The Glt1 glutamate receptor mediates the establishment and perpetuation of chronic visceral pain in an animal model of stress-induced bladder hyperalgesia

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ACKERMAN AL, JELLISON FC, LEE UJ, BRADESI S, RODRIGUEZ LV. The Glt1 glutamate receptor mediates the establishment and perpetuation of chronic visceral pain in an animal model of stress-induced bladder hyperalgesia. Am J Physiol Renal Physiol 310: F628–F636, 2016. First published December 23, 2015; doi:10.1152/ajprenal.00297.2015.—Psychological stress exacerbates interstitial cystitis/bladder pain syndrome (IC/BPS), a lower urinary tract pain disorder characterized by refractory discomfort referable to the lower urinary tract associated with urinary urgency/frequency and defecatory dysfunction. The perception of bladder fullness at low volumes is exceedingly painful, a phenomenon known as hyperalgesia or allodynia, pain in response to mildly noxious or innocuous stimuli, respectively.

Current evidence suggests IC/BPS may be a manifestation of afferent neurological dysfunction (6). Abnormal processing of sensory information related to the genitourinary tract results in bladder pain and voiding dysfunction via sensitization of visceral organ primary afferents, altered excitability of secondary order pain-transmitting neurons, and dysregulation of descending pathways modulating bladder function and nociception, leading to central augmentation (2).

It is now well accepted that stress facilitates the central augmentation involved in these abnormal nociceptive responses. A majority of patients with IC/BPS report symptom exacerbation following psychological and experimental stresses (28). While the exact interplay with neuronal dysfunction is unclear, stress in genetically susceptible individuals is associated with failure of local and descending inhibitory systems, magnifying pronociceptive visceral responses to typically nonnoxious stimuli (18).

Alterations in glial cell function may be fundamental in this enhancement of neuronal sensitivity and nociceptor activity through a complex interplay of cellular activation and production of soluble mediators. Several studies have identified altered neurotransmitter and cognate receptor expression within peripheral neurons, dorsal root ganglia, and the spinal cord in animal models of hyperalgesia. Glutamate is the key excitatory neurotransmitter and cognate receptor expression within nociceptors, modulating bladder function and nociception, participating in nociception (7). Regulation of glutamate levels in the synaptic cleft is mediated by transporters that reuptake extracellular glutamate; Glt1, expressed primarily on astrocytes, is responsible for 90% of glutamate clearance in the spinal cord (23). Glt1 is downregulated in multiple models of neuropathic pain (27) (17) as well as in a chemically induced cystitis model of IC/BPS (30), making this a promising therapeutic target in chronic bladder pain conditions. We sought to examine the role of glutamate processing in a physiological animal model of IC/BPS with high construct and face validity, induced by chronic exposure to stress through repetitive water-avoidance testing. These rodents exhibited enhanced nociceptive signaling in response to urinary bladder distention (22), generalized somatic hyperalgesia, colonic overactivity (4), and voiding dysfunction characterized by frequent small voids, increased mast cell activation in the bladder, and bladder hyperalgesia that is durable beyond the inciting stress (12).
MATERIALS AND METHODS

Animals. Female Wistar-Kyoto (WKY) rats 11–12 wk old (200–300 g) were purchased from Charles River Laboratories (Wilmington, MA). This strain is genetically predisposed to elevated levels of anxiety (16). Animals were maintained on a normal light-dark cycle; food and water were available ad libitum. Animals were allowed to accommodate for 2 wk before experimentation. Animals were housed in pairs in standard cages. All protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles (ARC 2011-143). All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

Chronic water avoidance stress protocol. Rats were placed on a cylindrical glass pedestal (8 × 8 × 11.5 cm3) in the center of a plexiglas cage (24 × 45 × 15 cm3). The container was filled with 25°C water to 1 cm below the top of the pedestal. Animals were exposed to water avoidance stress (WAS), a well-characterized psychological stressor, for 1 h/day for 10 days between 8:00 AM and 12:00 PM to minimize circadian effects (26). Control rats were handled for 1 min at the beginning and end of the experimental period (similar to rats exposed to WAS), but instead of exposing animals to water avoidance, they were placed in clean cages in isolation for 1 h. WAS-exposed rats were housed in pairs in the same facility as controls. No fewer than four rats per testing condition were used in any experiment. All rats were weighed before and after each protocol period to assess weight change from baseline over time. Fecal pellets were counted at the end of each session as a marker of stress and a validated method to estimate autonomic regulation of colonic motility (14).

