REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
24-10-2016	Journal Article	

4. TITLE AND SUBTITLE
Neuropathological and behavioral sequelae in IL-1R1 and IL-1Ra gene knockout mice after soman (GD) exposure

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of
Chemical Defense
8350 Ricketts Point Road
Aberdeen Proving Ground, MD
21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-P17-017

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Threat Reduction Agency
8725 John J. Kingman Road STOP 6201
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES Published in Neurotoxicology, 63, 43-56, 2017.

This project, CB3943, was sponsored by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

14. ABSTRACT
See reprint.

15. SUBJECT TERMS
Soman, Status epilepticus, Interleukin-1, Neurodegeneration, Neuroinflammation, Behavior

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	15	

NeuroToxicology 63 (2017) 43–56

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Neuropathological and behavioral sequelae in IL-1R1 and IL-1Ra gene knockout mice after soman (GD) exposure

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ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form 28 August 2017
Accepted 28 August 2017
Available online 1 September 2017

Keywords:
Soman
Status epilepticus
Interleukin-1
Neurodegeneration
Neuroinflammation
Behavior

ABSTRACT

Soman (GD) exposure results in status epilepticus (SE) that leads to neurodegeneration, neuroinflammation, and behavioral consequences including learning and memory deficits. The neuroinflammatory response is characterized by the upregulation of the pro-inflammatory cytokine, interleukin-1 (IL-1), which mediates the expression of other neurotoxic cytokines induced after GD exposure. However, the specific role of IL-1 signaling has not been defined in terms of the consequences of GD-induced SE. Therefore, the purpose of this study was to regulate IL-1 signaling and study the behavioral deficits and neurodegeneration that occur after convulsion onset. Wild type (WT), IL-1 receptor (IL-1R1) knockout (KO), and IL-1 receptor antagonist (IL-1Ra) KO mice were exposed to a convulsive dose of GD, and behavior was evaluated up to 18 days later. Activity was studied using the Open Field, anxiety was assessed in the Zero Maze, and spatial learning and memory were evaluated with the Barnes Maze. The animals were euthanized at 24 hours and 18 days to determine neuropathology in the piriform cortex, amygdala, thalamus, and CA1, CA2/3, and CA4 regions of the hippocampus. Unlike the IL-1Ra KO, the IL-1R1 KO showed less neuropathology compared to WT at 24 hours, but moderate to severe injury was found in all strains at 18 days. Compared to their saline controls, the exposed WT mice were significantly more active in the Open Field, and the IL-1R1 KO strain showed reduced anxiety in the Zero Maze Test. Compared to WT mice, IL-1R1 and IL-1Ra KO mice had spatial learning and memory impairments in the Barnes Maze. Therefore, the IL-1 signaling pathway affects neurodegeneration and behavior after GD-induced convulsions.

1. Introduction

Chemical warfare nerve agents (CWNA) induce seizure activity through overstimulation of the cholinergic system, where the accumulation of acetylcholine (ACh) in the synaptic cleft leads to convulsions and status epilepticus (SE) (McDonough and Shih, 1993; Shih and McDonough, 1997a, 1997b). Soman (pinacolyl methylphosphonofluoridate, GD) is a CWNA of particular interest because unlike other nerve agents, it causes a rapid phonylation reaction (aging) that inactivates acetylcholinesterase (AChE), which cannot be reversed (Svensson et al., 2001). The resulting SE that can occur from GD exposure leads to injury in multiple brain regions including the piriform cortex, amygdala, thalamus, and hippocampus (Johnson and Kan, 2010). The central nervous system (CNS) injury caused by pathogenic exposure to nerve agents results in leukocyte infiltration and macrophage activation (Johnson et al., 2011; Johnson and Kan, 2010). Chemokines guide peripheral neutrophils to damaged regions of the CNS, where upregulation of acute phase response cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα) (Johnson et al., 2011) can initiate and exacerbate a pro-inflammatory cascade. A similar response is seen in cerebral ischemia research, where IL-1 increases expression of pro-inflammatory mediators in microglia, suggesting IL-1 controls cytokine signaling in the CNS after injury (Basu et al., 2005). This pro-inflammatory process can exacerbate brain damage over time

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http://dx.doi.org/10.1016/j.neuro.2017.08.010

in models of nerve agent exposure, traumatic brain injury (TBI), and ischemic injury resulting in cognitive deficit (Budinich et al., 2013; Collombet et al., 2006b; Coubard et al., 2008; Filliat et al., 2007; Fox et al., 1998, 1999; Koo and Duman, 2009; Patel et al., 2014).

IL-1α and IL-1β (functionally identical and referred to as IL-1) bind to the IL-1 receptor (IL-1R1) and direct downstream inflammatory networks (Weber et al., 2010). A third protein, the IL-1 receptor antagonist (IL-1Ra), binds to the IL-1R1 to limit the activation of IL-1 signaling in a negative feedback loop (Weber et al., 2010). Upon injury, IL-1 is centrally released by activated astrocytes and microglia and binds to the IL-1R1, which is induced in neurons and astrocytes during SE (Vezzani et al., 2011). IL-1 signaling is a tightly regulated process, however, and IL-1Ra is also upregulated (Rothwell et al., 1997; Rothwell and Luheshi, 2000). However, in severe injury states, the upregulation of IL-1Ra is insufficient to block the increased release of IL-1, and the pro-inflammatory signaling continues, which can exacerbate brain damage (De Simoni et al., 2000; Dinarello, 1996; Pinteaux et al., 2006; Plata-Salaman et al., 2000; Vezzani et al., 2000b). Since seizures induce upregulation of IL-1 signaling (Vezzani et al., 1999), it is of interest to understand the role of IL-1 in the behavioral response and brain damage resulting from GD-induced SE.

Although IL-1 knockout (KO) mice have been used to study the role of IL-1 in ischemic insult, beta-amyloid induced brain injury, and seizurogenic research (Basu et al., 2005; Boutin et al., 2001; Craft et al., 2005; Lazovic et al., 2005; Pinteaux et al., 2006; Touzani et al., 2002; Vezzani et al., 2000b), these mice have not been used to study behavior or brain damage after GD-induced SE. Previous work shows conflicting evidence as to whether the absence of IL-1R1 improves spatial learning and memory after ischemic injury (Avital et al., 2003; Murray et al., 2013). Research in IL-1Ra KO mice is limited to describing brain damage only, not cognitive deficits (Pinteaux et al., 2006). This study investigated the role of IL-1 signaling in neuropathology and behavior after GD-induced convulsions, utilizing the Open Field to study activity, the Zero Maze for anxiety, and the Barnes Maze to analyze spatial learning and memory after GD exposure. Using IL-1R1 KO, IL-1Ra KO, and background matched wild type (WT) mice, it was found that all strains were equally prone to hyperactive behavior after GD exposure. Additionally, the absence of IL-1R1 attenuated 24 hour neuropathology in the piriform cortex, amygdala, and the CA4 region of the hippocampus, and anxiety-like behavior caused by GD, whereas the absence of IL-1Ra exacerbated brain damage in the piriform cortex and thalamus at 18 days and did not improve anxious behavior. Although the absence of functional IL-1R1 or IL-1Ra did not affect GD-induced deficits, the increased damage to the CA2/3 region of the hippocampus by the IL-1Ra KO mice may account for the greater deficits in learning and memory in this KO strain over time.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (000664; WT background strain), IL-1Ra KO (004754; IL-1Ra), and IL-1R1 KO (003245; IL-1R1) mice were obtained from and genotyped by Jackson Laboratories (Bar Harbor, ME). The background strain for the two KO mice strains is C57BL/6J. The total number of mice purchased for these experiments were as follows: WT: 119; IL-1Ra KO: 96; IL-1R1 KO: 85. Average pre-exposure weights for the WT, IL-1Ra KO, and IL-1R1 KO are as follows: 26.1 + 0.3 g, 19.0 + 0.5 g, and 23.8 + 0.5 g, respectively. No significant difference in weight gain was found among the three strains of mice after GD exposure through the course of the study (data not shown).

For behavior experiments, the number of animals utilized was as follows: WT: (N = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (N = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (N = 32, GD exposed n = 17, SAL control n = 15). For 24 hour and 18 day histopathology analysis, the number of animals utilized was as follows, respectively: WT (n = 21 &16), IL-1Ra KO (n = 14 & 17), IL-1R1 KO (n = 13 & 21). Animals were between 8 and 10 weeks old when exposed to GD. Mice were tail tattooed for identification and group housed by strain in polycarbonate cages in a temperature- and humidity-controlled room (68–79 °F, 30–70% humidity) for at least one week prior to exposure to nerve agent. The animals were maintained on a 12 hour light-dark schedule (lights on at 0600) with ad libitum access to food and water. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The animal care program at this institute is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

2.2. Soman (GD) convulsion model

On the day of exposure, animals received an intraperitoneal (IP) pre-treatment of the oxime HI-6 dichloride (1-(((4-(aminocarbonyl)pyridinio) methoxy) methyl)-2-((hydroxymino)methyl)pyridinium dichloride; BN44621, Starks Associates, Buffalo, NY; 50 mg/kg) in saline (SAL) five minutes prior to subcutaneous (SC) 1.6 LD50 GD administration (147 μg/kg). The oxime HI-6 is necessary to prevent respiratory distress and enhance survival (McDonough and Shih, 1993). Vehicle control animals received only HI-6 and SAL. GD (GD-U-2323-CTF-N, purity 98.8%) was obtained from the United States Army Medical Research Institute of Chemical Defense. Animals were observed for convulsion onset,

