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Authors

Alvarez, Pedro
Green, Paul G
Levine, Jon D

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Neonatal Handling Produces Sex Hormone-Dependent Resilience to Stress-Induced Muscle Hyperalgesia in Rats

Pedro Alvarez[1], **Paul G. Green**[1,2], and **Jon D. Levine**[1,3,*]

[1]Department of Oral and Maxillofacial Surgery, University of California, San Francisco, USA; Division of Neuroscience, University of California, San Francisco, USA

[2]Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, USA

[3]Department of Medicine, University of California, San Francisco, USA

Abstract

Neonatal handling (NH) of male rat pups strongly attenuates stress response and stress-induced persistent muscle hyperalgesia in adults. Since female sex is a well-established risk factor for stress-induced chronic muscle pain, we explored whether NH provides resilience to stress-induced hyperalgesia in adult female rats. Rat pups underwent NH, or standard (control) care. Muscle mechanical nociceptive threshold was assessed before and after water avoidance (WA) stress, when they were adults. In contrast to males, NH produced only a modest protection against WA stress-induced muscle hyperalgesia in females. While gonadectomy completely abolished NH-induced resilience in male rats but produced only a small increase in this protective effect in females. The administration of the antiestrogen drug fulvestrant, plus gonadectomy, did not enhance the protective effect of NH in females. Finally, knockdown of the androgen receptor by intrathecal antisense treatment attenuated the protective effect of NH in intact males. Together, these data indicate that androgens play a key role in NH-induced resilience to WA stress-induced muscle hyperalgesia.

Perspective—NH induces androgen-dependent resilience to stress-induced muscle pain. Therefore, androgens may contribute to sex differences observed in chronic musculoskeletal pain and its enhancement by stress.

Keywords

Androgens; musculoskeletal pain; nociceptor; sex difference; stress-induced pain; water avoidance stress; hypothalamic-pituitary-adrenal axis

[*]Corresponding author. Address: University of California San Francisco, 533 Parnassus Ave., Room B10, San Francisco, CA 94143-0440, USA. Tel.: +1 415 476 5108; fax: +1 415 476 6305. jon.levine@ucsf.edu (J. D. Levine).

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Introduction

Chronic musculoskeletal pain, which is overrepresented amongst women, is a common, debilitating and costly condition, which currently lacks effective treatment.[58, 61, 66] Psychological stress is well established to contribute to the etiopathogenesis of chronic musculoskeletal pain[13, 54, 62], as it can both trigger and aggravate these pain syndromes.[24, 54, 62] In spite of the importance of these two factors, the mechanisms underlying the interaction between sex and stress in chronic musculoskeletal pain remains poorly understood.

The response of the hypothalamic-pituitary-adrenal (HPA) axis to stressful stimuli is tuned during a critical sensitive neonatal period.[31] During this developmental period, maternal care is crucial for the setting of life-long HPA responses to stressors.[30, 33] Experiments in rodents have shown that perturbations of maternal care (e.g., by separating dams from pups) or interference with maternal behavior (e.g., by limiting the availability of nesting/bedding material) produce life-long increased responsiveness to stressful stimuli.[4, 42] In contrast, interventions such as brief handling of neonatal rats, which increases maternal care, produces a protective effect, resilience to stress, in the adult rat.[17, 27, 40, 49, 50] This neonatal handling (NH) protocol increases the quantity and quality of maternal care, which translates into a life-long attenuation in the response of the HPA axis to stressful stimuli.[17, 27]

The water avoidance stress (WA) protocol, which is a well-established rodent model of stress-induced pain[3, 7, 10, 19, 28, 53, 69], consists of repeated exposure to a psychological stressor, isolation on a small platform surrounded by water.[7] Increased plasma levels of corticosterone are observed after WA[22], concomitant to increases in nociceptor responsiveness[10] and expression of ion channels typically involved in nociception such as TRPV1, $Na_V1.7$ and $Na_V1.8$ in dorsal root ganglion (DRG) neurons.22, 23, 69 Since *in vivo* and *in vitro* treatments with corticosterone reproduce these changes, in a glucocorticoid receptor-dependent manner[23, 69], persistent activation of the HPA axis likely plays an important role in WA-induced hyperalgesia. Interestingly, we have observed that in the adult male rat NH markedly attenuates muscle hyperalgesia induced by WA.[3]

Dysfunctional responsiveness of the HPA axis[18, 39, 46, 57, 67] and exposure to early-life adversity[11, 46, 64, 65] are commonly observed in women affected by musculoskeletal pain. Therefore, studying early-life interventions aimed to produce resilience could reveal mechanisms underlying the vulnerability to stress-induced chronic pain. We tested the hypothesis that NH provides resilience to stress-induced muscle hyperalgesia in the adult female rat. Contrary to our hypothesis, the NH-induced protective (resilient) phenotype to stress-induced muscle hyperalgesia exhibited marked sex differences (i.e., fully present in males but almost absent in females), indicating that androgens play a central role in resilience to stress.