Assessment of tactile allodynia and referred hyperalgesia. Calibrated von Frey nylon monofilaments (Stoelting, Wood Dale, IL) were used in tests of tactile allodynia (24). Each animal was tested at baseline and after WAS during light hours (0600–1800). Each rat was placed in a metabolic cage with a wire mesh bottom. Behavioral accommodation was allowed for a minimum of 10 min or until cage exploration and grooming activity ceased.

Tactile allodynia was measured by touching the midplantar hindpaw with von Frey filaments of increasing force (0.4, 1, 2, 4, 8, and 15 g) (5). The filament was presented perpendicular to the plantar surface with sufficient force to cause bending of the filament and held for 6–8 s. Stimuli were presented at intervals >5 s, allowing for resolution of previous stimuli. Sharp withdrawal or licking of the stimulated hindpaw was recorded as a positive response. Ambulation was considered an ambiguous response; in such cases, the stimulus was repeated. The median 50% withdrawal threshold was determined using the up-down method (12).

Referred hyperalgesia of the bladder was tested using the percentage of withdrawal responses to filaments applied to the suprapubic area in ascending order of force, with small variations in the exact area stimulated to avoid desensitization (10). This method correlates well with abdominal visceromotor responses indicating pain seen with bladder distension in this model (12). Each filament was applied for 1–2 s for 10 iterations with an interstimulus interval >5 s. Sharp retraction of the abdomen, immediate licking or scratching of the area, and jumping were considered positive responses to filaments stimulation.

Drug administration. Ceftriaxone (CTX; Sigma, St. Louis, MO) and cefalothin (CLT; Sigma) were prepared in saline and administrated intraperitoneally at 200 mg (302 µmol·kg−1·day−1) from a volume of 2 mg/µl. Normal saline (vehicle) was used as a control for injections. Unless otherwise indicated, injections were administered immediately before the stress period was started.

To assess the effect of dihidrokainate (DHK) treatment on pain and voiding parameters in naïve rats, DHK was administered directly into the spinal canal via intrathecal catheters (ReCathCo, Allison Park, PA) placed as described previously (15). Briefly, animals were placed in a stereotactic unit (David Kopf Instruments, Tujunga, CA) and anesthetized with isoflurane. An ~1-cm incision was made inferior to the nuchal crest in the midline. The underlying muscle was spread in the midline and secured with a self-retaining retractor. Using a periosteal elevator, the cisternal membrane was exposed. A 22-gauge needle was used to make a small puncture in the cisternal membrane; flow of cerebrospinal fluid from this puncture site ensured appropriate placement of the incision. After flushing the intrathecal catheter (ReCathCo) with sterile saline, it was introduced into the puncture site and advanced slowly to the lumbar level. The catheter was immediately removed if any resistance or twitching from the animal was encountered. The catheter was secured in place to the muscle and allowed to exit the skin at the incision site. The catheter was then heat sealed to prevent cerebral spinal fluid (CSF) leakage. One hour before behavioral testing, DHK (20 µg/10 µl; EMD Millipore, Billerica, MA) or sterile saline (vehicle) was injected through the catheter, and the catheter was resealed to prevent leakage of CSF.

