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Central infusion of interleukin-1 receptor antagonist blocks the reduction in social behavior produced by prior stressor exposure

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Abstract

ARAKAWA, H., P. BLANDINO Jr. AND T DEAK. Central infusion of interleukin-1 receptor antagonist blocks the reduction in social behavior produced by prior stressor exposure. *PHYSIOL BEHAV* **(*) 000-000, 2009.-Pro-inflammatory cytokines such as interleukin-1beta (IL-1 β) in the brain modulate sickness behavior in rodents, in which animals show complex changes in behavior such as reduction of general activity, reduced social motivation, and fever response. The present studies examined the impact of lipopolysaccharide (LPS) and stressor (footshock) exposure on the later expression of social behavior in Sprague-Dawley rats using two separate behavioral paradigms. In Experiment 1, a traditional test for social interaction in which animals were allowed to investigate a juvenile rat in their home cages was conducted at 4 different time points following LPS or foot shock treatment. In Experiment 2, social investigation task which allowed animals to sniff the hole connected to other chamber where a stimulus animal was placed, but prevented physical contact, was used to measure social investigation at several time points following LPS or footshock treatment. Both systemic infusion of LPS (100 μ g/kg) and 2 hr footshock exposure (80 shocks, 1mA, 5 sec duration) elicited a time-dependent reduction of social interaction (Exp. 1) and investigation (Exp. 2); LPS-treated rats displayed a more profound reduction of social investigation from 2 hr to 6 hr after treatment, while rats exposed to footshock showed a reduction 6 hr after the footshock exposure. In Experiment 3, the footshock-induced reduction of social investigation was blocked by pretreatment with IL-1 receptor antagonist (IL-1Ra; 100 μ g icv) infusion. Together, these findings support a growing body of literature showing that stress-dependent changes in brain cytokines play a key role in mediating behavioral consequences of stressor exposure.

Keywords

Footshock; Pro-inflammatory cytokine; Sickness behavior; Social investigation; IL-1Ra; LPS; Rats; stress

Introduction

Systemic and central administration of pro-inflammatory cytokines such as interleukin-1beta (IL-1 β) and tumor necrosis factor alpha (TNF- α) to rats and mice initiates a complex pattern of behavioral changes, which are reminiscent of sickness [13,16], manifested as hyperthermia

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[41,49], motor depression [66], anorexia [5], and reduced social interaction [6,22,61]. A set of non-specific behavioral changes which are collectively named sickness behavior [14-15] is likely to be regulated according to a motivational perspective [17]. Elements of this complex suggest that some aspects of the syndrome may be mediated by activation of inflammatory signaling pathways.

The pro-inflammatory cytokine, IL-1 β acts on specific receptors encoded by two different genes. IL-1 receptor antagonist (IL-1Ra) is an endogenous inhibitor for IL-1 [25,31], which competes with IL-1 for occupancy of its cell surface receptors. Cytokine-induced deficits in social interaction can be selectively reduced by central administration of IL-1Ra [6,36]. This effect of the IL-1Ra is mediated by the type I receptors [2,46]. Importantly, the observation that intracerebroventricularly (i.c.v.) IL-1Ra fully blocked the behavioral effect of intraperitoneal (i.p.) IL-1 β suggests that peripheral IL-1 β triggers the synthesis and release of brain IL-1 β [11,42], since physiological quantities of IL-1 β protein do not pass through the blood-brain barrier in appreciable amounts [69]. In addition, lipopolysaccharide (LPS), a fragment from the cell wall of gram-negative bacteria [40], triggers the production of IL-1 β , interleukin-6 (IL-6), and TNF- α through activation of Toll-like receptor-4 (TLR-4) [33,55]. Both systemic and central administration of LPS increase levels of IL-1 β and related pro-inflammatory cytokines in several areas of the brain including the hippocampus, hypothalamus and diencephalic structures [55,64], induce a complex of behavioral changes comparable to cytokine-induced sickness behavior in the rat [7].