Material and methods

Animals

Primiparous timed-pregnant female Sprague Dawley rats (Crl:CD) were obtained from Charles River (Hollister, CA). Dams were housed with their litter in standard cages on postnatal days 0–1. On day 2, litters were assigned to handled (see below) or standard care (control) conditions. Behavioral experiments in adults were performed on 250–400 g (age: 50–75 days) male and female rats from these litters. Animals were housed in the Laboratory Animal Resource Center of the University of California, San Francisco, under a 12-hours light/dark cycle (lights on 7 am) and environmentally controlled conditions; ambient room temperature was 21–23 °C, and food and water were available *ad libitum*. Upon completion of experiments, rats were euthanized by CO_2 induced asphyxia followed by bilateral thoracotomy. Animal care and use conformed to NIH guidelines (NIH Guide for the Care and Use of Laboratory Animals) and to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

Gonadectomy and drug treatment

Gonadectomy was performed on postnatal day 25–26, under general anesthesia (isoflurane 2.5%) and infiltration of the incision area with local anesthetics (0.25% bupivacaine, Marcaine®, Hospira, Lake Forest, IL); all animals received preoperative carprofen (Rimadyl®, Zoetis, Kalamazoo, MI). Briefly, ovaries were accessed by means of bilateral cutaneous and peritoneum incisions. Once located, ovaries and their vascular bundles were ligatured with 4-0 silk suture (Perma-Hand Silk® Ethicon, Johnson & Johnson, Somerville, NJ). Ovaries were then excised, and the peritoneal and cutaneous incisions closed with 5-0 silk suture (Perma-Hand Silk® Ethicon, Johnson & Johnson, Somerville, NJ). The testes were accessed by means of a single cutaneous incision made through the scrotum. Their vascular bundles were tied off with 4-0 silk suture, and the testes removed. The cutaneous incision was closed with 5-0 silk suture.

The selective estrogen receptor degrader (SERD) fulvestrant (ICI 182,780, Sigma-Aldrich, St. Louis, MO) was dissolved in DMSO (5%) and corn oil (95%), and administered by means of osmotic pumps (ALZET® mini-osmotic pump model 2004, delivering 0.25 μl/hr, 200 μl reservoir; DURECT Corporation, Cupertino, CA) at a rate of 0.1 mg/kg/day, as previously reported.[15] To ensure adequate fulvestrant levels throughout the experiments, rats were implanted on postnatal day 25–26 (at the time of ovariectomy) and again on postnatal day 52–53. Control rats were implanted with an inert implant. Nociceptive thresholds were measured beginning 2 weeks after the last implantation surgery.

Neonatal handling

NH experiments were performed by using a well established neonatal protocol that induces resilience to stress in the adult rat.[3, 30, 36, 38] This protocol involves removing the pups from the home cage, placing them in a separate container and carefully handling them for 15 min.

Litters were handled daily on postnatal days 2–9. On postnatal day 21, pups were weaned and same-sex housed, 3 per cage.

Water avoidance-induced stress

The WA protocol in the rat,[7] which produces mechanical hyperalgesia in the skeletal muscle[3, 10, 19] was used as a psychological chronic stressor. Briefly, adult rats (aged 7–12 weeks) were placed on an acrylic platform (8 × 8 cm, 10 cm high) in the center of a clear plastic tank (45 cm length × 25 cm width × 25 cm height) filled with room temperature tap water to a depth of 9 cm for 1 hr/day for 10 consecutive days. One day after the last stress exposure, rats were tested for mechanical hyperalgesia in the gastrocnemius muscle.

Antisense oligodeoxynucleotides

The androgen receptor (AR) is expressed in dorsal root ganglion (DRG) neurons.[25] To evaluate the role of androgens, acting at their receptor on nociceptors innervating the gastrocnemius muscle, the expression of the AR was attenuated by administration of antisense oligodeoxynucleotide (AS ODN) directed against a unique sequence of the respective encoding mRNA, in the rat: 5′-GTG CAA TCA TTT CTG-3′. The corresponding GenBank Accession Number and ODN position within the cDNA sequence are NM_012502.1 and 2733–2747, respectively in *Rattus norvegicus*. The mismatch (MM) ODN sequence, 5′-GT**A** CAA TC**C** TTT **G**TG-3′, corresponds to the AR antisense sequence with 3 bases mismatched (indicated in bold typeface). The AS and MM ODNs were synthesized by Invitrogen (Carlsbad, CA). The AS ODN sequence has been shown to produce significant knockdown of AR.[21] We have previously shown that the intrathecal administration of AS ODN is able to block protein expression in sensory neurons and modulate muscle hyperalgesia.[2, 20] The ODNs were reconstituted in sterile 0.9% NaCl (10 μg/μl), aliquoted and stored at −20°C until use. Before injections, ODN aliquots were diluted in sterile 0.9% NaCl to a final concentration of 2 μg/μl. Rats were briefly anesthetized with 2.5% isoflurane and a 29-gauge hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) inserted into the subarachnoid space between the L4 and L5 vertebrae and a dose of 40 μg (volume 20 μl) of ODN injected. Intrathecal location of injections was confirmed by observing a tail-flick, as previously reported.[41] This procedure was repeated daily for 3 consecutive days and then every other day, for a total of 8 ODN injections.

Muscle mechanical nociceptive threshold

Mechanical nociceptive threshold was quantified in the gastrocnemius muscle using a Chatillon® digital force transducer (model DFI2, Amtek Inc., Largo, FL). Rats were placed in cylindrical acrylic restrainers designed to provide adequate comfort and ventilation, allow extension of the hind leg from the cylinder, and minimize restraint stress. All rats were acclimatized to the testing procedure, and testing performed in parallel across groups. Rats were adapted to the restrainer for 1 hr prior to starting each study and for 30 min prior to experimental manipulations. To measure nociceptive threshold, a 6 mm-diameter probe attached to the force transducer applied to the gastrocnemius muscle, to deliver a compression force that increased with time. The nociceptive threshold was defined as the force, in milliNewtons (mN), at which the rat withdrew its hind leg from the stimulus.

Results from each rat correspond to the mean of 3 readings per gastrocnemius muscle, taken at 5 min intervals. Rats were assigned randomly to experimental groups and behavioral readings were taken blind to treatment.

Statistics

Group data are expressed as mean ± SEM of n distinct rats. Statistical comparisons were made by means of two- or three- way repeated measures analysis of variance (ANOVA), followed by Bonferroni's or independent samples *t*-tests, as appropriate. Statistical significance was set at $P < 0.05$. Statistical Package for the Social Sciences (SPSS) version 24 (International Business Machines Corporation, Armonk, NY) and Prism 6.0h (GraphPad Software, Inc., La Jolla, CA) were used to perform data analysis and graph plotting.