Fig. 1. Downregulation of an astrocytic transporter responsible for glutamate clearance (Glt1) during water avoidance stress (WAS). A: protein samples isolated from sacral spinal cord homogenates from Wistar-Kyoto (WKY) rats subjected to the specified days of WAS were analyzed by immunoblotting with antibodies specific for Glt1 (top). The relative protein levels were quantitated by densitometry and normalized to actin levels for consistency (bottom), demonstrating the downregulation of Glt1 levels in the spinal cord with increasing duration of psychological stress. Spinal samples were also examined after intraperitoneal administration of 200 mg ceftriaxone (CTX), which exhibited enhanced Glt1 expression. 3T3, lysates from NIH/3T3 cells, which do not express Glt1, served as a negative control. *P = 0.016, **P = 0.007. B: percent responses to stimulation of the bladder (visceral hyperalgesia) and the hindpaw (tactile allodynia) with an 8-g von Frey filament at the time points specified demonstrate increasing pain responses with greater duration of WAS. A minimum of 4 animals/treatment group were examined (n = 8 for t = 0, 10). NS, not significant. *P < 0.0001.
To examine the effect of DHK treatment on CTX-mediated pain modulation, intrathecal catheters were placed and attached to pre-equilibrated osmotic pumps (DURECT, Cupertino, CA) containing DHK as specified in the manufacturer’s protocol to provide a release rate of 0.5 μl/h over 14 days (24 μg/day). Osmotic pumps were implanted in a subcutaneous pocket between the scapulae before skin closure. Animals were housed individually after surgical procedures and allowed to recover for 3 days before testing. CTX or vehicle was administered intraperitoneally机油 before each WAS protocol. Behavioral testing was performed as specified above after completion of the 10-day WAS protocol.

**Voiding frequency.** Baseline micturition parameters in all animals were obtained using a metabolic cage (Techniplast United States, Exton, PA) for 2-h sessions before and after exposure to WAS or control conditions. Animals had free access to food and water. Voiding frequency, intervals, and volumes, as well as fecal pellet excretion and water intake, were recorded.

**Immunoblotting.** After euthanasia, spinal cords were hydrox extruded with iced saline, and the lumbar L6—S2 spinal segments were dissected and sonicated in T-PER buffer (Pierce Biotechnology, Rockford, IL). After determination of total protein concentrations by standard BCA assay (Pierce), 20 μg protein were loaded onto an 8% SDS-PAGE gel and transferred to nitrocellulose membranes. Lysates from the NIH/3T3 cell line, which does not express Glt1, served as a negative control. After 1-h blocking in albumin, the membranes underwent overnight primary antibody (rabbit anti-Glt1 antibody, 1:1,000; rabbit anti-actin antibody, 1:1,000; Santa Cruz Biotechnology, Dallas, TX) and 1-h secondary antibody (horseradish peroxidase-conjugated rabbit IgG, 1:3,000, Santa Cruz Biotechnology) incubation, followed by X-ray film exposure with Enhanced Chemiluminescent Substrate (Pierce). Band intensity was quantitated by densitometry; relative protein levels were determined by normalizing experimental intensities to those determined for actin in each sample.

**Statistical analysis.** Quantitative data are expressed as means ± SE. To compare single paired means, we used the paired Student’s t-test. For tactile allodynia, linear regression analysis was used to evaluate the effects of force on pain levels. For bladder-specific visceral hyperalgesia testing, a nonlinear mixed effects model repeated measures (MMRM) multivariate analysis with random intercepts was used to account for multiple measurements. This model utilized a quadratic effect of force on withdrawal percentage, interacted with both WAS and treatment status, to allow for calibrated force stimulus (von Frey filament weight) to vary by both treatment condition and the presence or absence of preceding WAS. For multiple treatment conditions, one-way ANOVA followed by the Fisher least significant difference test was utilized. A P value <0.05 was considered to be a statistically significant difference between the experimental groups.

**RESULTS**

**Altered neurotransmitter processing after chronic stress.** After subjecting rats to chronic stress with daily WAS for 10 days, we examined the levels of Glt1 in lysates from the sacral bulb of the spinal cord. Quantitative analysis of these immunoblots revealed that Glt1 transporter levels are decreased in the spinal cord after chronic stress with water avoidance compared with control animals (Fig. 1A).