Exposure to footshock is a highly tractable stress model, well known to provoke increases in secretory activity at each level of the HPA axis [34-35]. The upregulation of CRF expression in the paraventricular nucleus of hypothalamus (PVN), one of the centers for HPA response, was observed following repeated footshock, suggesting that synaptic input to the CRF neuron is involved in the elicitation of increased central drive to the HPA axis that accompanies this model [62]. A single exposure to a 30 min intermittent footshock session elicits HPA secretory responses provokes transient expression of *c-fos* mRNA in the hypothalamus [34,44,52], which is similar with a response to IL-1 β infusion into the brain [26]. Importantly, 2-hr footshock treatment increased expression of IL-1 β in the hypothalamus [3-4,18].

Activation of inflammatory signaling pathways in response to stressor exposure relates mechanistically to both behavioral and physiological consequences of stressor exposure. For instance, central administration of IL-1 receptor antagonist has previously been shown to block certain features of learned helplessness [45], the impairment of memory consolidation produced by isolation of juvenile pups [39], and the reduction of neurogenesis in the hippocampus [39]. Considering that social interaction is one of the most commonly used behavioral tests to report sickness behavior in rat [6,9,19], along with the observation that stressor exposure powerfully reduces social interaction [64], we hypothesized that the footshock-dependent reduction of social interaction is mediated by induction of pro-inflammatory cytokines in the brain. We performed several experiments in which the impact of footshock exposure or LPS treatment was evaluated in each of two separate behavioral paradigms for the measurement of social interaction. In Experiment 1, we measured social interaction using the juvenile conspecific task as a means for establishing whether footshock exposure would dampen social interaction when adult male test subjects were exposed to novel (unmanipulated) juvenile conspecifics. The strength of this paradigm is that social encounters between the juvenile stimulus animal and the adult test subject are devoid of aggressive displays, thereby reporting changes in social behavior uncontaminated by other processes (aggression, territoriality, etc). Experiment 2 was similar in design, but employed a relatively new paradigm where adult subjects were permitted to interact exclusively through a hole in the apparatus, thereby preventing full physical contact [21]. Finally, Experiment 3 assessed

whether the reduction in social investigation produced by prior stressor exposure could be blocked by pretreatment with IL-1 receptor antagonist.

Materials and methods

Subjects and rearing condition

Adult male Sprague-Dawley rats were used as the subjects (270-360 g for Exp. 1 and 2, 250-346 g for Exp. 3). The subjects for Experiment 1 and 2 were born and bred in the animal facility in the Department of Psychology at SUNY-Binghamton using breeders purchased from Harlan (Indianapolis, IN, USA), while the subjects for Experiment 3 were purchased from Harlan at least 10 days prior to manipulation. Colony conditions were maintained at 22 ± 1 °C with a 14:10 light-dark cycle (lights on 06:00-20:00 h). The housing conditions for all experiments occurred in standard transparent Plexiglas cages, $43 \times 21.5 \times 20.5$ (H) cm, with access to food and water ad libidum. Rats were handled briefly for 3-5 min on each of 2 days prior to experimentation. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Binghamton University.

Surgical procedures

For Experiment 3, animals were anesthetized using inhalant isoflurane (dosed to effect) for stereotaxic placement of a 22 gauge stainless steel guide cannula (Plastics One, Roanoke, VA, USA) into the third ventricle, AP: -1.0-mm, ML: ± 0.0 -mm, DV: -6.0-mm. Rats were injected with 0.03 mg/kg of buprenorphine prior to, immediately after, and 24 hr post surgery for pain remittance. The top of the animal's head was shaved and a 20-mm midline incision was made across the top of the skull. After cleaning the periosteum, a 1-mm hole was drilled and the cannula was placed 6.0-mm below the skull surface. The guide cannula was fixed in place with dental cement (Plastics One) and three of jewelers' screws. Patency was maintained with a dummy cannula of 28 gauge stylette (Plastics One), which was removed at the time of injection. Post-operation, subjects were housed singly and allowed to recover at least for 10 days before behavioral testing.