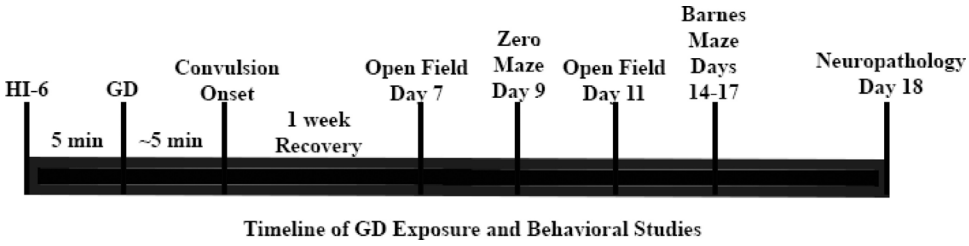

Fig. 1. Timeline of GD Exposure and Behavioral Studies.
On the day of GD exposure, animals are pre-treated with the HI-6 oxime and exposed to GD 5 minutes later. The animals convulse within 5 min of exposure and are given 1 week to recover with SC injections of SAL and wet mash to regain weight. The animals are behaviorally tested beginning day 7 post exposure and are euthanized on day 18 post exposure. GD, soman. SC, subcutaneous. SAL, saline.

characterized by full body tremor, and were observed intermittently throughout the day prior to return to their home cages. At the end of the exposure day, the animals were group housed with no more than five animals per cage. Beginning the day after exposure, all GD exposed and vehicle control animals were weighed each morning and given wet mash to compensate for weight loss and prepare for behavioral testing. These animals continued to receive wet mash until the last day of behavioral testing. If the animals did not gain weight from eating the wet mash or drinking water, they were given up to 0.2 ml of SAL twice a day (SC, morning and evening) until they gained weight. Only animals that gained weight post exposure via wet mash or wet mash and SAL injection were used for behavioral analysis. Animals were transferred to the behavior room four days after exposure and were group housed and tested in the same room one week after GD exposure.

2.3. Behavior testing

Behavior testing began one week after GD exposure for the WT: (n = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (n = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (n = 32, GD exposed n = 17, SAL control n = 15) mouse strains. Four IL-1R1 KO animals and four IL-1Ra KO animals failed to gain weight after GD exposure and died before behavioral testing began. All surviving animals included in the behavior studies gained weight during the week after GD exposure before testing began. Behavior testing occurred for the Open Field on days 7 and 11, the Zero Maze on day 9, and the Barnes Maze on days 14 to 17 after GD exposure (Fig. 1). For each behavioral test, all GD exposed animals were tested first, followed by all SAL controls. Three IL-1Ra KO animals experienced convulsions during the acclimation period before behavioral testing, and were not tested until the episode ended so as not to skew the behavior results. All behavior apparatuses were cleaned with 70% ethanol between testing sessions (Karlsson et al., 2005), and each apparatus was allowed to fully dry before the next test session began. Only those exposed animals that exhibited convulsions were used for behavioral analysis, as those not displaying convulsions fail to show neuropathology post exposure. For the Open Field, 10 to 12 animals were tested at a time in two cohorts starting at 1400, and testing lasted two to three hours. For the Zero Maze, the same number of animals were tested at a time in two cohorts starting at 1400, and testing lasted one to two hours. For the Barnes Maze, no more than 12 animals were tested a day starting in the morning in cohorts of three, and testing lasted four to six hours. The cohorts were spread out over a year and a half and groups of all three strains of animals were tested concurrently to avoid the confounding variable of seasonal effects.

2.3.1. Open field

The Stoelting (Wood Dale, IL) Open Field is a plexiglass box (40 × 40 × 35 cm) with opaque grey walls and floor that is used to test general activity and exploration (Budinich et al., 2013). It was illuminated with two free-standing fluorescent lights on either side of the Open Field, and overhead lights remained on for behavior testing. Total illuminance on the surface of the maze was 1.86 klux. Beginning at 1400 hours on days 7 and 11 post exposure, mice were placed in a clean polycarbonate cage with Sani-chip bedding (Harlan Teklad, Madison, WI) for 10 minutes to establish environmental acclimation to testing (Heinrichs and Koob, 2006) because the animals were housed in the same room used for behavioral testing. After acclimation, mice were placed in the center of the Open Field and monitored for 10 minutes with a camera linked to ANY-maze Behavioral Tracking Software (Stoelting). Total distance traveled was used to measure activity (Budinich et al., 2013; Karlsson et al., 2005; Prut and Belzung, 2003).

2.3.2. Zero Maze

Previous research has reported that the relationship between exploratory activity and anxious behavior is not well defined, and that locomotor activity is a poor measure of anxiety (Lister, 1990). More specific anxiety-based tests such as the Zero Maze can be applied to address this issue (Crawley, 2007; Tucker et al., 2016). The Stoelting Zero Maze is elevated 50 cm from the ground and consists of a circular, grey platform with a 5 cm-wide lane divided into two opposite open and closed quadrants, (Stoelting). The closed quadrants are surrounded by 15 cm high walls, which keep the area dark. The Zero Maze was illuminated with two free-standing fluorescent lights on either side of the maze, illuminating the open quadrants only so that the closed quadrants remained dark. Overhead lights remained on for behavioral testing. Total illuminance on the open quadrant surface of the maze was 2.86 klux. At 1400 hours beginning on day nine after exposure, the animals were acclimated for 10 minutes in a novel, clean cage, and then placed at the edge of the open quadrant, facing the entrance of a closed quadrant, and allowed to explore the Zero Maze for five minutes (Jacobson et al., 2007). The animals were monitored by a camera linked to ANY-maze software, which measured primary latency to exit the starting closed quadrant and time spent in the closed quadrants as measures of anxiety (Jacobson et al., 2007; Patel et al., 2014). The presence in each area of the maze was determined by where the center of the animal was located.

2.3.3. Barnes Maze

The Barnes Maze was used to study spatial learning and memory (Pompl et al., 1999; Sharma et al., 2010). It consists of a grey circular table 91 cm in diameter with 20 holes along the perimeter (5-cm diameter) elevated 91 cm from the ground (Stoelting). It was illuminated by two free-standing fluorescent lights on either side of the Barnes Maze, and overhead lights remained on for behavioral testing. Total illuminance on the surface of the maze was 3.46 klux. Beginning at 0900 on each day of testing, the animals were transported to the maze in an opaque cup that was overturned in the center of the Barnes Maze, so that the animal stood on the maze in the dark for 30 seconds. The cup was then lifted and the animal was allowed to explore the maze for five minutes. Spatial cues (Sharma et al., 2010) that the animals used to find the escape tunnel included posters with black and white geometric shapes placed on the walls surrounding the maze. On days 14 to 17 post exposure, each animal underwent four trials with 15 to 25 minutes between trials (Sharma et al., 2010). The animals were monitored by a camera linked to ANY-maze software, which recorded the latency to escape (measure of spatial learning and memory), the number of head pokes into the perimeter holes (measure of error), and the primary latency to head poke into the escape hole (Fox et al., 1999; Patel et al., 2014; Sharma et al., 2010). If the animal found the escape tunnel within the five-minute test (defined as remaining in the tunnel for 15 seconds), the test ended and the animal was allowed to remain in the tunnel for 30seconds to reduce anxiety (Fox et al., 1999). If the animal did not find the escape tunnel within the testing period, it was picked up and gently guided to the escape tunnel, where it was kept for one minute (Cheng et al., 2014; Fox et al., 1999).

2.4. Tissue collection

Pathology was assessed 24 hours after GD exposure for histopathology and 18 days after exposure following behavioral studies. At the study end, the animals were anesthetized with a sodium pentobarbital-based euthanasia solution (75–100 mg/kg, IP) and perfused with SAL for exsanguination. They were then perfused with 4% paraformaldehyde (FD Neurotechnologies, Columbia, MD), and post fixed in 4% paraformaldehyde for 48 to

72 hours and paraffin processed. Sections were serially cut at five microns from Bregma −1.06 mm to −1.34 mm using a Leica RM225 microtome and stained with hematoxylin and eosin (H&E) for neuropathology evaluation by a board certified pathologist blinded to the conditions of exposure and strain. The neuropathologist used a scale of zero to four to evaluate the piriform cortex, amygdala, thalamus and hippocampal sections (0: no damage; 1: minimal damage (1–10%); 2: mild damage (11–25%); 3: moderate damage (26–45%); 4: severe damage (>45%)) (Myhrer et al., 2006).

2.5. Statistical analyses

Assessment of mortality, the absence of convulsion, and the incidence of pathogenic convulsion across the three strains were evaluated by the Chi-Square Test. Physiological measures of body weight change overnight, and the time of onset to the presence of convulsive activity after GD treatment were analyzed with a one-way analysis of variance (1-way ANOVA), followed by Dunn's Multiple Comparison post hoc test. Neuropathology data was expressed as the median ± the first and third quartile and analyzed with the Mann Whitney test followed by a Dunn's Multiple Comparison post hoc test. Open Field, Zero Maze, and Barnes Maze data were analyzed using a 1-way ANOVA or a Treatment Group x Time Repeated Measures analysis of variance (2-way ANOVA), followed by Bonferroni post hoc comparisons. In the Zero Maze, latency data violated the homogeneity of variance assumption. Therefore, a Kruskal-Wallis test was performed followed by Dunn's Multiple Comparison post hoc test. In the Barnes Maze and Open Field Test, a significant treatment difference tests whether there is a difference between groups due to exposure to GD compared to SAL treatment, and a significant interaction effect suggests a difference between groups on some of the testing days. Statistical tests were significant where $p < 0.05$ and, where applicable, significant post hoc analysis was also given a value of $p < 0.05$.