Results

Effect of neonatal handling in gonad-intact rats

Three-way repeated measures ANOVA allowed evaluating within-subjects effect of time (pre-WA or post-WA, $F_{1,20}= 644.516$, $P < 0.001$), between-subjects sex (male or female, $F_{1,20}= 529.630$, $P < 0.001$), and treatment (control or NH, $F_{1,20}=160.157$, $P < 0.001$) effects. This analysis showed a significant time × sex × treatment interaction ($F_{1,20}= 41.715$, $P < 0.001$), indicating that the effect of the NH on muscle nociceptive threshold observed over time depends on sex.

Post-hoc analysis showed no significant differences in nociceptive threshold between control and NH males at baseline ($P = 0.376$). However, consistent with our previous observations (Alvarez et al., 2015), control males did differ from NH males in nociceptive threshold after WA (n = 6/group, $P < 0.001$). Similarly, while control females did not differ from NH females at baseline ($P = 0.382$), control and NH females exhibited differences in nociceptive threshold after WA (n = 6/group, $P = 0.006$, Fig. 1A). However, the magnitude of mean difference (Control post-WA *vs* NH post-WA) was much greater for males (729.1) than it was for females (154.0), indicating a sex difference in the protective effect of NH in gonad-intact rats (Fig. 1A).

Effect of neonatal handling in gonadectomized rats

Our three-way repeated measures ANOVA allowed evaluation of within-subjects effect of time (pre-WA or post-WA, $F_{1,20}= 1339.822$, $P < 0.001$), between-subjects sex (male or female, $F_{1,20}= 7.201$, $P = 0.013$) and treatment (control or NH, $F_{1,20}=258.373$, $P < 0.001$) effects. This analysis showed a significant time × sex × treatment interaction ($F_{1,20}= 12.912$, $P = 0.001$), indicating that the effect of the NH on muscle nociceptive threshold observed over time depends on sex, in gonadectomized rats.

Post hoc analysis showed that, in gonadectomized males, the control group (n = 6) differed from the NH group (n = 9) at baseline ($P < 0.001$). After WA exposure, gonadectomized control and NH males also showed significant differences in nociceptive threshold ($P < 0.001$, Fig. 1B). In gonadectomized females, the control group (n = 6) differed from the NH

group (n = 9) at baseline ($P < 0.001$). After WA exposure, gonadectomized control and NH females also exhibited significant differences in nociceptive threshold ($P < 0.001$, Fig. 1B).

Effect of long-term fulvestrant

Given that the attenuation of WA-induced hyperalgesia by NH is enhanced by ovariectomy, and that extra-ovarian sources of estrogen[60] could contribute to reduce the protective effect of NH, we explored the effect of long-term administration of the anti-estrogen drug fulvestrant in gonadectomized NH females. Two-way repeated measures ANOVA showed a significant effect for time (pre-WA, post-WA, $F_{2,14}= 87.40$, $P < 0.0001$), but not for treatment (control or fulvestrant, $F_{1,7}= 0.406$, $P < 0.0001$), or time × treatment interaction ($F_{2,14}= 0.217$, $P < 0.0001$) on muscle nociceptive threshold, suggesting that compared to control implants (n=4), fulvestrant (n=5) did not enhance the effect of gonadectomy in NH female rats, measured 1 day after last exposure to WA stress ($P > 0.05$; Fig. 2).

Effect of androgen receptor antisense

Since orchiectomy abolished the effect of NH in males we sought to explore whether a protective effect of male sex hormones (presumably testosterone) is dependent on its effect at its cognate receptor on nociceptors. Therefore, we administered AS ODN directed against AR mRNA intrathecally, to attenuate the expression of the AR receptor in sensory neurons. Two-way repeated measures ANOVA revealed a significant effect for treatment (AS ODN or MM ODN, $F_{1,7}= 13.12$, $P = 0.0085$), time (baseline, post ODN×3, postWA (post ODN×8), $F_{2,14}= 47.06$, $P < 0.0001$) and treatment × time interaction ($F_{2,14}= 15.18$, $P = 0.0003$) in NH male rats 1 day after last exposure to WA stress. Bonferroni's post-hoc test showed that, while the AS ODN intervention (n=5) did not significantly change baseline nociceptive threshold compared to MM ODN (control) treatment (n=4, $P > 0.05$, Fig. 3), it significantly decreased the nociceptive threshold after exposure to WA stress (i.e., increased muscle hyperalgesia) compared to MM ODN treatment ($P < 0.05$; Fig. 3).

Discussion

Early postnatal life is a critical period in terms of setting neuroendocrine responsiveness to stress later in life. We have previously shown that short periods of handling during postnatal days 2–9 (NH), produces a protective effect against stress-induced muscle hyperalgesia in the adult male rat.[3] The fact that WA stress enhances corticosterone plasma levels[22] and corticosterone treatments produce *in vivo* and *in vitro* enhancement of nociception[23, 69], persistent activation of the HPA axis likely plays a role in WA-induced hyperalgesia. Therefore, the antinociceptive effect of NH observed here is likely due to the prevention of the neuroendocrine stress response. Given that stress and female sex are risk factors for the development of chronic musculoskeletal pain, we explored whether NH could also provide protection against stress-induced muscle pain in female rats. Our findings indicate that NH-induced protection exhibit sex differences, with females expressing less protection, and that expression of resilience is androgen-dependent.