The accuracy of ventral cerebral cannulae was tested using angiotensin-II induced drinking behavior measure as previously described [19]. One day after testing of icv treatments on social investigation, drinking behavior was measured in each animal's home cage after icv infusion of 5 µg of angiotensin-II (Sigma, St. Louis, MO) in 2.5 µl of buffered saline. The amount of drinking was scored for 30 min beginning immediately after icv treatment. Data were analyzed only from rats demonstrating a robust dipsogenic response (>10 ml/30min) induced by angiotensin-II infusion.

Drug infusion

Lipopolysaccharide (LPS; from *E. coli* serotype 0111:B4) was purchased from Sigma Chemical Co. (St. Louis, MO). LPS was initially diluted in sterile, pyrogen free saline (0.9%) and aliquots were stored at -20°C until needed. On the day of experimentation, a frozen aliquot was thawed and diluted to 100 µg/ml in pyrogen free physiological saline. LPS injections were administered intraperitoneally (i.p.) at 100 µg/kg.

IL-1Ra (Amgen, Thousand Oaks, CA) was diluted with vehicle (sterile, pyrogen free saline) and administered i.c.v. At the time of microinjection, each rat was gently restrained by towel, and the stylus was removed, then 100 µg of IL-1Ra delivered in a volume of 2.5 µl, or pyrogen free saline (0.9%) vehicle was injected into the ventricle through the guide cannula over a time course of 60 sec, using a 10 µl Hamilton syringe connected to PE-10 tubing (Plastic One, VA) precut to the appropriate length. The injector (Plastic One) was left in place for another 30 sec

to allow for drug diffusion. The injector extended 1.0 mm below the end of the guide cannula into the ventricle.

Footshock exposure

For Experiment 1 and 2, rats were exposed to 80 inescapable footshocks (2.0 mA, 90 sec variable ITI, 5 sec each) over the course of approximately 2 hr [4]. The footshock chambers were standard operant chambers measuring $23.5 \times 20.5 \times 18$ (H) cm (Ralph Gerbrands, Model C, Arlington, MA, USA) that were adapted to deliver shock through the grid floor (18 bars spaced 1 cm apart on center, with a diameter of 1.0 mm each). All current was delivered to the grid floor by a shocker (BRS/LVE, Model SGS-004, Beltsville, MD, USA) driven by a personal computer interface.

After completion of Experiment 1 and 2, a new footshock system was acquired and used in Experiment 3. Animals received 80 inescapable footshocks (1.0 mA, 90 sec variable ITI, 5 sec each). The current was changed to 1.0 mA in Experiment 3 to account for greater efficiency of current delivery with the new system (1.0 mA produced a comparable behavioral response in preliminary validation studies as a setting of 2.0 mA using the older shock system). The foot shock chamber measured $30.5 \times 26.5 \times 33$ (H) cm (Habitest Chamber, Model H10-11R-TC-SF, Coulbourn Instruments, Allentown, PA, USA). The side walls of the chamber were constructed of stainless steel except the front doors that were constructed of clear Plexiglas. The floor consisted of steel rods through which a scrambled shock from a shock generator (LABLINC Model H01-01, and Precision Animal Shocker Model H13-15, Coulbourn Instruments, Allentown, PA, USA) could be delivered. The sound-attenuating chambers were illuminated by a 20-W white lightbulb and background noise was provided by individual ventilation fans. Each shock chamber and waste collection tray was cleaned with a wet paper towel after each session.

