential cognitive decline.* Psychol Aging, 2005. **20**(3): p. 402-11.
6. Vitaliano, P.P., et al., *Does caring for a spouse with dementia promote cognitive decline? A hypothesis and proposed mechanisms.* J Am Geriatr Soc. **59**(5): p. 900-8.
7. Henry, C.J., et al., *Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia.* J Neuroinflammation, 2008. **5**: p. 15.
8. Henry, C.J., et al., *Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines.* Brain Behav Immun, 2009.
9. Quan, N., *In-depth conversation: Spectrum and kinetics of neuroimmune afferent pathways.* Brain Behav Immun.
10. Cunningham, C., et al., *Systemic Inflammation Induces Acute Behavioral and Cognitive Changes and Accelerates Neurodegenerative Disease.* Biol Psychiatry, 2008.
11. Hinwood, M., et al., *Chronic stress induced remodeling of the prefrontal cortex: structural re-organization of microglia and the inhibitory effect of minocycline.* Cereb Cortex. **23**(8): p. 1784-97.
12. Ekdahl, C.T., Z. Kokaia, and O. Lindvall, *Brain inflammation and adult neurogenesis: the dual role of microglia.* Neuroscience, 2009. **158**(3): p. 1021-9.
13. Ekdahl, C.T., et al., *Inflammation is detrimental for neurogenesis in adult brain.* Proc Natl Acad Sci U S A, 2003. **100**(23): p. 13632-7.
14. Green, H.F., et al., *A role for interleukin-1beta in determining the lineage fate of embryonic rat hippocampal neural precursor cells.* Mol Cell Neurosci. **49**(3): p. 311-21.
15. Harezlak, J., et al., *Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment.* Aids. **25**(5): p. 625-33.
16. Glass, J.M., *Review of cognitive dysfunction in fibromyalgia: a convergence on working memory and attentional control impairments.* Rheum Dis Clin North Am, 2009. **35**(2): p. 299-311.
17. Bajaj, J.S., et al., *Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation.* Am J Physiol Gastrointest Liver Physiol. **303**(6): p. G675-85.

18. Engler, H., et al., *Effects of repeated social stress on leukocyte distribution in bone marrow, peripheral blood and spleen.* J Neuroimmunol, 2004. **148**(1-2): p. 106-15.
19. Avitsur, R., J.L. Stark, and J.F. Sheridan, *Social stress induces glucocorticoid resistance in subordinate animals.* Horm Behav, 2001. **39**(4): p. 247-57.
20. Wohleb, E.S., et al., *Peripheral innate immune challenge exaggerated microglia activation, increased the number of inflammatory CNS macrophages, and prolonged social withdrawal in socially defeated mice.* Psychoneuroendocrinology, 2012. **37**(9): p. 1491-505.
21. Wohleb, E.S., et al., *β-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat.* J Neurosci, 2011. **31**(17): p. 6277-6288.
22. Wohleb, E.S., et al., *Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior.* J Neurosci, 2013. **33**(34): p. 13820-13833.
23. Morris, R., *Developments of a water-maze procedure for studying spatial learning in the rat.* J Neurosci Methods, 1984. **11**(1): p. 47-60.
24. Sparkman, N.L., et al., *Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers.* J Neurosci, 2006. **26**(42): p. 10709-16.
25. Hein, A.M., et al., *Sustained hippocampal IL-1beta overexpression impairs contextual and spatial memory in transgenic mice.* Brain Behav Immun. **24**(2): p. 243-53.
26. Wohleb, E.S., et al., *Re-establishment of Anxiety in Stress-Sensitized Mice Is Caused by Monocyte Trafficking from the Spleen to the Brain.* Biol Psychiatry.
27. Ben Menachem-Zidon, O., et al., *Intrahippocampal transplantation of transgenic neural precursor cells overexpressing interleukin-1 receptor antagonist blocks chronic isolation-induced impairment in memory and neurogenesis.* Neuropsychopharmacology, 2008. **33**(9): p. 2251-62.
28. Videbech, P. and B. Ravnkilde, *Hippocampal volume and depression: a meta-analysis of MRI studies.* Am J Psychiatry, 2004. **161**(11): p. 1957-66.