1. Sex differences in NH protection against WA-induced muscle hyperalgesia

While NH fully prevented WA stress-induced muscle hyperalgesia in males, its protective effect was small, albeit statistically significant, in females. Sex differences of NH have been observed in other paradigms exploring its effect on behavioral and neuroendocrine responses to stress in adults. For example, in contrast to NH females, NH males exhibited shorter immobility time to chronic forced swimming stress suggesting enhanced capacity to cope with chronic stressors.[51] Furthermore, NH reduced restraint stress-induced increases in adrenocorticotropic hormone and corticosterone levels in adolescent male, but not female rats.[52]

Two mechanisms could be involved in the induction of sex differences observed in NH: first, evidence consistently shows that dams spend significantly more time caring for their male than female offspring[43, 44, 56], which could limit the effect of NH in females; second, sex hormones display important organizational effects on the developing HPA axis during early neonatal life.[37, 45, 48] Indeed, neonatal orchiectomy increases corticosterone secretion in response to stress, whereas hormone replacement in adults did not significantly reverse this change, compatible with the suggestion that the HPA axis is less responsive to testosterone in neonatally-gonadectomized males.[38] In this case, the induction of the protective effect of NH would depend on an appropriate sex hormone context during the critical period, namely the influence of androgens. In addition to these mechanisms, the expression of the NH phenotype is also likely due to ‘activational’ mechanisms, which depend on ongoing secretion of androgens (see below).

2. Reduction of the protective effect of NH by female sex hormones

In female rats, ovariectomy modestly enhanced NH-induced antihyperalgesia, suggesting that estrogens can attenuate the expression of the protective effect of NH. Estrogen is known to enhance HPA axis responsiveness, producing greater increased secretion of corticosterone and adrenocorticotrophin at baseline and in response to stress in females compared to males.[12, 26, 48] Furthermore, ovariectomized females have significantly lower basal and stress-induced corticosterone levels than gonad intact females.[59] These observations indicate that estrogen likely increases the responses of the HPA axis evoked by WA-induced stress, contributing to the reduced inhibitory effect of NH on WA-induced muscle pain. However, the administration of the antiestrogen fulvestrant, to gonadectomized females, did not further increase the protective effect of NH on WA-induced hyperalgesia. Thus, although estrogens partially counteract NH-induced antihyperalgesia, its absence does not uncover the NH protective effect observed in males, suggesting that it is not the presence of estrogens but the lack of androgens, which underlies the small protective effect of NH observed in females.

3. Androgens play a major role in expression of NH-induced resilience

Upon exposure to WA stress, gonadectomized NH males displayed muscle hyperalgesia of magnitude comparable to control (non-handled) WA-exposed males, supporting a major role for androgens in NH-induced resilience. Several mechanisms could be involved in the role of androgens in such resilience. While the effects of androgens may have been due to an organizational effect on the developing brain during the neonatal period[45], post pubertal orchiectomy eliminated the protective effect of NH, suggesting that ongoing secretion of

androgens is needed for the expression of NH-induced resilience to WA-induced muscle pain in the adult. Androgens may also act on the HPA axis allowing reduced stress responses to attenuate WA-induced hyperalgesia. Indeed, experiments in knockout mice show a role for AR in regulating anxiety-like behaviors and HPA axis responses.[9] Also, since ARs are expressed in sensory neurons,[25] androgens acting at this level may attenuate nociceptive inputs from the skeletal muscle nociceptors, contributing to the NH-induced phenotype. Indeed, masseter muscle inflammation-induced up-regulation of mu-opioid receptor (MOR) mRNA expression in the trigeminal ganglia is prevented by orchiectomy and restored by testosterone replacement.[68] Furthermore, TRPV1-positive trigeminal sensory neurons co-express AR, which transcriptionally regulates MOR expression in these neurons induced by inflammation.[29] These observations are consistent with our data from intrathecal antisense experiments, showing that the protective effect of NH is significantly attenuated after knockdown of the AR.

Finally, the contribution of androgens to NH-induced protection against stress-induced muscle hyperalgesia could also be due to an effect on neuroendocrine-immune responses triggered by stress. Indeed, testosterone inhibits macrophage activation and their production of pro-inflammatory cytokines,[14, 55] and induces the synthesis of the anti-inflammatory/antinociceptive cytokine IL-10.[14] Testosterone also modulates the immune response of lymphocytes, inhibiting the release of the pro-inflammatory cytokine interferon gamma and enhancing the release of anti-inflammatory IL-10.[5, 34] Importantly in this regard, there is a major contribution of pro-inflammatory cytokines to WA stress-induced hyperalgesia.[28, 47]

Conclusion

The resilience phenotype induced by NH in males is likely due to an interaction between decreased response of the HPA axis induced by enhanced maternal care in early-life and secretion of androgens. By acting on muscle nociceptors androgens contribute to the NH resilient phenotype to stress-induced muscle pain. In females, the resilience phenotype confers only a small protection to stress-induced muscle pain. Since sex hormones also play a role in neuropathic[8, 32, 63] and inflammatory[1, 6, 16, 35] pain, exploring the interaction between androgens and NH-induced resilience in preclinical models of these pain syndromes is an important future direction.