Social investigation apparatus

In Experiment 2 and 3, the social investigation apparatus consisted of two clear Plexiglas boxes measuring 65 cm $\times$ 24 cm $\times$ 15 cm sitting 12 cm apart, end to end [see 21,51]. In both ends of each box there was a 3.2 cm hole surrounded by photoelectric cells to detect movement through the hole. This permitted each animal to investigate the hole facing a conspecific or the hole that does not, thus yielding a measure of social interaction on one end and a measure of exploratory activity at the other. Each animal was placed in one of the social investigation boxes while its pair-mate was placed in the adjacent box for a total of thirty minutes. Testing occurred in a dimly lit room in the absence of an experimenter. The frequency and duration of photobeam breaks, indicative of head pokes through a hole, were automatically recorded at each of the social and nonsocial holes. Head pokes were scored separately for each animal in the pair.

Experimental procedure

Experiment 1—The aim of experiment 1 was to compare the effect of two types of stimulus which induce cytokine expression in the brain on social interaction as a highly sensitive index of sickness in rats. In Experiment 1-1, rats were injected i.p. with vehicle (sterile pyrogen-free saline) or 100 μ g/kg of LPS. For social interaction test, an unfamiliar juvenile rat aged 25-35 days was introduced into the subject's home cage for a 5 min test session at 2, 4, 6, and 24 hr after injection. Immediately after 5-min exposure, juvenile rats were returned to their cages and the subjects were kept undisturbed until the next session.

In Experiment 1-2, a different group of rats was exposed to a 2 hr session of intermittent footshock or remained in their home cages as controls. Rats were returned to their home cages immediately after footshock termination, and social interaction with a juvenile conspecific was

assessed at 2, 4, 6, and 24 hr after footshock termination. Using the same procedures described in the Exp. 1-1, each subject were confronted with an unfamiliar juvenile rat (aged 25-35 days) in their home cage for 5 min at four time point after footshock exposure.

Each session was videotaped and behavior was scored at a later time by observers blind to experimental treatment. The number of 5 sec intervals during which the adult rat displayed active social investigation (predominantly anogenital sniffing) directed towards the juvenile stimulus animals in a 5 min test session were counted.

Experiment 2—To assess the impact of LPS and footshock treatment on social motivation of male rats in the social investigation chamber, rats ($n = 10$ per group) were given 15 min of pre-exposure to the chamber to which testing occurred without a rat being present in the adjacent chamber and were returned to the home cage after pre-exposure. Six hr from the onset of pre-exposure, rats were returned to the apparatus and were tested against the previously housed cage mate for 30 min to assess baseline activity (baseline). The following day, pair-tested rats (previous cage mates) received like treatments and were exposed to 80 intermittent shocks over the course of approximately 2 hr or received LPS 100 $\mu\text{g/kg}$ i.p. and remained in the home cage for an equivalent time. A separate group of rats were used as unmanipulated controls. Social investigation was then tested in rats against their like-treated previous cage mate for 30 min at 2, 6 and 24 hr following the onset of footshock or LPS exposure. Time spent with poking their nose at each port was assessed independently.

Experiment 3—To explore the functional mechanism by which footshock exposure induces reduction of social investigation in male rats, Exp. 3 manipulated central IL-1 pathway by using icv infusion of IL-1Ra before and following footshock session. The experiment was conducted for 4 consecutive days; 2 days for pre-exposure (habituation) and 2 days for testing. On Day 1 and 2, the subjects were moved from colony room to testing room in their home cages and introduced into the adjacent test chamber for 30 min without stimulus animals in the paired chamber as a habituation, and thereafter were returned to their home cages. On day 3, all subjects were given icv injection of saline or IL-1Ra at both 30 min before footshock exposure and behavioral testing, and then returned to their home cages. Rats in footshock group were exposed to 80 intermittent shocks over the course of approximately 2 hr and then returned to their home cages, while rats in home cage group were remained in their home cages during that time. At four and twenty four hour following the onset of footshock exposure, all animals were moved to testing room and placed into the test chamber against their pair-tested rats for 30 min. Thereafter, animals were returned to their home cages. Between trials, the apparatus was cleaned by paper towels. The behaviors of each animal were video-recorded and assessed after completion of the experiment by an observer blind to treatment.

