Social Interaction Prevents the Development of Depressive-Like Behavior Post Nerve Injury in Mice: A Potential Role for Oxytocin

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Objective: To examine the salubrious role of social interaction in modulating the development of allodynia (increased sensitivity to typically innocuous physical stimuli) and depressive-like behavior post peripheral nerve injury in mice. The determination of potential mechanisms that mediate social influences on the behavioral and physiological response to peripheral nerve injury. **Methods:** Mice were pair housed or socially isolated for 2 weeks before spared nerve injury (SNI). Animals were cannulated; socially isolated animals were centrally treated with oxytocin; and socially paired animals were centrally treated with an oxytocin receptor antagonist. Animals were subsequently monitored for the development of mechanical allodynia and depressive-like behavior, and tissue was collected for analysis of the central levels of the cytokine interleukin 1 beta (IL-1β). **Results:** Depressive-like behavior was assessed via the Porsolt forced swim test, developed only among socially isolated mice with nerve injury. Socially isolated mice with nerve injury also were the only experimental group to exhibit increased frontal cortex IL-1β gene expression on day 7 post injury. Moreover, central treatment of socially isolated mice with oxytocin, a neuropeptide associated with social bonding, attenuated the effects of SNI on depressive-like behavior and reduced frontal cortex IL-1β protein levels in socially isolated animals. Conversely, pair-housed animals treated with a selective oxytocin receptor antagonist developed depressive-like behavior equivalent to that of socially isolated animals and displayed increased IL-1β protein levels within the frontal cortex. **Conclusion:** These data suggest that social interaction significantly alters the affective and neuroinflammatory responses to SNI through a mechanism that could involve oxytocin. **Key words:** social, oxytocin, neuropathic, depression, interleukin-β.

ELISA = enzyme-linked immunosorbent assay; **FST** = forced swim task; **ICV** = intracerebroventricular; **IL-1β** = interleukin-1β; **ISO** = isolated; **OTA** = oxytocin receptor antagonist; **PAG** = periaqueductal gray; **PFC** = prefrontal cortex; **POD** = postoperative day; **SNI** = spared nerve injury; **VEH** = vehicle.

INTRODUCTION

The etiology of chronic pain has been difficult to determine despite its high prevalence (1). Chronic pain is associated with decreased quality of life and a predisposition to several psychopathological conditions, including depression (2–4). When persistent pain results from a dysfunction, lesion, or injury of the nervous system, it is termed "neuropathic pain" and is considered one of the more disabling chronic pain conditions. Patients with neuropathic pain experience mechanical allodynia (pain in response to typically innocuous stimuli) and hyperalgesia (increased sensitivity to noxious stimuli); neuropathic pain and its associated symptoms are notoriously resistant to therapy and contribute to diminished quality of life (1,2). Recently, increased attention to the role of supraspinal structures associated with neuropathic pain has revealed diverse effects of chronic pain on limbic and cortical structures. Neuropathic pain is associated with amygdala hypertrophy, altered glial-neuronal interactions within the anterior cingulate, and neuroinflammatory responses within the prefrontal cortex (3–5). Similarly, neuropathic pain increases neurogenesis within the amygdala (6) and is associated with morphological and functional alterations within the prefrontal cortex (7). The impact of neuropathic pain on the structure and function of supraspinal structures provides a substrate through which chronic pain conditions can influence cognitive and affective processes. Persistent pain conditions are associated with an increased risk of mood disorders; >50% of the individuals with chronic pain conditions have symptoms of depression (8). One purpose of the current study is to determine whether the pathophysiology of peripheral nerve injury contributes to the development of depression.

Neuroinflammation is a possible link between neuropathic pain and depression. In addition to their role in immune signaling, proinflammatory cytokines are potent modulators of behavior and affect (9). Both exogenous and endogenous proinflammatory cytokines (e.g., interleukin 1 beta [IL-1β]) induce depressive-like behavior in nonhuman animals (10), and therapeutic administration of the proinflammatory cytokine interferon-α often elicits symptoms of depression in clinical patients (11). Elevated supraspinal levels of IL-1β are observed post neuropathic injury (5), and central administration of IL-1 receptor antagonist reduces the effects of neuropathic injury on depressive-like behavior (10). Together, these data suggest that increased central IL-1β signaling underlies the depressive-like responses that emerge post neuropathic injury in mice. Thus, factors that modulate IL-1β expression, in turn, have the potential to influence the development of depression after nerve injury.

Social environment is one of several factors that may modify the risk for developing neuropathic pain-induced depression. Social interaction alters neuroinflammatory responses to global and focal cerebral ischemia and cutaneous wound healing (12,13). However, despite several examples of social environment altering disease outcome via its effects on inflammatory processes, little is known regarding the physio-

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logical mechanisms of such action. One potential mediator is oxytocin, a nonapeptide that is synthesized in the hypothalamus and is released during social interaction (14,15). Oxytocin has been implicated in social recognition (16), approach, and avoidance behaviors (17). Oxytocin mediates the beneficial effects of social interaction on cutaneous wound healing in rodents (12) and prevents the behavioral and autonomic consequences of social isolation in voles (18). Additionally, oxytocin has both analgesic (19) and antidepressant properties (20), likely related to hypothalamic oxytocinergic projections to the amygdala, prefrontal cortex (PFC), and periaqueductal gray (PAG).