3. Results

3.1. Physiological outcomes

Table 1 indicates the various outcomes that were measured in WT, IL-1Ra KO, and IL-1R1 KO mice exposed to GD and split into five groups: mice that died up to 18 days after GD exposure, mice that survived exposure, convulsed, and had neuropathology, mice that survived exposure, convulsed, but had no neuropathology, mice that survived exposure, did not convulse, and had neuropathology, and mice that survived exposure, did not convulse, and had no neuropathology. All animals that died over the course of the study had convulsions after GD exposure and most died within two hours of exposure (38/48; 79%). Chi Square tests showed that the distribution of these outcomes did not vary across the groups $X^2(2) = 5.864$, $p = 0.210$ The IL-1Ra KO had the highest mortality rate after GD-induced convulsions (38.3%). The WT mice showed the highest percentage of animals that convulsed and survived (65.4%), and the IL-1Ra KO group had the highest percentage (10.0%) of animals that showed signs of convulsion, but no subsequent neuropathology 24 hours later. Animals that did not convulse did not show neuropathology, and the IL-1Ra KO group had the highest percentage of animals that did not convulse (10.0%). Table 2 shows convulsion onset data for all three mouse strains and weight changes 24 hours after GD exposure. The IL-1Ra KO group had the shortest convulsion onset time ($p < 0.05$), whereas the IL-1R1 KO group was longest to show convulsive activity. Compared to the weight loss seen post exposure in the WT mice, the IL-1R1 KO showed the greatest weight loss ($p < 0.001$), and the IL-1Ra KO mice showed the least weight loss post exposure.

Table 1
Convulsion and survival rates (% of total) for the three mouse strains after GD exposure.

Strain	Died	Survived			
		Convulsion		No Convulsion	
		Pathology	No Pathology	Pathology	No Pathology
Wild Type n = 52	26.9	65.4	5.8	0.0	1.9
IL-1R1 KO n = 42	28.6	61.9	7.1	0.0	2.4
IL-1Ra KO n = 60	38.3	41.7	10.0	0.0	10.0

The IL-1Ra KO mice had the highest mortality rate over the course of the study (18 days) after GD-induced convulsions. The WT group showed the highest percentage of convulsing animals that survived, and the IL-1Ra KO mice had the highest percentage of animals that convulsed without subsequent neuropathology. Animals that did not convulse did not show neuropathology, and the IL-1Ra KO group had the highest percentage of animals that did not convulse. GD, soman. WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. n, number of animals.

3.2. Neuropathology

Mice were scored for neuropathology at either 24 hours or 18 days. Mice not exposed to GD, regardless of strain, time, or brain region, did not show any neuropathology (data not shown); therefore all data presented are from GD exposed mice. Injury to the brain regions of interest is reflected in the H&E stained coronal sections (Fig. 2) that show injured and healthy tissue post exposure. Neuropathology results were compared between WT and the KO groups at 24hours and 18 days post exposure in six regions of the brain: piriform cortex (Fig. 3A), amygdala (Fig. 3B), thalamus (Fig. 3C), and the CA1 (Fig. 3D), CA2/3 (Fig. 3E), and CA4 (Fig. 3F) regions of the hippocampus. In the piriform cortex (Fig. 3A), the WT control strain developed severe neuropathology by 24 hours that was maintained up to 18 days. The neuropathology scores of the IL-1R1 KO strain were significantly less at 24 hours (** $p < 0.01$) compared to WT controls. However, at 18 days, neuropathology was more severe for this strain compared to the 24 hour score (#### $p < 0.0001$). There was no significant difference in neuropathology for the IL-1Ra KO strain compared to WT controls at 24 hours or 18 days, although neuropathology scores in the IL-1Ra KO group significantly increased over time in the piriform cortex (Fig. 3A) and thalamus (Fig. 3C). In the amygdala (Fig. 3B), the WT group had a significant reduction in neuropathology scores from 24 hours to 18 days (## $p < 0.01$), whereas the IL-1Ra KO was severely damaged at 24 hours and moderately damaged at 18 days. The IL-1R1 KO group again had less neuropathology at 24 hours compared to the WT mice (* $p < 0.05$), but scores did not significantly change for this group over time. In the thalamus (Fig. 3C), the WT group showed severe injury at 24 hours and moderate injury at 18 days, whereas both KO strains showed moderate injury at 24 hours that became severe injury at 18 days (# $p < 0.05$). Compared to the WT mice at 18 days, the IL-1Ra KO mice showed significantly more damage in the thalamus (* $p < 0.05$). In the CA1 region of the hippocampus (Fig. 3D), all mouse strains showed severe injury at 24 hours with no change at 18 days post exposure. The CA2/3 (Fig. 3E) was only mildly injured for the WT and IL-1R1 KO mouse strains at 24 hours, although the IL-1Ra KO group had a significantly higher neuropathology score than the WT group (* $p < 0.05$). By 18 days, the WT and IL-1R1 KO mice showed reduced, though non-significant damage, whereas the IL-1Ra KO group showed no such reduction (* $p < 0.05$). Finally, in the CA4 region of the hippocampus (Fig. 3F), the WT and IL-1Ra KO mice showed moderate and severe injury, respectively at 24 hours, whereas the IL-1R1 KO mice had significantly less injury than the WT mice (** $p < 0.01$). All mouse strains showed severe injury at 18 days post exposure in the

Table 2
Weight change and convulsion data for the three mouse strains after GD exposure.

Mouse Strain	Convulsion Onset Time (minutes)	Δ% Weight 24 hours after GD
WT (n)	3.67 ± 0.15 (51)	−17.45 ± 0.46 (36)
IL-1Ra KO (n)	2.77 ± 0.18 (57) *	−17.23 ± 1.12 (41)
IL-1R1 KO (n)	3.88 ± 0.28 (42)	−19.27 ± 0.64 (34) ***

The IL-1Ra KO mice had a faster convulsion onset time after GD exposure compared to WT mice ($p < 0.05$). The IL-1R1 KO mice had the greatest percentage of weight loss 24 hours after GD exposure compared to WT mice ($p < 0.001$). GD, soman. WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. n, number of animals.

CA4 region of the hippocampus, where the IL-1R1 KO group was significantly injured at 18 days (## $p < 0.01$) compared to 24 hours.

3.3. Open field

For the total distance traveled measure, a significant main effect of treatment ($F(1,32) = 14.13$, $p < 0.001$) was found between the exposed WT group and SAL control mice (Fig. 4A), where the exposed mice traveled a greater distance in the Open Field on days 7 and 11 compared to the SAL group ($p < 0.001$). There was a significant interaction ($F(1,34) = 4.296$, $p < 0.05$) between the exposed IL-1Ra KO group compared to its respective control, where the exposed group traveled farther on day 11 ($p < 0.05$, Fig. 4B). No significant difference in total distance traveled was found for the exposed IL-1R1 KO group compared to the IL-1R1 KO SAL control (Fig. 4C), nor was there a significant difference between the exposed groups for total distance traveled (Fig. 4D).

3.4. Zero Maze

In the Zero Maze, there was no significant difference in the time spent in the closed quadrants between the exposed WT group and its SAL control (Fig. 5A), the exposed IL-1Ra KO group and its SAL control (Fig. 5B) or the exposed IL-1R1 KO group and its SAL control (Fig. 5C). There was also no significant difference in time spent in the closed quadrants when the exposed KO groups were compared to the exposed WT group (Fig. 5D). Likewise, there was no difference between the groups in average number of entries into the closed arms or the total distance traveled (data not shown). However, the primary latency to exit the closed quadrant was affected by GD exposure (Fig. 6). Because the homogeneity of variance assumption was violated in analyzing the latency to exit from the closed quadrant in the Zero Maze, a Kruskal-Wallis test was performed and indicated the time to emerge from the closed quadrants for the six treatment groups was significantly different ($H(5) = 42.343$, $p < 0.0001$). Subsequent pairwise comparisons (Dunn's Method) indicated the latency to exit the closed quadrant was earlier for the mice that were not exposed to GD (median exits in seconds: WT SAL = 2.1, IL-1Ra KO SAL = 3.4, and IL-1R1 KO SAL = 3.3 s) compared to the groups of mice that sustained GD exposure (medians for WT exposed = 6.4, IL-1Ra KO exposed = 15.6, and IL-1R1 KO exposed = 9.1). Dunn's tests indicated the exposed and non-exposed WT ($p < 0.05$) and the exposed and non-exposed IL-1Ra KO ($p < 0.001$) groups were significantly different from one another.

3.5. Barnes Maze

The Barnes Maze was used to measure spatial learning and memory after GD exposure. There was a significant main effect of treatment ($F(1,30) = 17.89$, $p < 0.001$) for escape latency between the exposed WT and SAL groups, defined as the amount of time it took for animals to find and enter the escape hole (Fig. 7A). A significant interaction between testing day and treatment ($F(3,38) = 5.406$, $p < 0.05$) was found for the exposed IL-1Ra KO mice compared to their respective controls, where the exposed mice escaped faster on the first day of testing versus the control ($p < 0.01$, Fig. 7B). No significant differences in escape latency were found between the GD exposed IL-1R1 KO group and its respective SAL control (Fig. 7C). When comparing the exposed KO strains to the exposed WT group, there was a significant interaction effect ($F(6,47) = 4.370$, $p < 0.01$), where the WT mice escaped faster than the IL-1Ra KO mice on the fourth day of testing ($p < 0.05$, Fig. 7D).