Statistic analysis

For Experiment 1 and 2, a two-way analysis of variance (ANOVA) with repeated measure (time course; 2, 6, or 24 hr for Exp. 1 and 0, 2, 6, or 24 hr for Exp.2) and between-subject factor of manipulation (for Exp. 1; saline or LPS, and control or footshock, for Exp. 2; control, footshock, or LPS) was used. For Experiment 3, three-way ANOVAs with between-subject factor of drug (saline or IL1Ra) and footshock (control or footshock) and within-subject factor of time course (4 or 24 hr) were conducted. Post hoc comparisons used the Bonferroni test for repeated factors and the Tukey's HSD test for between-subject factors. A probability level of $p < .05$ was adopted as the level of statistical significance for all analyses.

Results

Experiment 1-1

Fig. 1a presents the mean duration of time for each group that rats spent active investigation, anogenital sniffing, to a juvenile stimulus animal in their home cage during a 5-min test. Administration of LPS to the adult subjects led to a robust suppression of social interaction directed at the juvenile conspecific that was evident 2, 4, and 6 hr after injection but not at 24 hr after injection, although saline-injected rats showed stable high investigation to a juvenile male throughout the four time points of test session. This was confirmed by a ANOVA, which found main effect of injection, $F(1,10) = 9.063$, $p < .05$. The interaction between injection condition and time was also significant, $F(3,30) = 8.202$, $p < .001$. These data support the sensitivity of our social interaction test by replicating previously published findings.

Experiment 1-2

Fig 1b shows the mean duration of time that rats spent active investigation toward a juvenile stimulus rat in their home cage during a 5-min test session. Rats remained in their home cage (control) or received 2 hr footshock session (footshock) and returned to their home cages immediately after footshock termination. Social interaction with a juvenile conspecific was assessed at 2, 4, 6 and 24 hr after stressor termination. As predicted, footshock exposure significantly reduced social investigation of a juvenile conspecific on 4 hr through 6 hr after, but not 24 hr after footshock exposure; main effect of stress condition: $F(1,11) = 6.67$, $p < .05$. Although this effect followed a similar temporal pattern as observed following LPS administration, it can be noted that the influence of footshock on social interaction was somewhat more modest as evidenced by a marginal interaction between stress condition and time, $F(3,33) = 1.16$, n.s.

Experiment 2

Figure 2 shows the mean duration of time that animals engaged poking to social hole or non-social hole for each group through time course of following manipulation. Animals given LPS infusion revealed decreased social hole pokes after 2 and 6 hrs, while rats exposed to footshock showed significant decrease of social hole poking after 6 hr from the onset of manipulation, compared to nonstressed control group. Twenty four hr later, however, social hole poking had fully recovered to baseline levels. A two way ANOVA conducted on these scores found significant main effects of manipulation, $F(2,27) = 6.146$, $p < .01$, and time course, $F(3,81) = 20.50$, $p < .01$. The interaction between manipulation and time course was also found, $F(6,81) = 5.291$, $p < .01$. Subsequent analysis confirmed the above group differences.

The mean duration of time that animals poked at non-social hole showed a different, but stable pattern from social hole poking. A two way ANOVA conducted on these scores revealed significant main effects of time course, $F(3,81) = 5.483$, $p < .01$, but not of manipulation, $F(2,27) = 3.276$, n.s. The interaction between manipulation and time course was significant, $F(6,81) = 2.143$, $p < .05$. Subsequent analysis found that rats given LPS showed decreased poke duration during 2 hr and 24 hr testing after injection ($p < .05$), while rats received footshock did not display lower duration of poking through the experiment period, compared to homecage control group. Again, in social investigation paradigm, footshock-induced behavioral modification was more modest than those induced by LPS injection, as evidenced by modest decreased poking to social hole, and no significant effect on non-social poking following footshock exposure.