The goals of the present study were 1) to determine whether social environment influences the behavioral sequelae of nerve injury; and 2) to identify biological mechanisms that could underlie such effects. The primary hypothesis was that social isolation before the induction of neuropathic injury would decrease the nociceptive threshold and increase the development of depressive-like behaviors. Additionally, we hypothesized that oxytocin would mitigate the physiological and behavioral changes after spared nerve injury (SNI), potentially through modulation of central IL-1β.

MATERIALS AND METHODS

Adult male C57/BL6 mice (Charles River, Wilmington, Massachusetts), weighing 23 g to 30 g, were maintained on a 14:10 light/dark cycle within a temperature and humidity-controlled vivarium. Water and food were available ad libitum throughout the study. The mice were either individually housed or pair housed with a female that was ovariectomized at least 2 weeks before pairing. Ovariectomized females were used as partners, rather than males, because often unrelated male mice will fight and cause wounding, which would be a confounding factor in the current study. The study was conducted in accordance with National Institutes of Health guidelines for the care and use of animals and under protocols approved by The Ohio State University Institutional Animal Care and Use Committee.

Experimental Protocols

Study 1: Housing Effects on Mechanical Allodynia, Depressive-Like Behavior, and IL-1β Messenger ribonucleic acid (mRNA) Levels

To assess the effects of housing condition on SNI, 30 animals were assigned randomly to one of four main experimental groups: sham surgery on isolated animals (Sham-ISO; $n = 5$); sham surgery on pair-housed animals (Sham-Pair; $n = 5$); SNI on isolated animals (SNI-ISO; $n = 10$); or SNI on pair-housed animal (SNI-Pair; $n = 10$). Mice were either socially isolated or housed with their ovariectomized partner (paired) beginning 2 weeks before SNI or sham surgery, and continuing through completion of the study. Baseline measures of mechanical allodynia were taken 1 day before surgery and reassessed on postsurgical days 1, 3, and 7. On postsurgical day 6, locomotor activity was assessed in an automated open-field chamber. The next day, von Frey testing was completed, and depressive-like behavior was assessed through the forced swim test (FST). Approximately 6 hours after the FST, animals were euthanized and tissue was collected for the quantification of central mRNA expression of the proinflammatory cytokines IL-1β, tumor necrosis factor (TNF)-α, and IL-6, and glial markers glial fibrillary acidic protein (GFAP) and CD11b (MAC-1) in both frontal cortex and PAG tissue samples. Serum samples were also collected for the determination of circulating IL-1β. Behavioral and physiological responses to peripheral nerve injury were chosen on the basis of previous research from our laboratory on the functional significant of central IL-1β signaling in the mediation of depressive-like behavior (10).

Study 2: Assessing a Potential Role of Central Oxytocin in Mediating the Effects of Social Housing on Mechanical Allodynia and Depressive-Like Behavior

To determine the potential functional significance of oxytocin, socially isolated mice were cannulated and treated daily with either the vehicle (VEH, 2 μL of artificial cerebral spinal fluid; $n = 8$), 0.1 μg of oxytocin ($n = 8$), or 1 μg of oxytocin ($n = 8$). Pair-housed mice were treated with 0.05 μg of the oxytocin receptor antagonist ($n = 9$) or VEH ($n = 8$). The doses for both oxytocin and the receptor antagonist were chosen based on previous research demonstrating their efficacy (12,21). The once daily infusions began 3 days before SNI and continued for 7 days post SNI. As in Study 1, baseline measures of mechanical allodynia were taken 1 day before surgery and reassessed on postsurgical days 1, 3, and 7. On postsurgical day 6, locomotor activity was assessed in an automated open-field chamber. The next day, at approximately 30 minutes after intracerebroventricular (ICV) injection, depressive-like behavior was assessed through the FST and mechanical allodynia was assessed with von Frey monofilaments. The mice were euthanized 6 hours later, and brain tissue was collected to verify cannula placement and to assess IL-1β protein levels in the frontal cortex. To confirm that the IL-1β mRNA concentrations of Study 1 were apparent at the protein levels, we chose to measure frontal cortex IL-1β protein levels in Study 2. To reduce animal use, Sham-SNI mice were not included in Study 2 because housing had no effect on allodynia or depressive-like behavior among this treatment group in Study 1, and because ICV treatment of Sham-SNI mice with IL-1 receptor antagonist does not affect depressive-like behavior in the FST (10).

Cannulation and Drug Administration

One week before SNI, the mice were anesthetized with 1% to 1.5% isofluorane in oxygen-enriched air and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, California). A sterile, stainless steel cannula (2.00 mm below the pedestal, Plastics One, Roanoke, VA) was positioned at +0.02 mm posterior and +0.95 mm lateral to bregma, and secured with glue. The oxytocin (Sigma Chemical Company, St. Louis, MO) and oxytocin receptor antagonist (desGly-NH_2-d$(CH_2)_5$[D-Tyr^2, Thr^4]OVT; provided by Dr. Maurice Manning of The University of Toledo) were dissolved in artificial cerebrospinal fluid. The once daily 2 μL injection of oxytocin, oxytocin receptor antagonist, and VEH was given between 7 AM and 8 AM over the course of 60 seconds. Accuracy of cannula placement was determined via cresyl violet staining on the final day of the study. Due to blockage, looseness, or surgical misplacement of the cannula, seven mice were excluded from the study.