Primary latency was measured, which is defined as the amount of time an animal took to locate the hole (i.e. perform an initial nose poke) without entering the hole. A significant interaction ($F(3,29) = 4.833$, $p < 0.01$) between the GD exposed WT mice and their SAL controls was observed, where the control mice had a shorter primary latency to nose poke the escape hole versus the exposed mice on the first day of testing ($p < 0.05$, Fig. 8A). When the exposed IL-1Ra KO mice were compared to IL-1Ra KO SAL control mice, a significant main effect of treatment ($F(1,37) = 9.735$, $p < 0.01$) was found where the SAL control mice had a shorter primary latency (Fig. 8B). A significant interaction effect ($F(3,26) = 5.257$, $p < 0.01$) occurred between the GD exposed IL-1R1 KO and its respective SAL control, where the SAL control had a shorter primary latency on the first day of testing ($p < 0.001$) compared to the exposed group (Fig. 8C). A significant treatment by day interaction ($F(6,47) = 4.392$, $p < 0.001$) was found between the exposed KO strains and WT group (Fig. 8D). Here, the WT had a faster primary latency than the IL-1R1 KO mice on the first day of testing ($$ $p < 0.01$) and the IL-1Ra KO mice on the third day of testing (* $p < 0.05$).

The number of errors, defined as total number of nose pokes, was used as an additional measure of learning behavior in the Barnes Maze. There was a significant interaction effect ($F(3,29) = 10.49$, $p < 0.0001$), where the WT SAL control committed more errors on the first day of testing compared to the exposed WT group ($p < 0.0001$, Fig. 9A). Comparison of the IL-1Ra KO groups indicated there was also a significant interaction effect ($F(3,37) = 6.514$, $p < 0.001$), where the IL-1Ra KO SAL group committed more errors on the first ($p < 0.0001$), second, and fourth days of testing ($p < 0.05$) versus the exposed IL-1Ra KO group (Fig. 9B). A significant main effect of treatment ($F(1,26) = 8.014$, $p < 0.01$) was found between the GD exposed IL-1R1 KO group and its respective control (Fig. 9C) indicating a greater number of errors was committed by the IL-1R1 KO SAL control group across all days of testing. No significant difference in errors committed was found between the GD exposed mouse strains (Fig. 9D).

4. Discussion

Although it is known that nerve agent exposure leads to brain damage and changes in behavior, this study is the first to analyze the effect of GD exposure on physiology, behavioral changes, and brain damage in WT mice and KO mice with genetically altered IL-1 signaling pathways. Whereas in the healthy brain, low constitutive expression of the pleiotropic protein IL-1 plays a role in long term potentiation and memory formation (Suzuki et al., 1999), GD-induced SE results in a transient increase in IL-1, which participates

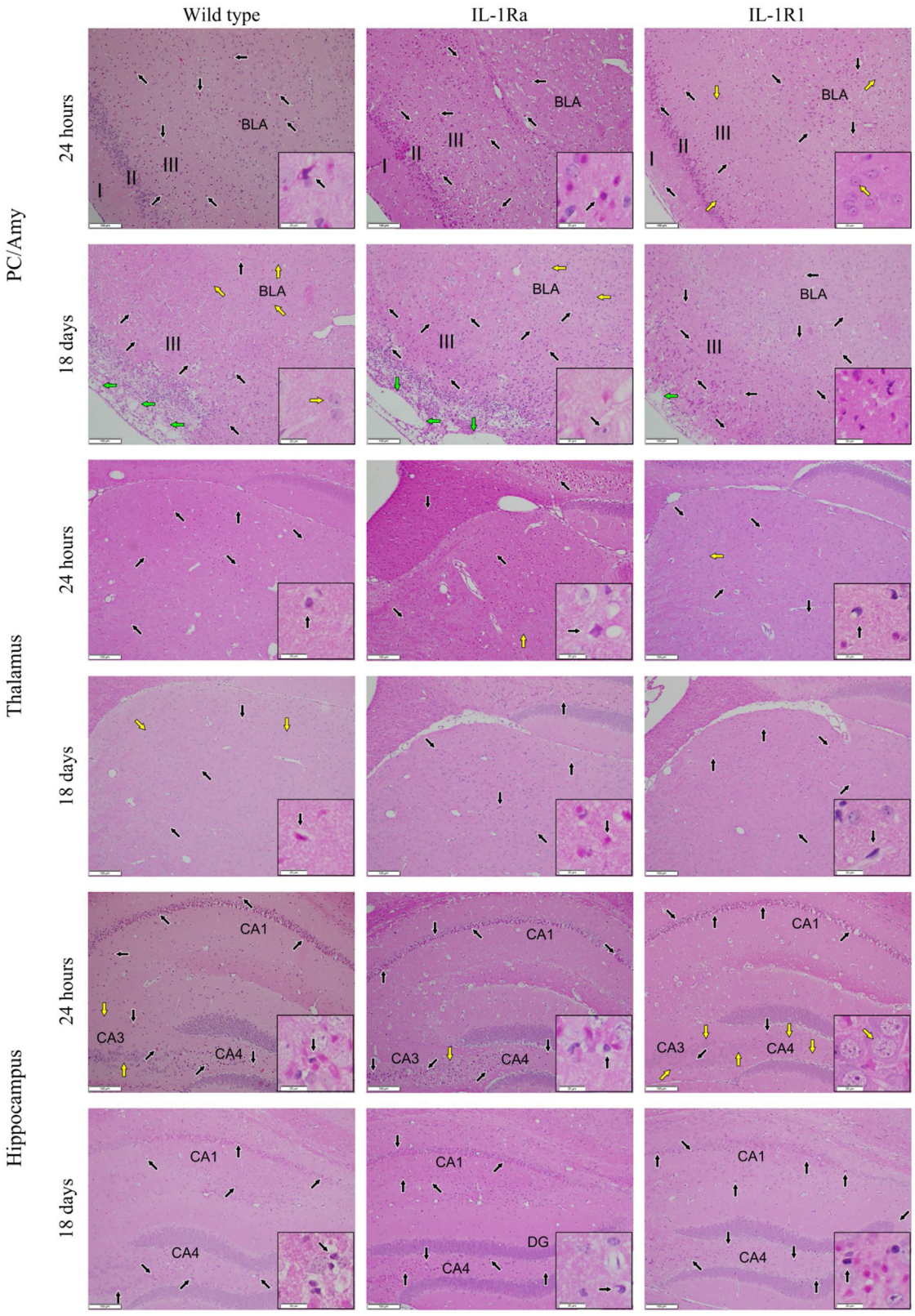

Fig. 2. H&E coronal sections depicting neuropathology 24 hours and 18 days after GD-induced convulsions.
H&E stained coronal sections (10 x images, scale: 100 μm, with superimposed 40 x images, scale 20 μm) demonstrating neuropathology 24 hours and 18 days after convulsive exposure to GD in WT, IL-1Ra, and IL-1R1 KO mice. The black arrows indicate eosinophilic, dying neurons and cellular debris, yellow arrows indicate healthy neurons, and green arrows show lesion formation. PC/Amy, Piriform Cortex/Amygdala. BLA, basolateral amygdala. IL-1Ra, Interleukin-1 receptor antagonist. IL-1R1, Interleukin-1 Receptor Type 1. WT, Wild Type. SAL, saline.