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References

1. Aloisi AM, Ceccarelli I, Fiorenzani P, De Padova AM, Massafra C. Testosterone affects formalin-induced responses differently in male and female rats. Neurosci Lett. 2004; 361:262–264. [PubMed: 15135943]
2. Alvarez P, Bogen O, Green PG, Levine JD. Nociceptor interleukin 10 receptor 1 is critical for muscle analgesia induced by repeated bouts of eccentric exercise in the rat. Pain. 2017; 158:1481–1488. [PubMed: 28628078]

3. Alvarez P, Levine JD, Green PG. Neonatal handling (resilience) attenuates water-avoidance stress induced enhancement of chronic mechanical hyperalgesia in the rat. Neurosci Lett. 2015; 591:207–211. [PubMed: 25637700]
4. Baram TZ, Davis EP, Obenaus A, Sandman CA, Small SL, Solodkin A, Stern H. Fragmentation and unpredictability of early-life experience in mental disorders. Am J Psychiatry. 2012; 169:907–915. [PubMed: 22885631]
5. Bebo BF, Schuster JC, Vandenbark AA, Offner H. Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. J Immunol. 1999; 162:35–40. [PubMed: 9886367]
6. Borzan J, Fuchs PN. Organizational and activational effects of testosterone on carrageenan-induced inflammatory pain and morphine analgesia. Neuroscience. 2006; 143:885–893. [PubMed: 17008018]
7. Bradesi S, Schwetz I, Ennes HS, Lamy CM, Ohning G, Fanselow M, Pothoulakis C, McRoberts JA, Mayer EA. Repeated exposure to water avoidance stress in rats: a new model for sustained visceral hyperalgesia. Am J Physiol Gastrointest Liver Physiol. 2005; 289:G42–53. [PubMed: 15746211]
8. Calabrese D, Giatti S, Romano S, Porretta-Serapiglia C, Bianchi R, Milanese M, Bonanno G, Caruso D, Viviani B, Gardoni F, Garcia-Segura LM, Melcangi RC. Diabetic neuropathic pain: a role for testosterone metabolites. J Endocrinol. 2014; 221:1–13. [PubMed: 24424289]
9. Chen CV, Brummet JL, Lonstein JS, Jordan CL, Breedlove SM. New knockout model confirms a role for androgen receptors in regulating anxiety-like behaviors and HPA response in mice. Horm Behav. 2014; 65:211–218. [PubMed: 24440052]
10. Chen X, Green PG, Levine JD. Stress enhances muscle nociceptor activity in the rat. Neuroscience. 2011; 185:166–173. [PubMed: 21513773]
11. Coppens E, Van Wambeke P, Morlion B, Weltens N, Giao Ly H, Tack J, Luyten P, Van Oudenhove L. Prevalence and impact of childhood adversities and post-traumatic stress disorder in women with fibromyalgia and chronic widespread pain. Eur J Pain. 2017; 21:1582–1590. [PubMed: 28543929]
12. Critchlow V, Liebelt RA, Bar-Sela M, Mountcastle W, Lipscomb HS. Sex difference in resting pituitary-adrenal function in the rat. Am J Physiol. 1963; 205:807–815. [PubMed: 4291060]
13. Crofford LJ. Psychological aspects of chronic musculoskeletal pain. Best Pract Res Clin Rheumatol. 2015; 29:147–155. [PubMed: 26267008]
14. D'Agostino P, Milano S, Barbera C, Di Bella G, La Rosa M, Ferlazzo V, Farruggio R, Miceli DM, Miele M, Castagnetta L, Cillari E. Sex hormones modulate inflammatory mediators produced by macrophages. Ann N Y Acad Sci. 1999; 876:426–429. [PubMed: 10415638]
15. El Hajj MC, Ninh VK, El Hajj EC, Bradley JM, Gardner JD. Estrogen receptor antagonism exacerbates cardiac structural and functional remodeling in female rats. Am J Physiol Heart Circ Physiol. 2017; 312:H98–H105. [PubMed: 27769996]
16. Fanton LE, Macedo CG, Torres-Chávez KE, Fischer L, Tambeli CH. Activational action of testosterone on androgen receptors protects males preventing temporomandibular joint pain. Pharmacol Biochem Behav. 2017; 152:30–35. [PubMed: 27461546]
17. Fenoglio KA, Chen Y, Baram TZ. Neuroplasticity of the hypothalamic-pituitary-adrenal axis early in life requires recurrent recruitment of stress-regulating brain regions. J Neurosci. 2006; 26:2434–2442. [PubMed: 16510721]
18. Fischer S, Doerr JM, Strahler J, Mewes R, Thieme K, Nater UM. Stress exacerbates pain in the everyday lives of women with fibromyalgia syndrome--The role of cortisol and alpha-amylase. Psychoneuroendocrinology. 2016; 63:68–77. [PubMed: 26431802]
19. Green PG, Alvarez P, Gear RW, Mendoza D, Levine JD. Further validation of a model of fibromyalgia syndrome in the rat. J Pain. 2011; 12:811–818. [PubMed: 21481648]
20. Green PG, Chen X, Alvarez P, Ferrari LF, Levine JD. Early-life stress produces muscle hyperalgesia and nociceptor sensitization in the adult rat. Pain. 2011; 152:2549–2556. [PubMed: 21864980]
21. Hamy F, Brondani V, Spoerri R, Rigo S, Stamm C, Klimkait T. Specific block of androgen receptor activity by antisense oligonucleotides. Prostate Cancer Prostatic Dis. 2003; 6:27–33. [PubMed: 12664061]