SNI

The SNI and sham surgeries were performed on anesthetized mice (isofluorane), using sterile surgical techniques and a previously described procedure for inducing nerve injury in mice (22). The right hind limb was immobilized in a lateral position and slightly elevated. The three peripheral branches (sural, common peroneal, and tibial nerves) of the sciatic nerve were exposed. The tibial and common peroneal nerves were ligated, using a 6.0 silk suture and transected (1.5-mm sections were removed). The sural nerve was carefully preserved. The sham procedure consisted of the same surgery without ligation or transection of any nerves; instead, a 3-mm long thread of 6.0 silk was placed longitudinally at the level of the trifurcation.

Behavioral Testing

Behavioral testing was conducted during the dark phase of the daily light-dark cycle. The mice were habituated to the room for 15 minutes before testing. The individual who conducted and scored the behavioral tests was uninformed of experimental assignments, and all animals were tested, using the same apparatus under consistent environmental conditions. The apparatus was thoroughly cleaned with 70% ethanol between mice. The time points used in this study were based on previous work on neuropathic pain and behavior (10).

Assessing Allodynia

Baseline von Frey monofilament testing (Stoelting Co., Wood Dale, Illinois) took place 1 day before SNI or sham surgery. Subsequent testing took place on days 1, 3, and 7 post surgery. Testing procedures were conducted as previously described by Bourquin and colleagues (22). Assessment began with 8 mg of monofilament and was followed by increasingly firm monofilaments until a positive response was determined. A positive response was defined as a flexion response (paw withdrawal), occurring twice in ten applications of the respective filament being applied to the lateral side of both hind paws. After a positive response, the threshold (in milligrams) was noted, and no further monofilaments were applied. A significant decrease in threshold post surgery is interpreted as the development of allodynia.

Open Field

General activity and anxiety-like behavior were assessed during a 60-minute session in an open-field apparatus (40 cm × 40 cm × 37.5 cm), using Flex Field photobeam activity (San Diego Instruments, San Diego, California). The apparatus was enclosed in a sound attenuating chamber equipped with a ventilating fan. Data were analyzed to determine general locomotor activity and relative amount of activity occurring in the periphery versus the center of the apparatus (anxiety-like behavior).

Forced Swim Task (FST)

Mice were placed into an opaque cylinder tank (24 cm diameter, 53 cm height) filled to a depth of 30 cm with water maintained at 25°C to 27°C. The water was changed after each animal, and the tank was thoroughly cleaned. Swimming behavior was recorded for 5 minutes and scored for time spent actively swimming versus floating (no leg or tail movement contributing to forward movement). Quantification of float versus swim time was performed with Observer software (Version 5, Exeter Software, Setauket, New York). An increase in floating is interpreted as an increase in depressive-like behavior (23).

Serum Corticosterone Concentration

Trunk blood samples were collected at the time of euthanasia and placed on ice. Clots were removed and the samples were centrifuged at 6000 rpm for 30 minutes at 4°C; sera samples were collected and stored at −80°C until assayed. Corticosterone concentrations were determined by using an I^{125} corticosterone kit (MP Biomedical, Solon, Ohio). The standard curve was run in triplicate, and samples were run in duplicate.

Gene Expression

On postsurgical day 7, the mice were euthanized via rapid cervical dislocation and the PFC and PAG were rapidly dissected from the brain under ribonuclease-free conditions. The PFC was chosen because several studies have reported immunological, morphological, and functional changes in the cortex of rodents post nerve injury (5, 7, 10), whereas the PAG was chosen because it is an important structure in descending pain modulation (24). Total ribonucleic acid (RNA) was extracted from the tissue, using a homogenizer (Ultra-Turrax T8, IKA Works, Wilmington, North Carolina) and an RNeasy Mini Kit (Qiagen, Valencia, California). Extracted RNA was suspended in 30 μL of ribonuclease-free water, and RNA concentration was determined by a spectrophotometer (NanoDrop ND-1000, Wilmington, Delaware). Inventoried primer and probe (Applied Biosystems, Foster City, California) were utilized for the quantification of IL-1β, IL-6, TNF-α, GFAP and MAC-1 mRNA levels. A TaqMan 18S rRNA primer and probe set (labeled with VIC dye, Applied Biosystems, Foster City, California) was used as a control gene for relative quantification. Amplification was performed on an ABI 7000 Sequencing Detection System by using Taqman Universal polymerase chain reaction master mix. IL-6, TNF-α, MAC-1 and GFAP mRNA levels were not significantly different among experimental groups in Study 1 ($p > .05$) and, therefore, were not assessed in Study 2.

Protein Quantification

On postsurgical day 7, the mice were euthanized via rapid cervical dislocation, and tissue was collected. Samples from the frontal cortex were dissected from whole brain. The tissue was then homogenized in radioimmunoprecipitation assay buffer with protease inhibitors (Pierce, Rockford, Illinois). Brain tissue lysates and serum samples were diluted 1:10 and assayed by using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Carlsbad, California) according to the manufacturer's protocol. Once we determined that only one of our immune measures of interest was significantly different among groups (IL-1β), we switched from polymerase chain reaction (semiquantitative measure of gene expression) to ELISA (quantitative measure of protein) for Study 2. Due to the methodological constraints of the ELISA assay, there was only enough tissue homogenate to assess one protein, and IL-1β was chosen based on the mRNA data from Study 1.