Experiment 3

Figure 3 depicts the mean duration of time that rats spent to poke at both non-social and social holes during 4 and 24 hr later testing period for each group. Four hour after the onset of footshock exposure, rats exposed to footshocks showed reduced investigation at social hole but not non-social hole, compared to control rats remained in their homecages. This reduction of social investigation at the social hole following exposure to footshock was prevented by IL-1Ra infusion. A three-way ANOVA conducted on the scores of social hole investigation found significant main effect of footshock, $F(1,25) = 11.897, p < .01$, but not of drugs, $F(1,25) = 1.390$, n.s. or time course, $F(1,25) = 1.214$, n.s. The interaction between footshock and drugs was significant, $F(1,25) = 6.937, p < .05$, and the footshock $\times$ time course interaction was also significant, $F(1,25) = 3.924, p < .05$, but the interaction between drug and time course was not significant, $F(1,25) = 0.61$, n.s. The footshock $\times$ drug $\times$ time course interaction was not significant, $F(1,25) = 1.616$, n.s. Subsequent post hoc analysis revealed that poking duration at social hole in rats given saline administration was lower in footshock animals than control animals ($p < .01$), while that in rats given IL1Ra infusion was similar in shocked animals with control animals. At the 4 hr after footshock session, footshock animals showed lower duration of social poking than homecage controls ($p < .001$), while at the 24 hr after footshock session, these differences was disappeared.

Neither footshock exposure nor IL-1Ra administration had any significant effect on poking duration at non-social hole. A three-way ANOVA revealed a significant main effect of time course, $F(1,25) = 10.413, p < .01$, but not of footshock, $F(1,25) = 0.007$, n.s., or drugs, $F(1,25) = 0.028$, n.s. All the interaction was not significant; footshock $\times$ drug, $F(1,25) = 3.118$, footshock $\times$ time course, $F(1,25) = 0.047$, drug $\times$ time course, $F(1,25) = 0.125$, and footshock $\times$ drug $\times$ time course, $F(1,25) = 1.094$. These indicated the specificity of the effect of footshock exposure on investigatory behavior to social hole, as expressed by no condition differences in non-social poking.

Discussion

The time course of acute sickness behavior induced by properties of cytokines has been examined in locomotor activity, social activities and food intake that develop in cytokine-treated animals [14-15]. Adult animals presented with juveniles of the same species spontaneously investigate these social stimuli using mainly olfactory cues [72]. This form of social behavior keeps being expressed at a high level as long as unfamiliar juveniles are presented or inter-trial-interval is kept for more than 2 hr [1,68]. Systemic administration of LPS, IL-1 β and TNF- α to adult rats decreases the duration of social investigation in this paradigm [74]. These effects appear slowly since any behavioral changes do not develop before 1-3 h after injection. They last for 4-6 h and usually dissipate by 24 h [53].

Experiment 1 demonstrated temporal pattern of social investigation in male rats toward a juvenile rat following LPS injection, which is consistent with the previous findings; rats given LPS infusion showed clear reduction of social investigation from 2 hr to 6 hr after injection, while control rats maintained high level of investigation throughout 4 time points of test session. Interestingly, footshock treated rats revealed a similar, but modest, temporal pattern in the social investigation toward a juvenile rat as observed following LPS administration. Though the parallels between footshock exposure and the consequences of LPS administration were remarkably similar across experiments, one must interpret these data with caution because the time course and magnitude of LPS effects on social behavior are highly dependent on dose. In this regard, we do not intend to equate the consequences of footshock exposure and LPS administration. The goal, instead, was to provide converging lines of evidence that changes in social interaction produced by stressor exposure may be sickness-like in nature, and that common neural mechanisms (expression of IL-1 β in brain) may be operating in both conditions.