22. Hong S, Fan J, Kemmerer ES, Evans S, Li Y, Wiley JW. Reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in the water avoidance stressed rat. Gut. 2009; 58:202–210. [PubMed: 18936104]
23. Hong S, Zheng G, Wu X, Snider NT, Owyang C, Wiley JW. Corticosterone mediates reciprocal changes in CB 1 and TRPV1 receptors in primary sensory neurons in the chronically stressed rat. Gastroenterology. 2011; 140:627–637.e4. [PubMed: 21070780]
24. Hurwitz EL, Morgenstern H, Yu F. Cross-sectional and longitudinal associations of low-back pain and related disability with psychological distress among patients enrolled in the UCLA Low-Back Pain Study. J Clin Epidemiol. 2003; 56:463–471. [PubMed: 12812821]
25. Keast JR, Gleeson RJ. Androgen receptor immunoreactivity is present in primary sensory neurons of male rats. Neuroreport. 1998; 9:4137–4140. [PubMed: 9926862]
26. KITAY JI. Sex differences in adrenal cortical secretion in the rat. Endocrinology. 1961; 68:818–824. [PubMed: 13756461]
27. Korosi A, Shanabrough M, McClelland S, Liu ZW, Borok E, Gao XB, Horvath TL, Baram TZ. Early-life experience reduces excitation to stress-responsive hypothalamic neurons and reprograms the expression of corticotropin-releasing hormone. J Neurosci. 2010; 30:703–713. [PubMed: 20071535]
28. Lee JY, Kim N, Kim YS, Nam RH, Ham MH, Lee HS, Jo W, Shim Y, Choi YJ, Yoon H, Shin CM, Lee DH. Repeated Water Avoidance Stress Alters Mucosal Mast Cell Counts, Interleukin-1β Levels with Sex Differences in the Distal Colon of Wistar Rats. J Neurogastroenterol Motil. 2016; 22:694–704. [PubMed: 27466288]
29. Lee KS, Zhang Y, Asgar J, Auh QS, Chung MK, Ro JY. Androgen receptor transcriptionally regulates μ-opioid receptor expression in rat trigeminal ganglia. Neuroscience. 2016; 331:52–61. [PubMed: 27320211]
30. LEVINE S. Infantile experience and resistance to physiological stress. Science. 1957; 126:405.
31. LEVINE S, LEWIS GW. Critical period for effects of infantile experience on maturation of stress response. Science. 1959; 129:42–43. [PubMed: 13615320]
32. Lin SM, Tsao CM, Tsai SK, Mok MS. Influence of testosterone on autotomy in castrated male rats. Life Sci. 2002; 70:2335–2340. [PubMed: 12150198]
33. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science. 1997; 277:1659–1662. [PubMed: 9287218]
34. Liva SM, Voskuhl RR. Testosterone acts directly on CD4+ T lymphocytes to increase IL-10 production. J Immunol. 2001; 167:2060–2067. [PubMed: 11489988]
35. Macedo CG, Fanton LE, Fischer L, Tambeli CH. Coactivation of μ- and κ-Opioid Receptors May Mediate the Protective Effect of Testosterone on the Development of Temporomandibular Joint Nociception in Male Rats. J Oral Facial Pain Headache. 2016; 30:61–67. [PubMed: 26817034]
36. Macrì S, Würbel H. Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. Horm Behav. 2006; 50:667–680. [PubMed: 16890940]
37. McCormick CM, Furey BF, Child M, Sawyer MJ, Donohue SM. Neonatal sex hormones have ‘organizational’ effects on the hypothalamic-pituitary-adrenal axis of male rats. Brain Res Dev Brain Res. 1998; 105:295–307. [PubMed: 9541747]
38. McCormick CM, Kehoe P, Kovacs S. Corticosterone release in response to repeated, short episodes of neonatal isolation: evidence of sensitization. Int J Dev Neurosci. 1998; 16:175–185. [PubMed: 9785114]
39. McLean SA, Williams DA, Harris RE, Kop WJ, Groner KH, Ambrose K, Lyden AK, Gracely RH, Crofford LJ, Geisser ME, Sen A, Biswas P, Clauw DJ. Momentary relationship between cortisol secretion and symptoms in patients with fibromyalgia. Arthritis Rheum. 2005; 52:3660–3669. [PubMed: 16258904]
40. Meaney MJ, Mitchell JB, Aitken DH, Bhatnagar S, Bodnoff SR, Iny LJ, Sarrieau A. The effects of neonatal handling on the development of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. Psychoneuroendocrinology. 1991; 16:85–103. [PubMed: 1961847]