Statistical Analysis

The data are expressed as mean ± standard error of the mean. Testing of statistical significance was performed, using analysis of variance (ANOVA). When a significant overall treatment effect was reported, post hoc analyses were conducted, using the Tukey test. Group differences were considered statistically significant at $p < .05$. In the case of the von Frey data, conditions of normality were not met, so the data were log transformed, which succeeded in normalizing the data. All von Frey data were analyzed, using repeated-measures ANOVA to assess effects of time and group. Corticosterone concentrations, FST, and open-field data were analyzed, using one-way ANOVA.

RESULTS

Study 1. Social Isolation Exacerbates Mechanical Allodynia and Depressive-Like Behavior

Housing condition (social isolation versus pair housing) was a significant determinant of mechanical allodynia only after nerve injury. Paw withdrawal thresholds were similar among all experimental groups at baseline ($F(1,26) = 0.75$, $p > .05$) and did not change significantly from baseline post Sham-SNI surgery ($F(3,27) = 0.33$, $p > .80$). As expected, repeated-measures ANOVA revealed that the two nerve injury groups exhibited a decrease in paw withdrawal threshold after SNI ($F(3,57) = 22.81$, $p < .01$) (Fig. 1*a*). The socially isolated mice that sustained nerve injury (SNI-ISO) displayed significantly reduced paw withdrawal thresholds compared with pair-housed neuropathic animals (SNI-Pair) as revealed by a significant housing × time interaction (Fig. 1*a*) ($F(3,78) = 14.21$, $p < .01$; post hoc = $p < .05$) with socially isolated animals displaying significantly increased levels of mechanical allodynia. Together, these data suggest that exposure to social isolation exacerbates SNI-induced allodynia.

Social isolation precipitated depressive-like behavior and neuroinflammation post nerve injury. The SNI-ISO group spent significantly more time floating during the FST than the sham and SNI-Pair groups ($F(1,26) = 29.62$, $p < .01$; post hoc = $p < .05$) (Fig. 1*b*), which is indicative of increased depressive-like behavior among the SNI-ISO group. There were no significant differences in behavior in the FST among the SNI-Pair, Sham-Pair, and Sham-ISO groups. Thus, pair housing prevents the development of depressive-like behavior after nerve injury. A similar pattern was apparent for IL-1β mRNA expression in the frontal cortex on postsurgical day 7; SNI-ISO mice had significantly greater PFC IL-1β mRNA than the two sham groups and the SNI-Pair group (Fig. 2*a*) ($F(1,26) = 9.74$, $p < .01$), which did not differ significantly from one another ($p > .05$). We (10) previously demonstrated that there is a causal relationship between central IL-1β expres-

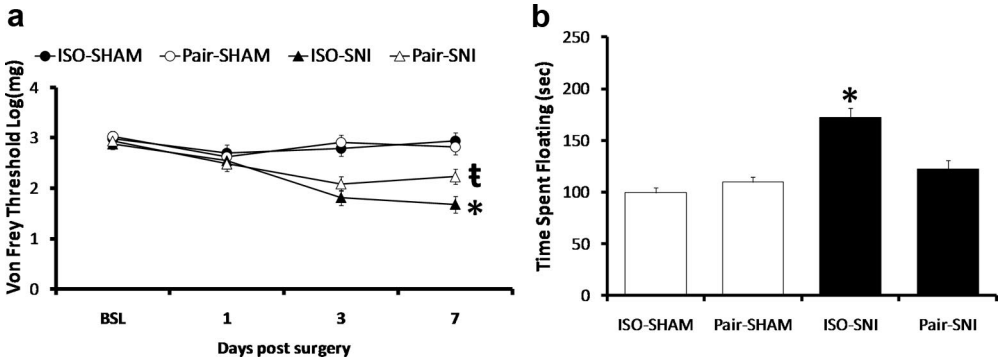

Figure 1. ***a***) Paw withdrawal thresholds were similar among all groups at baseline (*BSL*) and did not change significantly post sham surgery ($p > .05$). Spared nerve injury (*SNI*) significantly decreased paw withdrawal threshold by day 3 and continued through 7 days post surgery. Chronic social isolation further decreased paw withdrawal threshold on post-SNI day 7 relative to Pair-SNI and sham. ***b***) SNI significantly increases depressive-like behavior in the Porsolt forced swim test among socially isolated, but not socially paired, animals compared with sham animals at 7 days post surgery. Data are presented as mean ± standard error of the mean. * indicates significantly different from ISO-Sham, Pair-Sham, Pair-SNI ($p < .05$); ‡ indicates significantly different from ISO-Sham, Pair-Sham, ISO-SNI ($p < .05$).