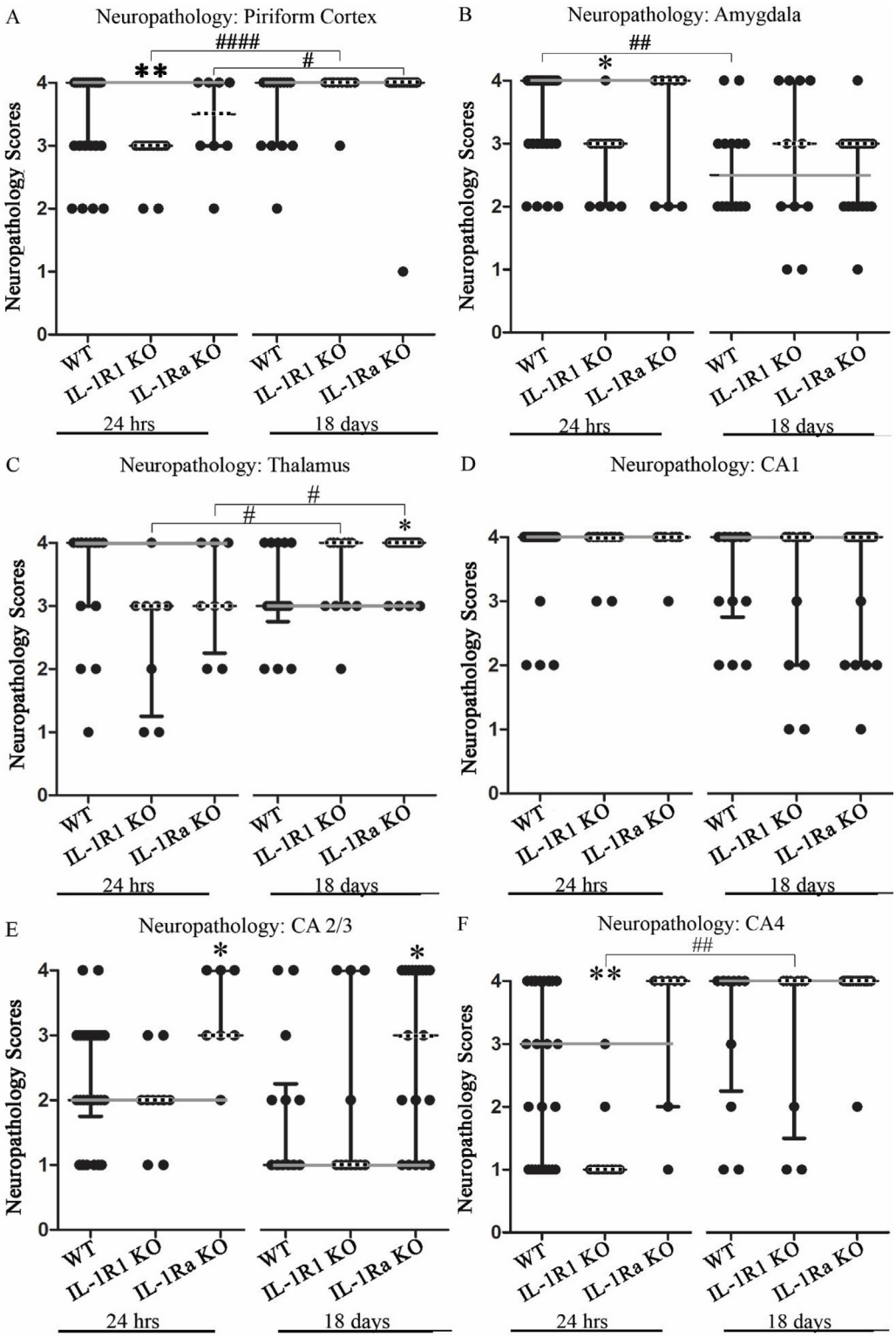

Fig. 3. Neuropathology scores 24 hours and 18 days after GD exposure in the WT, IL-1Ra KO, and IL-1R1 KO mouse strains.
The solid line represents the score of the WT strain, and the dashed lines the score of the KO mouse strains. A score of 0: no damage, 1: minimal damage, 2: mild damage, 3: moderate damage, 4: severe damage. Statistical differences within strain between 24 hours and 18 days are represented by # (# $p < 0.05$; ## $p < 0.01$; #### $p < 0.0001$); differences between the IL-1Ra and IL-1R1 KO strains compared to the WT strain at 24 hours and 18 days, respectively, are represented by * (* $p < 0.05$; ** $p < 0.01$). WT, Wild Type. SAL, saline. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. Bars represent the median and first and third quartiles.

in a positive feedback loop resulting in long term brain injury (Bartfai et al., 2007; Collombet et al., 2005; Johnson and Kan, 2010; Murray et al., 2013; Srinivasan et al., 2004). In the brain, IL-1 is released by activated astrocytes and microglia, binding to the IL-1R1, which is induced in neurons and astrocytes during SE (Vezzani et al., 2011). It should also be noted that GD exposure increases a systemic inflammatory response that may further contribute to blood brain barrier breakdown, hippocampal inflammation and

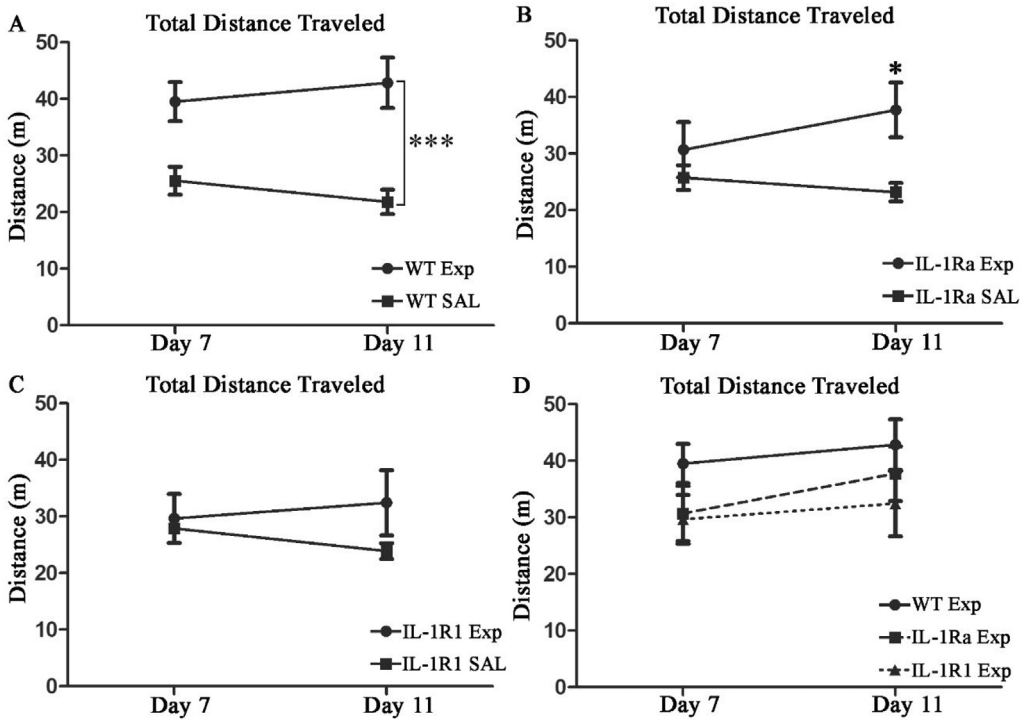

Fig. 4. Total distance traveled in the Open Field as a measure of activity.
The exposed WT group traveled farther in the Open Field compared to their SAL controls on days 7 and 11, showing hyperactive behavior after GD exposure (A). Similarly, the exposed IL-1Ra KO group traveled farther on day 11 compared to their SAL controls (B). No significant difference in total distance traveled was found between the IL-1R1 KO and its SAL control or between the GD exposed mouse strains (D). * represents significant differences between the exposed groups and their respective SAL controls (* $p < 0.05$, *** $p < 0.001$). WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. SAL, Saline. WT: (N = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (N = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (N = 32, GD exposed n = 17, SAL control n = 15). Bars represent the mean ± s.e.m.

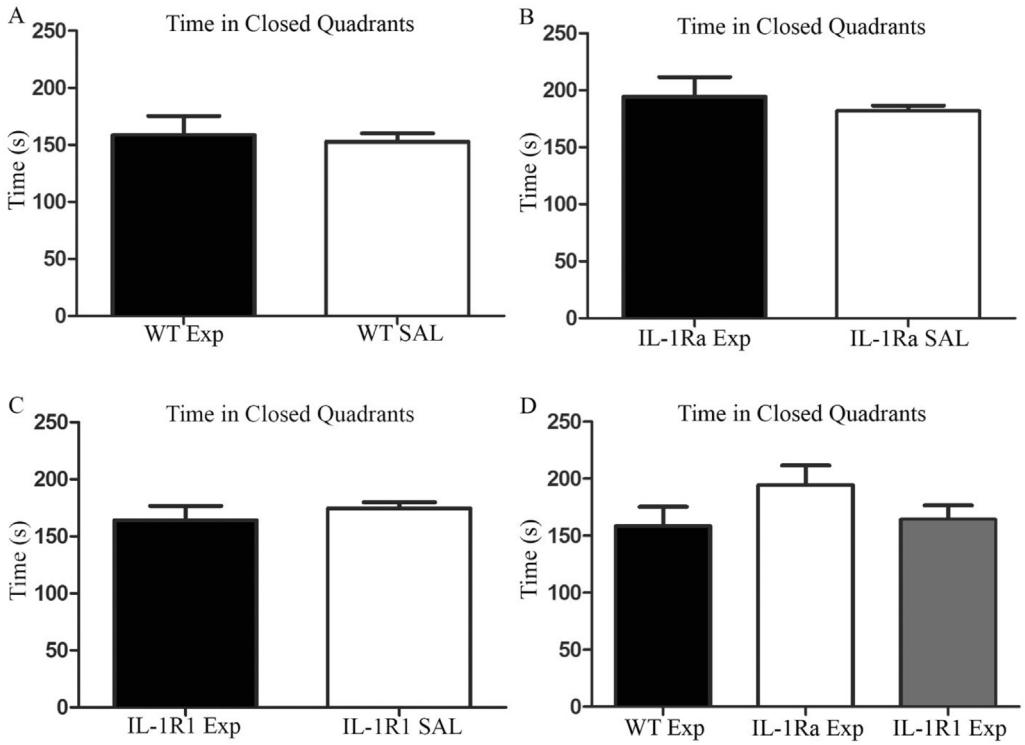

Fig. 5. Time spent in the closed quadrants of the Zero Maze.
The Zero Maze was run on day 9 post exposure. No significant differences were found between the GD exposed strains and their respective controls for time spent in the closed quadrants (A, B, C). No difference in time spent in the closed quadrants was found between the GD exposed groups (D). WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. SAL, Saline. WT: (N = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (N = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (N = 32, GD exposed n = 17, SAL control n = 15). Bars represent the mean ± s.e.m.

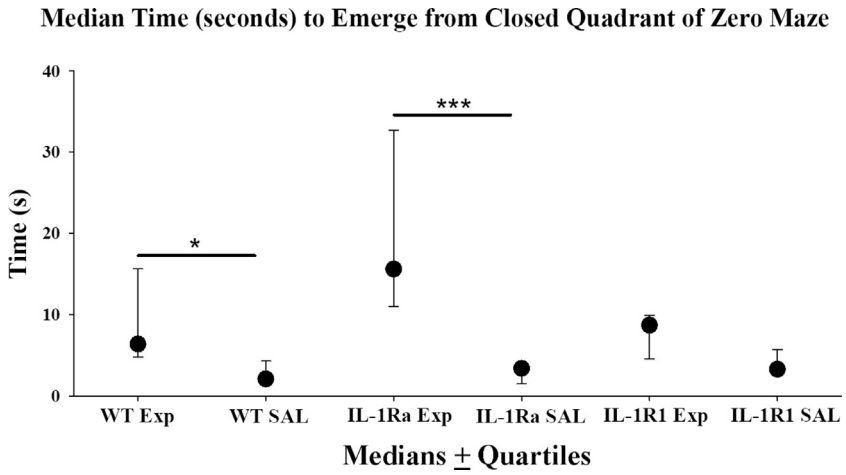

Fig. 6. Latency to first exit from the closed quadrant of the Zero Maze.
The Zero Maze was run on day 9 post exposure. Significant differences were found between the WT and IL-1Ra KO GD exposed strains compared to their respective controlsfor latency to exit the closed quadrant where testing began. The WT and IL-1Ra KO SAL groups had a shorter latency to exit the closed quadrant compared to their respective GD-exposed groups. * represents significant differences between the exposed groups and their respective SAL controls (* $p < 0.05$, *** $p < 0.001$). WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. SAL, Saline. WT: (N = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (N = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (N = 32, GD exposed n = 17, SAL control n = 15). Bars represent the median ± the quartiles.