41. Mestre C, Pélissier T, Fialip J, Wilcox G, Eschalier A. A method to perform direct transcutaneous intrathecal injection in rats. J Pharmacol Toxicol Methods. 1994; 32:197–200. [PubMed: 7881133]
42. Molet J, Maras PM, Avishai-Eliner S, Baram TZ. Naturalistic rodent models of chronic early-life stress. Dev Psychobiol. 2014; 56:1675–1688. [PubMed: 24910169]
43. Moore CL, Morelli GA. Mother rats interact differently with male and female offspring. J Comp Physiol Psychol. 1979; 93:677–684. [PubMed: 479402]
44. Moore CL, Wong L, Daum MC, Leclair OU. Mother-infant interactions in two strains of rats: implications for dissociating mechanism and function of a maternal pattern. Dev Psychobiol. 1997; 30:301–312. [PubMed: 9142506]
45. Negri-Cesi P, Colciago A, Celotti F, Motta M. Sexual differentiation of the brain: role of testosterone and its active metabolites. J Endocrinol Invest. 2004; 27:120–127. [PubMed: 15481811]
46. Nicolson NA, Davis MC, Kruszewski D, Zautra AJ. Childhood maltreatment and diurnal cortisol patterns in women with chronic pain. Psychosom Med. 2010; 72:471–480. [PubMed: 20467005]
47. Nozu T, Miyagishi S, Nozu R, Takakusaki K, Okumura T. Repeated water avoidance stress induces visceral hypersensitivity: Role of interleukin-1, interleukin-6, and peripheral corticotropin-releasing factor. J Gastroenterol Hepatol. 2017; 32:1958–1965. [PubMed: 28299830]
48. Panagiotakopoulos L, Neigh GN. Development of the HPA axis: where and when do sex differences manifest. Front Neuroendocrinol. 2014; 35:285–302. [PubMed: 24631756]
49. Panagiotaropoulos T, Papaioannou A, Pondiki S, Prokopiou A, Stylianopoulou F, Gerozissis K. Effect of neonatal handling and sex on basal and chronic stress-induced corticosterone and leptin secretion. Neuroendocrinology. 2004; 79:109–118. [PubMed: 15004433]
50. Panagiotaropoulos T, Pondiki S, Papaioannou A, Alikaridis F, Stamatakis A, Gerozissis K, Stylianopoulou F. Neonatal handling and gender modulate brain monoamines and plasma corticosterone levels following repeated stressors in adulthood. Neuroendocrinology. 2004; 80:181–191. [PubMed: 15591794]
51. Papaioannou A, Gerozissis K, Prokopiou A, Bolaris S, Stylianopoulou F. Sex differences in the effects of neonatal handling on the animal's response to stress and the vulnerability for depressive behaviour. Behav Brain Res. 2002; 129:131–139. [PubMed: 11809504]
52. Park MK, Hoang TA, Belluzzi JD, Leslie FM. Gender specific effect of neonatal handling on stress reactivity of adolescent rats. J Neuroendocrinol. 2003; 15:289–295. [PubMed: 12588518]
53. Prusator DK, Greenwood-Van Meerveld B. Sex differences in stress-induced visceral hypersensitivity following early life adversity: a two hit model. Neurogastroenterol Motil. 2016; 28:1876–1889. [PubMed: 27385091]
54. Reme SE, Dennerlein JT, Hashimoto D, Sorensen G. Musculoskeletal pain and psychological distress in hospital patient care workers. J Occup Rehabil. 2012; 22:503–510. [PubMed: 22466375]
55. Rettew JA, Huet-Hudson YM, Marriott I. Testosterone reduces macrophage expression in the mouse of toll-like receptor 4, a trigger for inflammation and innate immunity. Biol Reprod. 2008; 78:432–437. [PubMed: 18003947]
56. Richmond G, Sachs BD. Maternal discrimination of pup sex in rats. Dev Psychobiol. 1984; 17:87–89. [PubMed: 6698313]
57. Riva R, Mork PJ, Westgaard RH, Lundberg U. Comparison of the cortisol awakening response in women with shoulder and neck pain and women with fibromyalgia. Psychoneuroendocrinology. 2012; 37:299–306. [PubMed: 21764519]
58. Rollman GB, Lautenbacher S. Sex differences in musculoskeletal pain. Clin J Pain. 2001; 17:20–24. [PubMed: 11289085]
59. Seale JV, Wood SA, Atkinson HC, Harbuz MS, Lightman SL. Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. J Neuroendocrinol. 2004; 16:989–998. [PubMed: 15667454]
60. Simpson ER. Sources of estrogen and their importance. J Steroid Biochem Mol Biol. 2003; 86:225–230. [PubMed: 14623515]

61. Sluka KA. Peripheral and central mechanisms of chronic musculoskeletal pain. Pain Manag. 2013; 3:103–107. [PubMed: 24504260]

62. Sluka KA, Clauw DJ. Neurobiology of fibromyalgia and chronic widespread pain. Neuroscience. 2016; 338:114–129. [PubMed: 27291641]

63. Stinson C, Deng M, Yee MB, Bellinger LL, Kinchington PR, Kramer PR. Sex differences underlying orofacial varicella zoster associated pain in rats. BMC Neurol. 2017; 17:95. [PubMed: 28514943]

64. Walker EA, Keegan D, Gardner G, Sullivan M, Bernstein D, Katon WJ. Psychosocial factors in fibromyalgia compared with rheumatoid arthritis: II. Sexual, physical, and emotional abuse and neglect. Psychosom Med. 1997; 59:572–577. [PubMed: 9407574]

65. Weissbecker I, Floyd A, Dedert E, Salmon P, Sephton S. Childhood trauma and diurnal cortisol disruption in fibromyalgia syndrome. Psychoneuroendocrinology. 2006; 31:312–324. [PubMed: 16274933]

66. Wijnhoven HA, de Vet HC, Picavet HS. Prevalence of musculoskeletal disorders is systematically higher in women than in men. Clin J Pain. 2006; 22:717–724. [PubMed: 16988568]

67. Wingenfeld K, Heim C, Schmidt I, Wagner D, Meinlschmidt G, Hellhammer DH. HPA axis reactivity and lymphocyte glucocorticoid sensitivity in fibromyalgia syndrome and chronic pelvic pain. Psychosom Med. 2008; 70:65–72. [PubMed: 18158367]

68. Zhang X, Zhang Y, Asgar J, Niu KY, Lee J, Lee KS, Schneider M, Ro JY. Sex differences in μ-opioid receptor expression in trigeminal ganglia under a myositis condition in rats. Eur J Pain. 2014; 18:151–161. [PubMed: 23801566]

69. Zheng G, Hong S, Hayes JM, Wiley JW. Chronic stress and peripheral pain: Evidence for distinct, region-specific changes in visceral and somatosensory pain regulatory pathways. Exp Neurol. 2015; 273:301–311. [PubMed: 26408049]

Highlights

- Neonatal handling (NH) markedly reduces stress-induced muscle pain in adult male rats
- NH has minimal effect on stress-induced muscle pain in adult females
- Gonadectomy blocks the NH effect on stress-induced pain in males
- Knockdown of the androgen receptor attenuates NH protection in gonad intact males
- Resilience to stress-induced muscle pain by NH is androgen-dependent