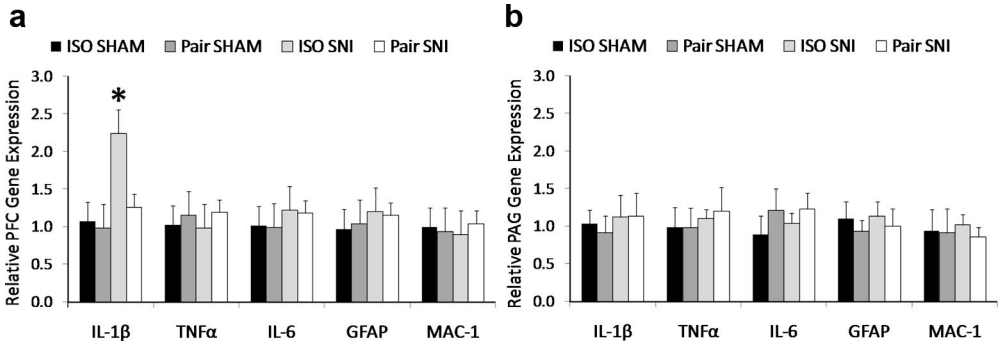

Figure 2. ***a***) Spared nerve injury (*SNI*) engenders an increase in frontal cortex interleukin 1 beta (IL-1β) messenger ribonucleic acid within socially isolated, but not socially paired, animals at 7 days post surgery. Social housing and nerve injury did not influence the expression of tumor necrosis factor (*TNF*)-α, IL-6, glial fibrillary acidic protein (*GFAP*), MAC-1 within the frontal cortex. ***b***) Neither housing conditions nor nerve injury influenced the mRNA expression of IL-1β, TNF-α, IL-6, GFAP, or MAC-1 with the periaqueductal gray (*PAG*). Data were collected 7 days post surgery. Data are presented as mean ± standard error of the mean. PFC, prefrontal cortex. * indicates significantly different from ISO-Sham, Pair-Sham, Pair-SNI ($p < .05$).

sion after nerve injury and the development of depressive-like behavior. Neither peripheral nerve injury nor housing conditions alter serum IL-1β levels ($F(1,26) = 0.82$, $p > .05$) (Supplemental Digital Content 1, http://links.lww.com/PSYMED/A10), or central mRNA expression of TNF-α, IL-6, MAC-1, and GFAP in the PFC or PAG ($p > .05$) (Fig. 2). Similarly, IL-1β mRNA levels within the PAG were similar in all groups ($p > .05$) (Fig. 2*b*). The current data suggest that social environment may modulate the development of depressive-like behavior through its effects on IL-1β expression after SNI.

All four experimental groups exhibited similar levels of general locomotor activity ($F(1,26) = 0.14$, $p > .05$) and anxiety-like behavior in an open-field apparatus ($F(1,26) = 0.64$, $p > .05$) (Supplemental Digital Content 2, http://links.lww.com/PSYMED/A11). On postoperative day 7, all four experimental groups exhibited similar serum corticosterone concentrations ($F(1,26) = 1.31$, $p > .05$; ISO-Sham: 48.56 ± 4.88 ng/mL; Pair-Sham: 45.25 ± 4.57 ng/mL; ISO-SNI: 55.00 ± 4.57 ng/mL; Pair-SNI: 45.93 ± 4.88 ng/mL). Thus, it does not seem that locomotor deficits, increased anxiety, or altered corticosteroid exposure contributed to expression of depressive-like behavior after SNI in socially isolated mice.

Study 2a. Administration of Oxytocin to Socially Isolated Mice Prevents the Development of Depressive-Like Behavior After SNI

A 3 (0.1 μg, 1.0 μg oxytocin versus VEH) × 4 (baseline, postoperative day [POD]1, POD3, POD7) repeated-measures ANOVA revealed that daily oxytocin administration had no influence on mechanical allodynia ($F(6,63) = 1.02$, $p > .05$) (Fig. 3*a*). Daily central administration of 1 μg of oxytocin to socially isolated SNI mice attenuated the development of depressive-like behavior ($F(2,21) = 30.74$, $p < .001$; post hoc: $p < .05$) (Fig. 3*b*) as compared with socially isolated vehicle-treated and 0.1 μg of oxytocin-treated mice. Furthermore, PFC IL-1β protein concentrations were significantly reduced in socially isolated neuropathic animals treated with 1 μg of oxytocin compared with VEH and 0.1 μg of oxytocin-treated animals ($F(2,21) = 7.03$, $p < .05$; post hoc: $p < .05$) (Fig. 3*c*). Additionally, vehicle-treated socially isolated neuropathic animals displayed elevated prefrontal IL-1β levels compared with their vehicle-treated socially paired neuropathic counterparts ($F(1,14) = 4.73$, $p < .05$), confirming the mRNA data provided in Study 1. Treatment with 0.1 μg of