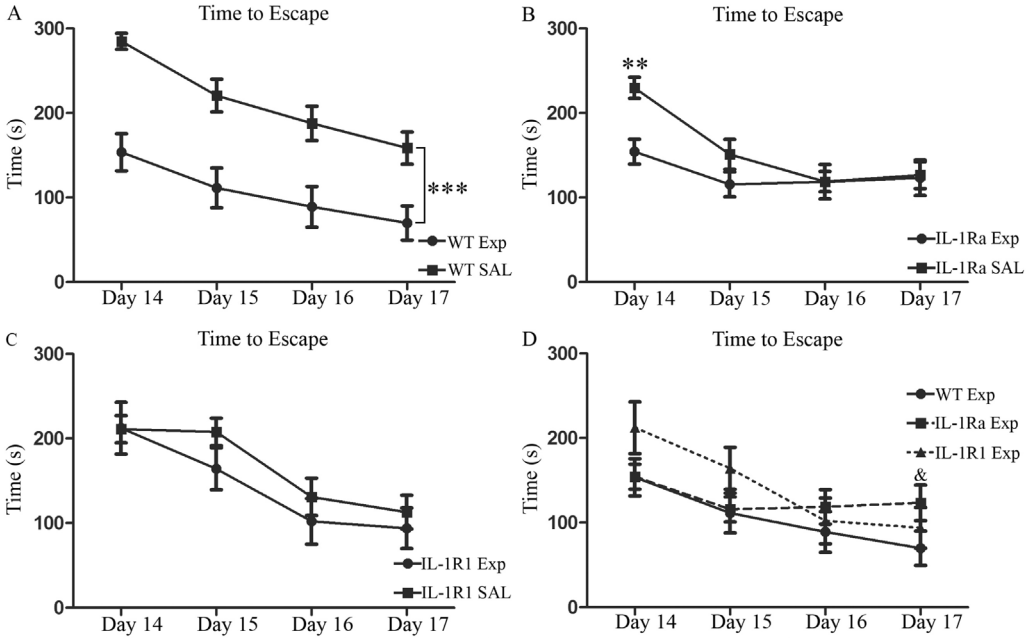

Fig. 7. Escape Latency in the Barnes Maze.
The GD exposed WT group escaped faster than the SAL controls over the four testing days in the Barnes Maze (A). The exposed IL-1Ra KO group escaped faster on day 1 only compared to its respective control (B). No significant difference in time to escape was found between the exposed IL-1R1 KO group and its SAL control (C). When comparing the exposed WT and IL-1Ra KO groups, the WT mice escaped faster on day 4 (D). * represents significant differences between the exposed groups and their respective SAL controls (** $p < 0.01$, *** $p < 0.001$). & represents significant differences between the exposed WT and exposed IL-1Ra KO group (& $p < 0.05$). WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. SAL, Saline. WT: (N = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (N = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (N = 32, GD exposed n = 17, SAL control n = 15). Bars represent the mean ± s.e.m.

cognitive defects (Carpentier et al., 1990; Cibelli et al., 2010; Grange-Messent et al., 1999; Petrali et al., 1991; Terrando et al., 2011, 2013; Wan et al., 2007). Regardless, the lack of a functional IL-1 signaling system, such as in the IL-1R1 KO mice, may help protect this strain from the consequences of nerve agent exposure. IL-1 signaling can be inhibited by IL-1Ra, which is produced endogenously and competes with IL-1 for binding to the IL-1R1 (Rothwell et al., 1997; Rothwell and Luheshi, 2000). IL-1Ra KO mice are more susceptible to neurodegeneration and neuroinflammation due to uncontrolled IL-1 signaling (Craft et al., 2005; Pinteaux et al., 2006), which may explain the worse outcomes seen in this model for the IL-1Ra KO strain.

WT, IL-1Ra KO, and IL-1R1 KO animals were exposed to GD and observed for convulsion onset. Although seizure activity was not measured in this study, previous work has shown that GD-induced SE leads to brain damage (Eisenkraft et al., 2013) and convulsion always precedes SE. In this model, we observed that convulsion induces brain damage in the piriform cortex, amygdala, thalamus, and hippocampus (Fig. 3) and a high percentage of mice developed convulsion and brain injury (Table 1). Acute brain injury resulting

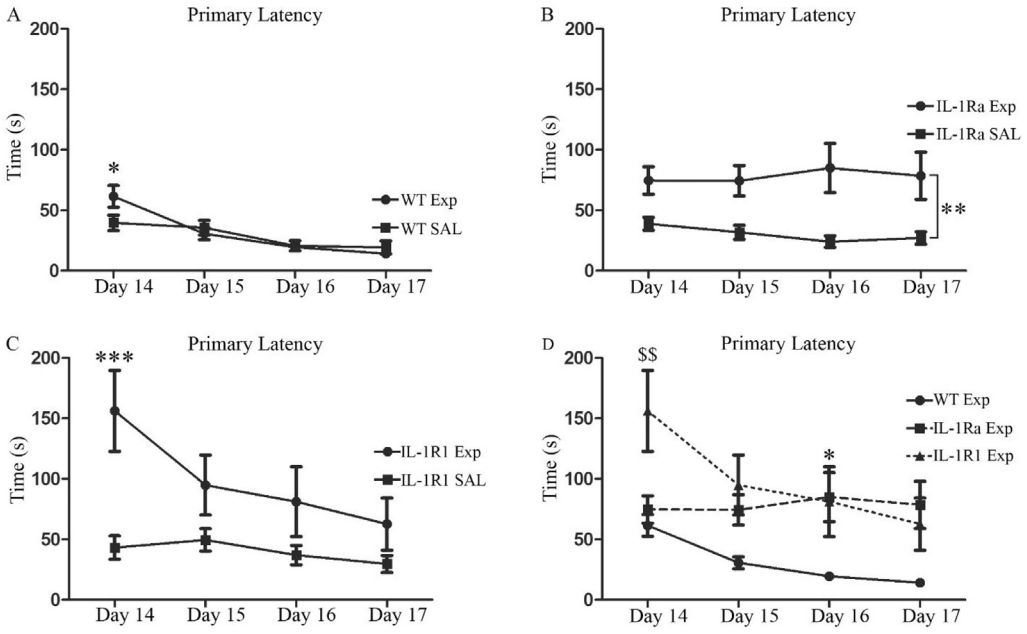

Fig. 8. Primary Latency to locate the escape hole in the Barnes Maze.
The WT SAL group had a shorter primary latency to find the escape hole compared to the exposed WT on Day 1 (A). The IL-1Ra KO SAL control had a shorter primary latency compared to the IL-1Ra KO exposed group (B). The IL-1R1 KO SAL group had a shorter primary latency on day 1 compared to the exposed group (C). The exposed WT group had a shorter primary latency on day 1 compared to the exposed IL-1R1 KO group and day 3 compared to the exposed IL-1Ra KO group. In Fig. A–C, * represents significant differences between the exposed groups and their respective SAL controls (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). In Figure D, $ refers to the difference in primary latency between the exposed IL-1R1 KO group and the WT group ($$ $p < 0.01$); * refers to the difference in primary latency between the exposed IL-1Ra KO group and the WT group (* $p < 0.05$). WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. SAL, Saline. WT: (N = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (N = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (N = 32, GD exposed n = 17, SAL control n = 15). Bars represent the mean ± s.e.m.

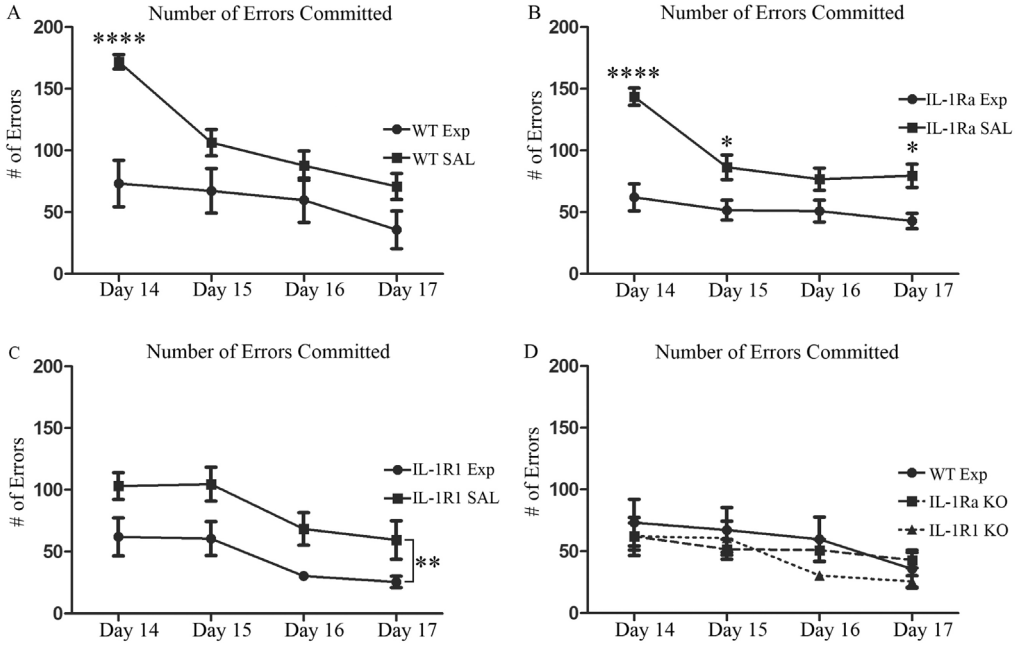

Fig. 9. The number of errors committed in the Barnes Maze.
The WT SAL control committed more errors than the GD exposed WT group on day 1 (A). The IL-1Ra KO SAL control committed more errors on days 1, 2, and 4 compared to the exposed IL-1Ra KO group (B). Across all days, the IL-1R1 KO SAL group committed more errors than the IL-1R1 KO exposed group. (C). There was no significant difference in errors committed between the exposed WT group compared to the exposed KO strains (D). * represents significant differences between the exposed groups and their respective SAL controls (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$). WT, Wild Type. IL-1Ra, Interleukin-1 Receptor Antagonist. IL-1R1, Interleukin-1 Receptor Type 1. SAL, Saline. WT: (N = 42; GD exposed n = 21, SAL control n = 21), IL-1Ra KO (N = 43; GD exposed n = 25, SAL control n = 18), and IL-1R1 KO (N = 32, GD exposed n = 17, SAL control n = 15). Bars represent the mean ± s.e.m.