In social interaction test as a standard test for assessing the magnitude of sickness behavior, adult animals are allowed to investigate a juvenile male directly [8,28]. This behavior is robust and highly motivated in rodents [30], the magnitude of which depends on the age, sex, and reproductive condition of the stimulus animals [28,72]. Thus, in Experiment 2, direct comparison of social investigation in the social investigation paradigm between rats given LPS and those received footshock stress session was performed. The apparatus used here in which animals were allowed to sniff the compartments where the stimulus animals were placed is considered to dissociate the impact of consequence of social interaction with the pure social motivation [21,51]. LPS treated rats showed a decrease in social investigation from 2 hr through 6 hr after the injection, while shocked rats displayed reduced social investigation at 6 hr after the shock session. LPS-treated, but not footshock-treated, rats showed a tendency to decrease non-social investigation throughout 24 hr after the session (during 2 hr and 24 hr time point after infection), as compared to home cage controls. The present results in the social investigation paradigm, therefore, suggest that LPS and footshock exposure exert similar yet distinct profiles of behavioral change in this paradigm. While the similarities in behavioral outcomes may reflect the fact that both footshock and LPS injection are both stressors, albeit different in nature, that activate some common physiological responses (brain cytokine expression, HPA axis activation, etc), differences in behavioral outcomes between the two heterotypic manipulations are largely to be expected. For instance, LPS administration produces a much broader profile of inflammatory changes in brain with respect to shock exposure, which increased predominantly IL-1 gene expression [50]. Indeed, our data support the extant literature inasmuch as IL-1Ra has been shown to be more effective in blocking the ability of IL-1 to induce sickness behavior than to completely reverse the effects of systemic LPS [though see 37].

The temporal pattern of brain IL-1 β expression in rats injected intraperitoneally with LPS is mediated by vagal afferents [8]. Immunoreactive *c-Fos* expression as a marker of the cellular activation occurs during the first 2–4 h after LPS injection involves perivascular phagocytic cells in the circumventricular organs and choroid plexus [37]. IL-1 β expression in these structures is accompanied by expression of *Fos* protein in the projection areas of the vagus nerves, including the nucleus tractus solitarius, medial preoptic area, supraoptic nucleus, central amygdala, and paraventricular nucleus of hypothalamus (PVN). From there, the IL-1 β immunoreactive signal gradually spreads into the brain where it recruits adjacent microglial cells [58–59]. Section of the vagus nerve attenuated the decrease in social exploration [8,10], fever response [71], and the induction of IL-1 β mRNA in the mouse in response to i.p. infusion of LPS [43]. The intermittent footshock also induces temporal pattern of IL-1 β expression in rat brain [18] that also involves noradrenergic input [4].

Experiment 3 demonstrated a critical contribution of IL-1 β in reduction of social investigation induced by footshock exposure. In the same behavioral paradigm with Exp. 2, male rats were exposed to social investigation chamber and allowed to investigate both social and non-social holes. Compared to saline-injected rats, shocked animals showed clear reduction in investigation to social holes 4 hr but not 24 hr following footshock session, while rats given icv infusion of IL-1Ra did not show such reduction of investigation following footshock session. Interestingly, IL-1Ra infusion per se did not produce any behavioral modification in the social investigation chamber, indicating that induction of IL-1 β in the brain following footshock session may be responsible for reduction of social investigation with footshock exposure. IL-1Ra specifically binds to the type I IL-1 receptor with an affinity similar to that of IL-1 β [23]. Most biological effects of IL-1 β are mediated by the type I IL-1 receptor [12]. This indicates that reduction of social investigation following footshock exposure may be mediated by IL-1 β expression in the brain, which is consistent with the previous findings showing that both central and peripheral administration of IL-1 β induces reduction of social investigation, which is blocked by central administration of IL-1Ra [6,7]. Conversely,