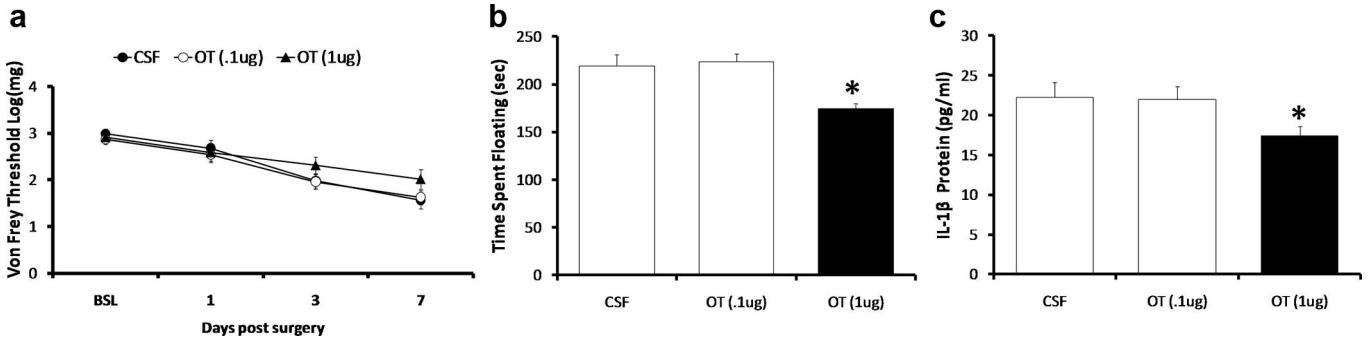

Figure 3. ***a***) Neither dose of oxytocin had a significant influence on paw withdrawal thresholds. Paw withdrawal thresholds were similar among all groups at baseline. ***b***) Daily administration of 1 μg of oxytocin decreased the depressive-like effect of social isolation on spared nerve injury although no differences were detected for the 0.1μg dose. ***c***) Treatment of socially isolated animals with 1 μg of oxytocin led to significant reductions in interleukin 1 beta (*IL*-1β) protein levels in the frontal cortex compared with vehicle-treated animals 7 days post surgery. Treatment with 0.1 μg of oxytocin had no apparent effect on IL-1β levels. Data are presented as mean ± standard error of the mean. * indicates significantly different from cerebrospinal fluid and oxytocin (0.1 μg, $p < .05$). *CSF* = cerebrospinal fluid; *OT* = oxytocin.

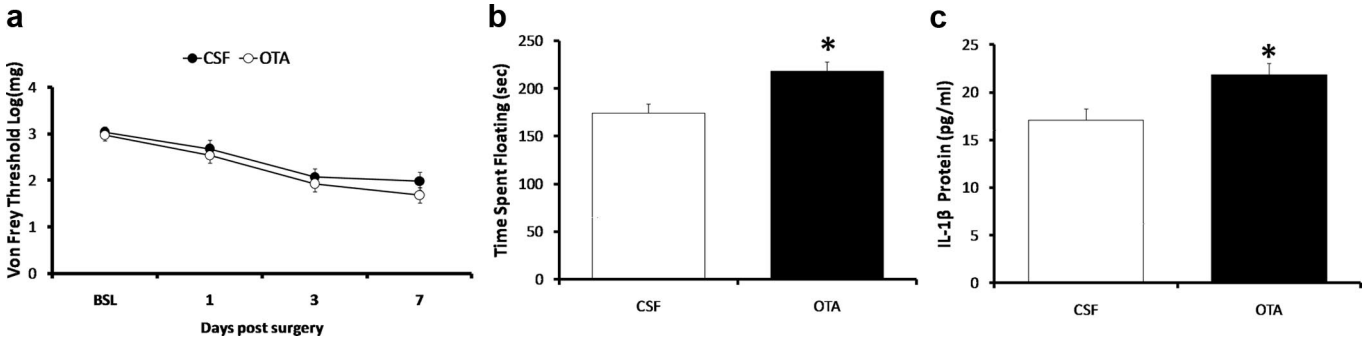

Figure 4. ***a***) Daily treatment of pair-housed spared nerve injury mice with 0.05 μg of oxytocin receptor antagonist had no influence on mechanical allodynia; ***b***) at 7 days post surgery, oxytocin receptor antagonist administration to pair-housed spared nerve injury mice led to significant increases in time spent floating during the Porsolt forced swim test, an indication of depressive-like behavior; and ***c***) significantly increased frontal cortex interleukin 1 beta (*IL*-1β) protein concentrations. Data are presented as mean ± standard error of the mean. * indicates significantly different from cerebrospinal fluid ($p < .05$). *CSF* = cerebrospinal fluid; *OTA* = oxytocin receptor antagonist.

oxytocin had no effects on von Frey threshold, depressive-like behavior, or IL-1β levels ($p > .05$), suggesting a dose-dependent response of oxytocin post SNI. Central administration of 1 μg of oxytocin had no effects on locomotor activity ($F(1,15) = 0.02$, $p > .05$) or anxiety-like behavior in the open field ($F(1,15) = 3.23$, $p > .05$) (Supplemental Digital Content 2, http://links.lww.com/PSYMED/A11).

Study 2b. Administration of an Oxytocin Receptor Antagonist (OTA) to Pair-housed Mice Allows the Development of Depressive-Like Behavior Post SNI

Daily central administration of an OTA (0.05 μg) to pair-housed SNI mice had no effect on mechanical allodynia ($F(3,45) = 0.99$, $p > .05$) (Fig. 4*a*) as determined by a 2 (OTA versus VEH) × 4 (baseline, POD1, POD3, POD7) repeated-measures ANOVA. However, OTA administration significantly increased depressive-like behavior ($F(1,15) = 7.36$, $p < .05$) (Fig. 4*b*) and IL-1β protein levels in the frontal cortex ($F(1,15) = 9.02$, $p < .01$) (Fig. 4*c*) relative to the vehicle-treated pair-housed SNI mice. OTA administration did not alter locomotor activity ($F(1,15) = 0.61$, $p > .05$) and had no effect on overall central tendency ($F1,15) = 0.42$, $p > .05$) (Supplemental Digital Content 2, http://links.lww.com/PSYMED/A11).

Together, Study 2 suggests that the administration of exogenous oxytocin protects socially isolated mice against the development of depressive-like behavior and PFC IL-1β expression after nerve injury. In contrast, the administration of OTA to pair-housed mice results in increased depressive-like behavior and PFC IL-1β expression. Interestingly, oxytocin administration decreased but did not eliminate allodynia after SNI in socially isolated mice, whereas OTA had no influence on allodynia after SNI among socially paired mice.