from GD-induced SE stimulates microglia and astrocytes in the CNS, and activated macrophages and infiltrating leukocytes in the periphery to upregulate inflammatory cytokines, including IL-1 (de Araujo Furtado et al., 2010; Dinarello et al., 2012; Johnson et al., 2011; Johnson and Kan, 2010) contributing to long term brain injury (Basu et al., 2005). In the current study, IL-1Ra KO animals have increased IL-1 signaling after GD exposure because the missing IL-1Ra is not available to block this signaling. These animals show increased brain damage in the piriform cortex between 24 hours and 18 days. Compared to the exposed WT animals, there is damage in the thalamus and CA2/3 region of the hippocampus at 18 days (Fig. 3). Additionally, the IL-1Ra KO mice display a cognitive deficit in the Barnes Maze for escape latency on day 14 compared to the IL-1Ra KO SAL group (Fig. 7B), on day 17 compared to the exposed WT group (Fig. 7D), and primary latency to escape over all testing days compared to the IL-1Ra KO SAL group (Fig. 8B). These behavioral results may be due to increased long-term brain damage in the CA2/3 region of the hippocampus, which is essential for spatial learning and memory. Conversely, IL-1R1 KO animals only showed less acute brain injury in the piriform cortex and amygdala (Fig. 3A, B) without improved long-term behavioral function. This may be because the behavioral tests were conducted outside the window of acute neuroprotection.

Many differing physiological reactions following GD exposure were observed between the WT controls and the two KO strains. The IL-1R1 but not the IL-1Ra KO strain sustained a greater percentage of weight loss at 24 hours compared to the exposed WT group (Table 2). This difference was not the result of differential reactions to handling, injection, or stress as no significant difference was observed between WT controls and either KO strain for weight loss after SAL injection (data not shown). Previous research has shown that early weight loss correlates with neuronal degeneration in the hippocampus after GD exposure in mice (Filliat et al., 2007). However, in the current study, the absence of a functional IL-1 signaling system in the IL-1R1 KO strain significantly enhanced weight loss while acutely protecting certain hippocampal regions (CA4) along with the piriform cortex and amygdala. Conversely, enhanced IL-1 activity in the IL-1Ra KO strain had no effect on weight loss, though the CA2/3 region of the hippocampus was significantly more injured than WT controls. Convulsion onset was also different between the three mouse strains. The onset of convulsions after GD exposure occurred significantly faster for the IL-1Ra KO mice compared to the exposed WT animals (Table 2), whereas no difference was observed between the IL-1R1 KO and WT groups. Previous work in rats exposed to intrahippocampal injection of IL-1β has shown that this cytokine produces a pro-convulsant response (Vezzani et al., 1999). The additive, convulsive effects of GD and an overly active IL-1 signaling system (Dhote et al., 2007; Johnson and Kan, 2010; Williams, 2003) may explain why the IL-1Ra KO mice convulsed sooner than the WT control mice.

Neuropathology was observed in the exposed WT animals and was consistent with previous work in mouse GD models (Collombet et al., 2011, 2006b, 2008; Filliat et al., 2007). In this study, neuropathology scores were significantly different between the three strains investigated at 24 hours, except in the CA1 region of the hippocampus, where severe neuropathology was observed across all strains at 24 hours and 18 days, and the thalamus at 24 hours, where the IL-1R1 and IL-1Ra KO mice were moderately injured (Fig. 3). Our model of nerve agent intoxication leads to cholinesterase inhibition and acetylcholine buildup, glutamatergic hyperexcitation, and SE. It is the result of these events that induces brain damage in the piriform cortex, amygdala, thalamus, and hippocampus induced by increases in acetylcholine, excitatory amino acids, and excessive calcium influx. The overexcitation of cholinergic receptors after GD intoxication would explain similar neuropathology scores between the KO animals in the thalamus (Fig. 3C) at 24 hours, where regardless of KO strain, the same damage is seen. Additionally, the upregulation of IL-1 post injury has been reported to increase neuronal excitability in the CA1 region of the hippocampus (Vezzani et al., 2011). The acute and long term brain damage seen in the CA1 region of the hippocampus may be the result of overexcitation of the cholinergic receptors (de Araujo Furtado et al., 2010; Eisenkraft et al., 2013; Johnson and Kan, 2010; McDonough and Shih, 1997; Shih and McDonough, 1997b) resulting in primary excitotoxic injury before the acute inflammatory reaction is initiated, and the upregulation of IL-1 resulting from seizure activity due to GD exposure (Svensson et al., 2005, 2001; Williams, 2003).

WT mice had moderate to severe neuropathology in five of six brain regions investigated at 24 hours (Fig. 3). Similar results were seen for the IL-1Ra KO mice, although the CA2/3 (Fig. 3E) also showed significantly more damage than WT controls possibly due to high concentrations of IL-1R1 in the hippocampus (Vezzani et al., 1999). The IL-1R1 KO strain, however, showed significant reduced neuropathology scores in three of the six regions investigated (piriform cortex, amygdala, and CA4) compared to WT controls. Previous work in IL-1R1 KO mice exposed to ischemic insult has shown a reduced infarct volume 24hours after injury in the cerebral cortex (Basu et al., 2005; Touzani et al., 2002). This suggests that IL-1 signaling plays a role in the progression of acute neuropathology following convulsive GD exposure and that inhibition of the IL-1 signaling pathway can attenuate that progression at 24 hours after injury. Since GD exposure induces seizure activity, which increases brain damage post exposure, a lack of IL-1R1 attenuates IL-1 signaling. Therefore, in the current study, the IL-1R1 KO animals may have shown less brain damage compared to the exposed WT animals because they convulsed less (Figs. 3A, 3B, 3F). The neuropathology in IL-1Ra KO mice in this study is consistent with studies using IL-1Ra KO mice in other CNS injury models such as ischemia, stroke, and β-amyloid induced damage, where these animals exhibit neurodegeneration and have higher numbers of activated microglia compared to WT controls (Craft et al., 2005; Loddick et al., 1997; Pinteaux et al., 2006). Endogenous IL-1Ra inhibits the IL-1R1, the functional receptor for IL-1 (Avital et al., 2003; Vezzani et al., 2000b), thus reducing the pro-inflammatory cascade and reducing neuropathology. Although seizures also increase endogenous levels of IL-1Ra, upregulation of the inhibitor is not adequate to reduce the overwhelming increase in IL-1 after injury (De Simoni et al., 2000; Plata-Salaman et al., 2000; Vezzani et al., 2000b). In this model, uninhibited IL-1 signaling likely helps initiate and maintain seizure activity by enhancing glutamatergic neurotransmission (Vezzani et al., 1999, 2000b) and, therefore, neuronal cell death via excitotoxicity (Baille et al., 2005; McDonough and Shih, 1997).

In WT mice, few changes were observed from 24 hours to 18 days after GD exposure except for a significant decline in neuropathology scores in the amygdala (Fig. 3B). Moderate/severe damage was still evident in the piriform cortex, thalamus, CA1, and CA4 regions of the hippocampus, and minimal/mild damage in the amygdala and CA2/3 region of the hippocampus (Fig. 3). Similarly, degenerating eosinophilic neurons were found in the CA2/3 region of the hippocampus, the dentate gyrus, and thalamus in rats from days 1 to 30 after exposure to a convulsive dose of GD (Collombet et al., 2007; McDonough and Shih, 1997). These data show that in this model of GD-induced SE, brain injury was acute and persistent up to at least 18 days. These data also show that, at least in the amygdala, recovery is part of the natural progression of this injury mechanism and is consistent with previous findings (Collombet et al., 2008). While IL-1 signaling inhibition was neuroprotective at 24 hours, these benefits are not sustained to 18 days as there were no significant differences between the WT and IL-1R1 KO strains at 18 days (Fig. 3). In fact, neuropathology scores significantly increased from 24 hours to 18 days in the IL-1R1 KO in the piriform cortex,

thalamus, and CA4 (Fig. 3A, C, D). These results suggest that the absence of IL-1R1 attenuates acute brain damage but this reduction is transient. Exposed IL-1Ra KO mice had moderate to severe injury in all brain regions studied at 24 hours, with some regions receiving significantly higher neuropathology scores at 18 days than WT (Fig. 3). These data indicate that IL-1 signaling inhibition can reduce acute but not chronic brain injury. IL-1 signaling after GD exposure is inhibited in IL-1R1 KO mice and may delay injury after SE, where other pro-inflammatory pathways such as TNF signaling are concurrently active (Lambertsen et al., 2005; Lehtimaki et al., 2003) and may contribute to the appearance of brain damage at later time points. Inflammatory responses are not relegated to just very early time points as previous research in GD-exposed mice has shown the most intense microglia and astrocyte activation between three and seven days after injury (Baille et al., 2005; Collombet et al., 2005). This inflammatory response participates in secondary brain damage, but can also initiate cell regeneration after brain injury (Collombet et al., 2011, 2006a). In the amygdala at 18 days, the wild type mice showed significant improvement in neuropathology scores, and the IL-1Ra KO mice showed decreased neuropathology scores from severe to moderate injury over time (Fig. 3B), whereas the IL-1R1 KO maintained the same score. This indicates that IL-1 signaling is important in both the initial injury and recovery of the brain post exposure (Mori et al., 2014). By inhibiting IL-1 signaling in the IL-1R1 KO, regeneration is also being inhibited, whereas the IL-1 signaling that occurs in the IL-1Ra KO is allowing regeneration to occur over time. This may explain the improved scores in the amygdala of IL-1Ra KO mice at 18 days (Fig. 3B). This study shows that the absence of IL-1 signaling does not prevent this brain damage over time.