peripheral administration of LPS enhances the expression of IL-1 β in the brain [29,42], and reduces social investigation, which failed to be blocked by central administration of IL-1Ra [7,36]. One exception which was reported recently [38] demonstrated that icv infusion of IL-1Ra 4hr after ip LPS administration attenuated depressive effect on social investigation. This indicates that the timing of IL-1Ra administration may be critical for LPS induced inflammatory cascades on sickness behavior, as peripheral LPS administration results in increased plasma and central levels of multiple proinflammatory cytokines in a time-dependent manner [74]. Additionally, the differences between LPS- and footshock-induced behavioral depression suggest that LPS induced reduction of social motivation may be mediated by several types of proinflammatory cytokines which are activated following peripheral LPS administration, while footshock induced inhibition of social investigation may be specifically mediated by the induction of IL-1 β in the brain.

One alternative interpretation of these data is that the reduction in social interaction produced by footshock and/or LPS administration may reflect cytokine-mediated anxiety [eg. 65]. Our data do not directly address this question, but it should be noted that use of the social interaction test is considered to be a viable test of anxiety predominantly when subjects are tested in an unfamiliar (i.e., anxiogenic) environment [27,70]. In the present experiments, all subjects were well-acclimated to the test apparatus prior to manipulation as a method to ensure that the observed changes would indicate alterations in social processes rather than anxiety. Future studies will be necessary to clarify this distinction, but these are beyond the scope of what we sought to accomplish in the present work.

Increased expression of IL-1 β has been observed in the hypothalamus after certain stressor such as tailshock [47-48], footshock [4,18], and immobilization [63], but not other stressor including maternal separation [32], predator exposure [54], or forced swim [18]. From an evolutionary perspective, we have argued that increased cytokine expression in brain occurs only after stressors that pass a certain threshold of stress intensity, and provides a mechanism by which periods of intense arousal (incurred by stressor exposure) promote a period of recuperation [20]. Since activation of inflammatory signaling pathways in the brain are also associated with a diverse range of neurodegenerative diseases, such as stroke, Alzheimer's disease and Parkinson's disease [60,73] as well as psychological disorders such as depression [24], the demonstration that behavioral consequences of stressor exposure were ameliorated by administration of anti-inflammatory peptide may have very specific treatment implications.

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Figure 1.

A. Rats ($n=6$ per group) were injected i.p. with vehicle or 100 µg/kg LPS. At 2, 4, 6 and 24 hr after injection, a juvenile conspecific was placed into the cage, and the number of 5 sec intervals during which the adult test subject engaged in social investigation of the juvenile conspecific during a 5 min test was scored. LPS administration to the adult test subjects significantly reduced investigation of a juvenile rat at 2, 4 and 6 hr after injection. * indicates $p<.05$. B. Rats ($n=6-7$ per group) were exposed to footshock and returned to their home cages immediately afterwards. At 2, 4 and 6 hr after stressor termination, an unfamiliar juvenile conspecific was introduced into the test subject's home cage for the assessment of social interaction. The number of 5 sec intervals during which the adult test subject engaged in social investigation of the juvenile conspecific during a 5 min test was significantly suppressed at 4 and 6 hr after stressor termination. * indicates $p<.05$.

Fig. 2.

Time-course of the social investigation of rats given 2 hr footshock session (Footshock), or LPS (100 μ g/kg) injection, or remained in their home cage (Control). The duration (sec) of poking to the social hole (social holes) and the non-social hole (Non-social holes) during 30 min session was counted as the mean $\pm$ S.E.M in each time point, baseline and 2, 6, and 24 hr following treatment. Significant differences between treatment groups compared to control in each time point; * $p < .05$.

Fig. 3.

Impact of icv IL-1ra infusion on social investigation of rats given 2-hr footshock session. The duration (sec) of poking to the social hole (social hole) and the non-social hole (Non-social hole) during 30 min session at 4 and 24 hr following the end of footshock session was presented as the mean $\pm$ S.E.M. Significant differences between treatment groups ; * $p < .05$, ** $p < .01$.