DISCUSSION

The current study suggests that social environment may be an important factor that influences whether depressive-like behavior develops after peripheral nerve injury. Socially isolated mice developed depressive-like behavior and increased IL-1β expression in the PFC after SNI, whereas pair-housed mice did not. Increased IL-1β neurotransmission has been implicated in the development of depressive-like behavior post peripheral nerve injury (10), suggesting that IL-1β may be part of the physiological mechanism underlying social influences on the development of depression after SNI. The upstream physiological link between social environment and depression-like behavior may be oxytocin, a hormone released

during affiliative social interaction (17). Administration of exogenous oxytocin to socially isolated animals prevented the increase in IL-1β protein expression and the development of depressive-like behavior after SNI, whereas administration of a highly selective OTA to pair-housed mice led to a significant increase in both IL-1β protein levels and depressive-like behavior after SNI. Together, these data provide the first evidence that social interaction modulates the development of depressive-like behavior after nerve injury and that the mechanism may involve oxytocin acting through an IL-1β mediated pathway.

As expected, the SNI procedure precipitated increases in mechanical allodynia beginning 3 days post surgery (Fig. 1*a*). Exposure to chronic social isolation exacerbated the SNI-induced increase in mechanical allodynia on day 7 post surgery (Fig. 1*a*). Furthermore, the present study replicated previous findings, demonstrating that nerve injury increases depressive-like behavior in mice (6,10) (Fig. 1*b*) and frontal cortex expression of IL-1β (5,10) (Fig. 1*c*). However, the development of depressive-like behavior and central IL-1β response is dependent on the social housing condition; socially isolated mice that underwent SNI spent significantly more time floating in the swim test compared with pair-housed mice that underwent SNI and both groups of sham-operated mice. Behavior in the swim test was indistinguishable for mice that were socially paired for at least 2 weeks before SNI and sham-operated mice in both housing conditions. Similarly, socially isolated, compared with socially housed, mice displayed a nearly 80% increase in IL-1β mRNA within the frontal cortex, representing an additional pathway through which social interaction modulates the neurobehavioral response to peripheral nerve injury. Thus, the paired group was protected from the elevations in cortical IL-1β mRNA and associated depressive-like behavior after nerve injury (Fig. 1, *b* and *c*). Also, housing did not influence depressive-like behavior in the absence of the nerve injury. There was no significant difference in time spent floating in the FST among socially isolated and pair-housed sham-operated mice. As previously reported (10), the measurement of depressive-like behavior in the current study was not confounded by SNI-induced alterations in locomotor behavior or anxiety because all experimental groups displayed comparable levels of general locomotor activity and anxiety-like behavior in an open field.

The data from Study 2 suggest that the physiological substrate through which the social environment influences the development of depressive-like behavior post SNI may be oxytocin. Paired and socially isolated mice were cannulated and treated daily with oxytocin, OTA, or artificial cerebrospinal fluid beginning 3 days before SNI and continuing through completion of the study. The cannulation and injection procedure did not obscure the relationship between social environment and the development of post-SNI depressive-like behavior; socially isolated mice treated with the vehicle spent significantly more time floating during the Porsolt swim test than pair-housed mice treated with the vehicle (Figs. 3*b* and 4*b*). Infusions of oxytocin (1 μg) into the lateral ventricle of socially isolated mice decreased depressive-like behavior relative to socially isolated mice treated with a lower dose of oxytocin (0.1 μg) or the vehicle. Furthermore, pair-housed SNI animals treated with OTA exhibited increased depressive-like behavior in the Porsolt swim test relative to pair-housed mice that had been treated with the vehicle (Fig. 3*b*). In this task, pair-housed mice treated with OTA were indistinguishable from vehicle-treated socially isolated mice. Thus, central blockade of oxytocinergic signaling eliminated the protective effect of pair housing against the development of depressive-like behavior after nerve injury. Together, these data suggest that social interaction may protect against the development of depressive-like behavior after nerve injury through a mechanism involving oxytocin.

The concomitant increase in depressive-like behavior (Fig. 1*b*) and prefrontal IL-1β gene expression (Fig. 1*c*), paired with the modulation of SNI-induced depressive-like behavior and frontal cortex IL-1β levels post treatment with oxytocin and OTA (Figs. 3*c* and 4*c*), supports the hypothesis that social interaction modulates SNI-induced depressive-like behavior via oxytocin-mediated reductions in the central IL-1β response. Numerous studies (25–27) have implicated inflammatory mediators, including IL-1β, in the pathophysiology of depression. Individuals suffering from medical conditions characterized by inflammation and increased central IL-1β production have significantly higher rates of major depression (9,28). Additionally, the risk of depression is elevated in individuals with specific polymorphisms in genes within the IL-1 family (29). Furthermore, we (10) have recently demonstrated a causal relationship between increased brain IL-1β expression and the development of depression post SNI in mice. Together, the findings that 1) the administration of oxytocin to socially isolated animals reproduces the effect of social pairing on SNI-induced depressive-like behavior and 2) that administration of the OTA to pair-housed animals reversed the effects of social interaction on SNI-induced depressive-like behavior suggesting that oxytocin mediates the salubrious effects of social interaction affective responses to peripheral nerve injury. Moreover, the finding that manipulation of oxytocinergic signaling modulates frontal cortex IL-1β levels suggests that the effects of the oxytocin manipulations on depressive-like behavior may be associated with IL-1β signaling. Apart from the data presented in this study, the anti-inflammatory properties of oxytocin have been well described (30,31). Thus, in conjunction with previous reports on the powerful effects of social interaction on health outcome, these data shed light onto the neuropeptide and neuroimmunological underpinnings that allow for the transduction of social information to physiological processes.