Although seizure activity was not recorded in this study, the IL-1Ra KO animals not only convulsed the fastest after GD exposure (Table 2), but they also had the highest percentage of animals that convulsed and died post exposure (Table 1). Previous studies have reported that endogenous IL-1β is induced in seizure models (Ravizza and Vezzani, 2006), enhancing neuronal excitability, and contributing to seizure activity by promoting glutamatergic function (Vezzani et al., 2000b, 2004). In the IL-1Ra KO mice, IL-1 can readily bind to IL-1R1 after GD exposure, inducing seizure activity. Additionally, the hippocampus is the second richest brain region in the IL-1R1 (Vezzani et al., 2000a), which may have contributed to the overall damage witnessed in the CA1 region of the hippocampus of the WT and IL-1Ra KO mice after GD exposure (Fig. 3D). The results of the current study demonstrate the fine balance of IL-1 signaling and the dual role IL-1 can play after injury. This has been well established in previous research, where seizure activity induced by kainic acid or bicuculline in mice has been shown to trigger IL-1 release and prolong hippocampal seizures (Vezzani et al., 1999, 2000b). Although seizure activity induces IL-1Ra upregulation, it is increased in the CNS to a lesser extent, and therefore, is less effective in attenuating seizure activity. This was shown when control mice and IL-1R1 KO mice were exposed to bicuculline and treated with an intracerebral injection of human recombinant IL-1Ra (Vezzani et al., 2000b). Unlike the control mice, the IL-1R1 KO was not protected from continued convulsions because the drug could not bind to the missing IL-1R1, indicating the tight regulation of the IL-1 signaling pathway (Rothwell et al., 1997; Rothwell and Luheshi, 2000; Spulber and Schultzberg, 2010; Vezzani et al., 2000b; Weber et al., 2010).

Memory processes require the integration of a variety of CNS regions, including the amygdala and hippocampus, the thalamus, and other limbic structures including the piriform cortex. This study and others have shown profound injury in these regions after GD exposure (Collombet et al., 2005, 2006b, 2008; Filliat et al., 2007), and GD-induced injury can affect behavioral performance (Collombet et al., 2006b; McDonough et al., 1995). This study used the Open Field to test activity, the Zero Maze for evaluating anxiety, and the Barnes Maze to test spatial learning and memory. The Open Field test measures how an experimental condition can affect the rodent's activity as a reaction to a novel environment (Mamczarz et al., 2010; Prut and Belzung, 2003). In the Open Field, a pattern developed among the WT (Fig. 4A) and IL-1Ra KO (Fig. 4B) mouse groups, where the exposed animals traveled farther compared to their individual SAL controls. Although distance traveled is a measure of exploratory behavior (Mamczarz et al., 2010; Walsh and Cummins, 1976), these GD-exposed animals showed consistent increased activity compared to the controls, characterized by increased movement in a novel environment and movement near the edges of the Open Field (Budinich et al., 2013; Simon et al., 1994). Previous work in rats exposed to a high dose of GD have shown that the animals tend to run around the walls of the apparatus when introduced to the Open Field, indicating increased activity (Raffaele et al., 1987). In this study, there was no difference in the total distance traveled between the exposed WT or KO mouse groups, indicating that the absence of IL-1Ra or IL-1R1 did not significantly affect the activity of the animals after GD exposure.

The Zero Maze was used to assess anxiety-like behavior, where the time spent in the closed quadrants and the primary latency to emerge from the initial closed quadrant were used as measures of anxiety (Jacobson et al., 2007). Although no significant differences for time in the closed quadrants were found between each strain and its respective control (Fig. 5), the WT and IL-1Ra KO mice that were exposed to GD took a significantly longer time to initially exit the closed quadrant (Fig. 6). These results suggest that GD exposure in the WT and IL-1Ra KO mouse strains makes the animals less willing to venture into an open, brightened environment, suggesting anxiety to a novel task (Coubard et al., 2008). However, GD did not significantly increase the median time for first exit in the IL-1R1 KO mice (Fig. 6). Similar effects have been reported in other paradigms, where IL-1R1 KO mice were tested for anxiety responses after a treatment in the Elevated Plus Maze (EPM), the predecessor of the Zero Maze that also tests for anxiety-like behavior (Jacobson et al., 2007), and the light-dark box. In one study, uninjured IL-1R1 KO mice spent more time in the open areas of the EPM compared to WT mice, indicating that the absence of IL-1R1 decreases anxiety (Koo and Duman, 2009). Another study with this strain reported that the stressor, repeated social defeat, failed to reduce the time for IL-1R1 KO mice to enter the closed quadrant of the light-dark box test (Wohleb et al., 2011).

The Barnes Maze was used to evaluate spatial learning and memory in the WT and KO mouse strains. Surprisingly, GD-exposed WT mice had significantly shorter escape latencies than their SAL controls in the Barnes Maze at all time points investigated (Fig. 7A). These results seem to contradict previous work, where GD-exposed mice were found to exhibit deficits in performance, such as swimming slower and traveling a longer distance to the submerged platform in the Morris Water Maze (MWM), another test of spatial learning and memory, compared to SAL controls (Collombet et al., 2011; Filliat et al., 2007). However, these discrepancies may be explained by a fundamental difference between the MWM and the Barnes Maze. The water in the MWM is an aversive and stressful stimulus, while the bright light of the Barnes Maze is much less aversive, putting less pressure on the mice to escape (Sharma et al., 2010). While the light might not be an aversive stimulus coaxing the SAL control animals to escape, the exposed animals may have a photophobic response to the lights making their escape times shorter. Brighter light conditions are considered to be more stressful to rodents versus low light conditions (Lacroix et al., 2000; Mamczarz et al., 2010).

Additionally, photophobia has been associated with inflammation and traumatic brain injury and, therefore, may be active in this model as well (Du et al., 2005; Thiels et al., 2008). Interestingly,

Fig. 7D shows the WT and IL-1R1 KO mice escape faster over time (days 14 to17 in WT mice; days 14 to16 in IL-1R1 KO mice). Conversely, the IL-1Ra KO had an increased latency to escape (Fig. 7D) over time. Furthermore, injury in the CA2/3 region of the hippocampus was worse in the IL-1Ra KO at 18 days compared to other strains (Fig. 3E). Increased IL-1 signaling likely led to increased damage in this region, which affects spatial learning and memory and prolonged the escape latency of the IL-1Ra KO mice.

Because of the potential confounding of escape latency by exploratory behavior being a stronger motivator than light aversion, the primary latency to initially find the escape tunnel was analyzed as an alternate metric of learning and memory (Harrison et al., 2006; O'Leary and Brown, 2013). Exposed WT and IL-1R1 KO groups took longer than their SAL controls to initially find the escape hole on day one only (Fig. 8A, C). However, there was a persisting significant difference between the exposed IL-1Ra KO and its control (Fig. 8B). This suggests greater impairment of spatial learning and memory when IL-1 signaling is unchecked in this model and may be related to the severity of CA2/3 injury (Hicks et al., 1994; Smith et al., 1993) in this strain compared to the WT and IL-1R1 KO strains.

Another measure of spatial learning and memory is the number of errors committed while attempting to find the escape hole. The WT SAL control on day 14 and the IL-1Ra KO SAL controls on days 14, 15, and 17 committed more errors than their respective exposed mice (Fig. 9A, B), and the IL-1R1 KO SAL control committed more errors than the IL-1R1 KO strain across days after GD exposure (Fig. 9C). In the absence of an aversive stimulus, the number of errors committed on the maze is interpreted as an indicator of exploratory behavior rather than cognitive impairment (Fox et al., 1998; Koopmans et al., 2003; Sharma et al., 2010). These results indicate that the SAL controls were more exploratory than the exposed animals, regardless of strain, and that the absence of IL-1R1 or IL-1Ra does not significantly affect exploratory behavior.

5. Conclusion

This study showed that IL-1 signaling has an effect on neurodegeneration resulting from GD-induced SE. Whereas excessive IL-1 signaling after convulsive GD exposure results in brain damage and cognitive deficits, reducing IL-1 signaling can attenuate acute brain injury. Conversely, IL-1 signaling also appears to be important in the recovery phase after GD-induced SE as well, where a lack of IL-1 signaling can prevent repair at later time points. Future work with IL-1Ra KO and IL-1R1 KO mice exposed to GD will investigate the effect of IL-1 signaling on the up- or down-regulation of inflammatory cytokines, such as IL-6 and TNFα, that are regulated by IL-1 signaling and have an effect on the neuroinflammatory response post exposure. A better understanding of neuroinflammation after GD exposure may provide more insight on the effect of IL-1 signaling on the long-term neurodegeneration and behavioral deficit observed after nerve agent intoxication.

Funding

This project, CB3943, was sponsored by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division. The funding source had no role in study design, collection, analysis and interpretation of data, the writing of the report, or the decision to submit the article for publication.

Acknowledgment

Thank you to Dr. Cara Olsen for her assistance with the statistical analyses for this project.

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