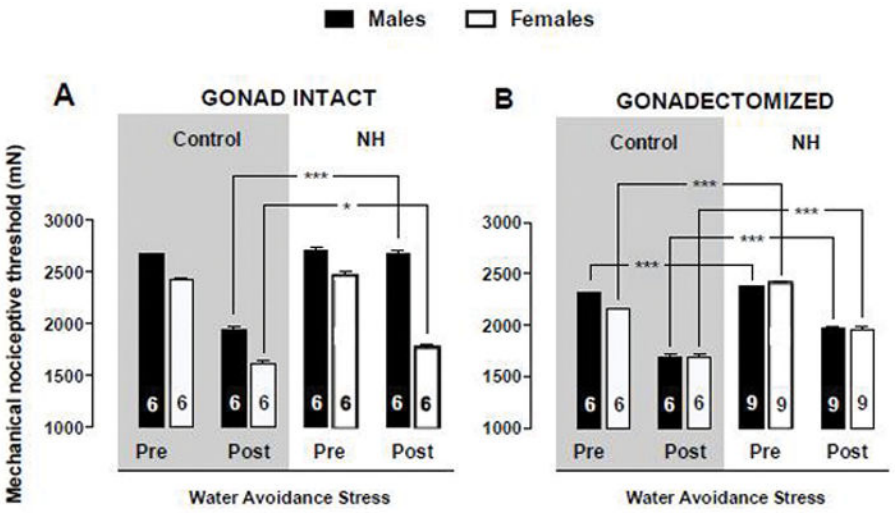

Figure 1. Sex differences in neonatal handling-induced protection against muscle hyperalgesia produced by water avoidance stress, in adult rats

Baseline muscle mechanical nociceptive thresholds were measured pre-exposure to water avoidance stress (**Pre**), in naïve (**Control**) and neonatally handled (**NH**) adult male (solid bars) and female (open bars) rats. Nociceptive thresholds were also obtained one day after the last exposure to water avoidance stress (**Post**). (**A**) Gonad intact rats: a greater protective effect of NH is observed in adult male, gonad intact rats; (**B**) Gonadectomy attenuates the protective effect of NH on water avoidance stress-induced muscle hyperalgesia, in adult rats. $*P < 0.05$; $***P < 0.001$. Numbers in each bar indicate sample size.

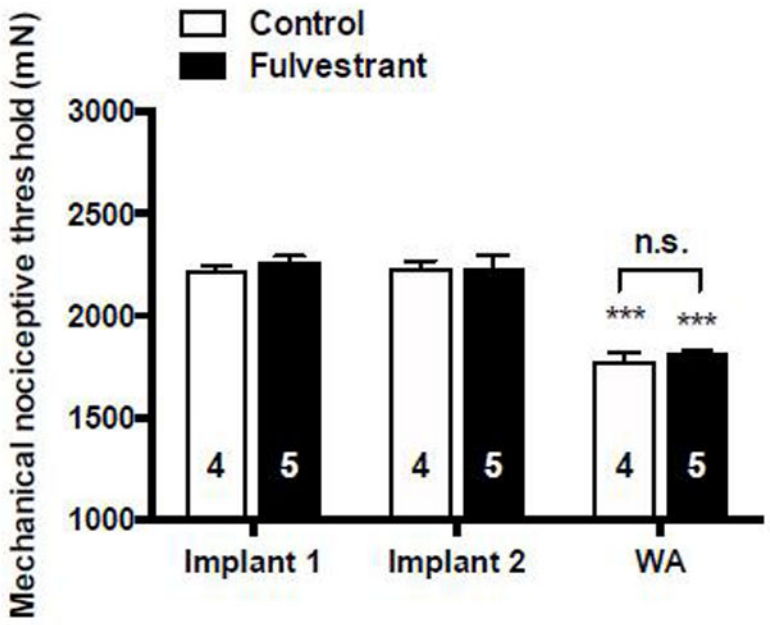

Figure 2. Systemic fulvestrant does not increase the protective effect of neonatal handling in gonadectomized female rats

Neonatally handled female rats gondectomized at postnatal days 24–25, receiving control treatment or fulvestrant, exhibited comparable muscle mechanical nociceptive thresholds 2 weeks after each implant of osmotic pumps delivering the respective treatments. After 10 days of exposure to water avoidance stress (**WA**) a significant decrease in nociceptive threshold was observed in both experimental groups. ***$P < 0.001$. Numbers in each bar indicate sample size.

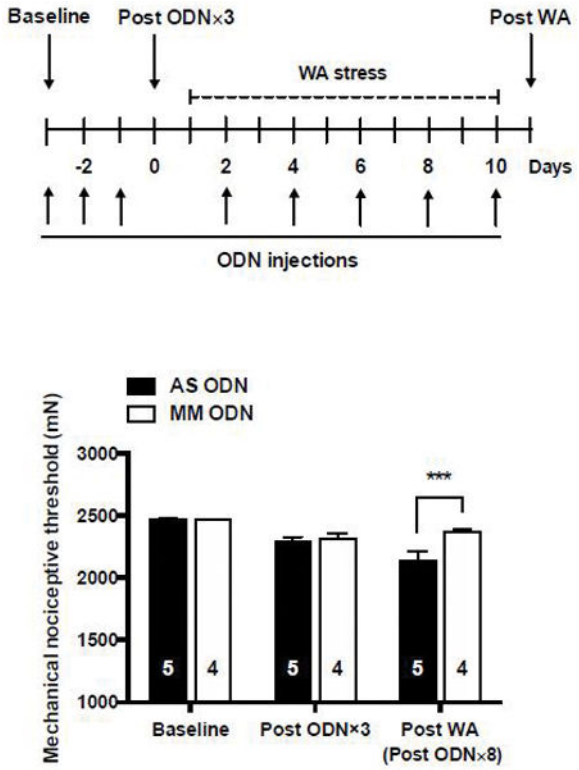

Figure 3. Knockdown of androgen receptor (AR) by intrathecal antisense attenuates the protective effect of neonatal handling in gonad intact adult male rats

Experimental groups did not exhibit differences in mechanical nociceptive threshold at baseline or after 3 intrathecal injections (**Post ODN×3**) of antisense (**AS**) or mismatch (**MM**) oligodeoxynucleotides (**ODN**). In contrast, the full AS treatment (**Post ODN×8**) attenuated the protective effect of neonatal handling on water avoidance stress (**WA**)-induced muscle hyperalgesia in adult male rats. ***$P < 0.001$. Numbers in each bar indicate sample size.