In contrast to the potent effects of social housing on depressive-like behavior, modifying oxytocin neurotransmission had no significant effect on allodynia after SNI. The limited effects of oxytocin on paw withdrawal after SNI were unexpected, given that several other studies (19,32) have demonstrated antinociceptive effects of oxytocin. One potential explanation for this discrepancy is the route of administration.

Whereas the present study utilized ICV injections, the majority of work on the influence of oxytocin on pain is focused at the level of the spinal cord and, as a result, utilizes intrathecal manipulations. Moreover, it is possible that social interaction influences pain and behavioral responses through independent mechanisms. Recent conceptualizations of pain differentiate between the sensory and emotional components, with the former dependent on processing in the sensory cortex and the latter a result of activity within limbic structures (33). Furthermore, preliminary evidence (34) suggests that oxytocin may act via vasopressin receptors to modulate allodynia. One potential explanation for the differential effects of oxytocin on depressive-like behavior and mechanical allodynia may have resulted from localization of injection site or dosage used. Regardless, the oxytocin manipulations presented in the current study were more efficacious in modulating the affective components of the pain response than the sensory components. It should be noted that the distinction between the affective and sensory components of the pain response has not been fully elucidated and future work is necessary to better describe the differences between these aspects of pain.

Although Study 1 demonstrates that social environment influences allodynia, depressive-like behavior, and frontal cortex IL-1β, additional studies will be necessary to more accurately examine social housing-dependent alterations in oxytocinergic signaling and their potential effects on peripheral nerve injury outcome. For example, future studies will need to address the precise neuroanatomical substrates through which social housing conditions may influence oxytocin signaling. Furthermore, it will be important to better understand the time course of the social housing effects on neuropathic injury outcome and whether such changes are related to oxytocin levels Also, because oxytocin was not administered to pair-housed mice with nerve injury in the present study, it is not known whether there are potential additive effects of exogenous oxytocin and social interaction, as has been reported in some clinical studies (35). However, recent data (18) suggest that central oxytocin administration has no effect on depressive-like behavior in healthy, pair-housed animals. The absence of a socially isolated group treated with the OTA precludes any statements regarding the importance of baseline endogenous oxytocin in modulating IL-1β expression and depressive-like behavior among socially isolated mice with nerve injury. However, the OTA used in this study has previously been shown to be highly selective for the oxytocin receptor (e.g., 95 times more potent as an OTA than as vasopressin antagonist (36)—a fact that supports the hypothesis that the pharmacological effects reported in this study are not due to interaction with other receptors (e.g., vasopressin). Therefore, the converging pharmacological evidence provided in the current study does suggest that oxytocin is a good candidate for further mechanistic exploration of the social influences on SNI outcome. A second important initiative will involve determining a site of action in the brain for both oxytocin and IL-1β after SNI. Previous work (10) from our laboratory established a causal relationship between central IL-1β and depressive-like behavior post SNI, and the current study revealed increased IL-1β data in the PFC, but future studies will require extensive mapping of IL-1β expression after SNI and site-specific microinjections of IL-1 receptor antagonist to determine which brain regions are involved in mediating the effects of IL-1β on depressive-like behavior. It will also be important to assess the long-term consequences of social housing conditions on depressive-like behavior post peripheral nerve injury and to include other pain measures, such as thermal hyperalgesia. Full characterization of the influences of social environment on SNI outcome and identification of underlying mechanisms may provide important new insights into the treatment and management of neuropathic pain.

Despite a large literature documenting the beneficial effects of social support on human health, little is known regarding underlying mechanisms. The current data compliment previous studies (13) suggesting that oxytocin may mediate the effects of social interactions on a wide range of health conditions modeled in rodents. Although a link has not been established conclusively between oxytocin and human health, there is a wealth of data suggesting that it modulates social processes in people. For example, oxytocin decreases amygdala activation to threatening stimuli, increases trust, promotes the encoding of positive social memories (37,38), facilitates positive communication between spouses (39), and interacts with social support to decrease physiological stress reactivity (35). Furthermore, specific human oxytocin receptor gene polymorphisms are associated with loneliness (40), adult separation anxiety disorder (41), and empathy (42). Thus, if oxytocin modulates social behavior in humans, the potential exists for it to influence health outcomes as well.

In sum, this study demonstrates that social interaction is an important determinant of the physiological and behavior responses to peripheral nerve injury. However, the physiological and behavioral trajectories after peripheral nerve injury were not fixed responses; increased expression of IL-1β and depressive-like behavior emerged only among socially isolated mice. Additionally, the current study revealed the potential role of oxytocin in mediating social influences on SNI-associated symptomology. Providing exogenous oxytocin counteracted the effect of social isolation on the development of depressive-like behavior and IL-1β response to nerve injury, whereas treatment with a selective OTA eliminated the protective effect of pair housing on SNI. Together, the data suggest that social factors influence the development of depressive-like behavior after nerve injury through possible alterations in oxytocin and neuroimmune signaling.

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