The time course of Glt1 downregulation and its association with pain symptoms in these animals were then assessed. Animals were subjected to 0, 1, 3, 5, 8, or 10 days of WAS, then tested for tactile allodynia and visceral hyperalgesia im-

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**Fig. 3. Dihydrokainate (DHK) administration induces visceral and tactile allodynia similar to WAS.** A: percent withdrawal responses to suprapubic stimulation with von Frey filaments increased significantly after 10 days of WAS (visceral hyperalgesia). B: thresholds for withdrawal after hindpaw presentation (tactile allodynia) were significantly lower after WAS. A single intrathecal dose of DHK generated a pain phenotype indistinguishable from animals after WAS. Four animals per treatment group were examined. *P <0.001.
inversely associated with the development of both tactile allodynia and visceral hyperalgesia. Glt1 inhibition via a single DHK administration was sufficient to generate a clinical phenotype of lowered pain thresholds in behavioral testing indistinguishable from that seen after WAS (Fig. 3, *P < 0.001). Interestingly, neither a single dose nor continuous 14-day infusion of intrathecal DHK was able to induce the increases in voiding frequency and decreases in voided volume seen after chronic WAS (Fig. 4).

CTX prevents development of WAS-induced allodynia. CTX, a β-lactam antibiotic that increases Glt1 expression and activity in the brain and spinal cord, has good penetration into the CNS and a duration of action of several days. Intraperitoneal injection has been shown to attenuate mechanical allodynia in rats in different models of neuropathic pain (20) (8). To determine whether reversal of Glt1 downregulation could abrogate the induction of visceral pain by chronic stress, we administered CTX or vehicle (saline) throughout the 10-day WAS protocol. We first confirmed that daily intraperitoneal injection of 200 mg/kg CTX was able to upregulate Glt1 in the sacral spinal cord to levels averaging 171 ± 12.7% (*P = 0.007) of that seen at baseline, even after the 10-day WAS period (Fig. 1A). In behavioral testing, this CTX-mediated increase in Glt1 levels prevented the induction of visceral hyperalgesia and tactile allodynia seen after WAS (Fig. 5, A and B; *P < 0.001). Pain responses in CTX-treated animals were not significantly different from those seen in control animals that did not experience psychological stress (*P = 0.64). CTX also prevented the increased voiding frequency seen in these animals when administered throughout the inciting WAS period (Fig. 2C, *P = 0.003). CTX treatment of naive animals before any handling had no significant effect on pain responses (*P = 0.262) or voiding parameters (*P = 0.990) (Fig. 6).

Intrathecal DHK induces similar pain responses as chronic stress. To recreate the downregulation of Glt1 in the absence of psychological stress, we utilized DHK, a selective Glt1 inhibitor that does not cross the blood-brain barrier. After placement of indwelling intrathecal catheters, naive nonstressed animals were given a single intrathecal dose of vehicle (sterile saline) or DHK (20 μg) 1 h before assessment of tactile allodynia and visceral hyperalgesia. Glt1 inhibition via a single DHK administration was sufficient to recreate the downregulation of Glt1 in the absence of psychological stress. 

Immediately, Spinal cord samples from these animals were then analyzed for Glt1 by immunoblotting (Fig. 1A). Protein levels at 3, 5, 8, and 10 days of WAS were significantly decreased to 41, 29, 13, and 27% of baseline Glt1 expression, respectively (*P < 0.05). The decrease in Glt1 at each time point was inversely associated with the development of both tactile allodynia (*P < 0.001) and referred hyperalgesia (*P = 0.001) in behavioral testing (Fig. 1B). After 10 days WAS, concurrent with Glt1 downregulation, animals also exhibited voiding alterations (Fig. 2, A and B) characterized by increased voiding frequency (6.5 ± 0.7 vs. 0.8 ± 0.5 voids/2 h, *P = 0.002) and small voids (152 ± 151 vs. 1,196 ± 311 ml/void, *P = 0.02) as previously observed (26).

![Graph showing number of voids per 2 hours for different treatment groups vs. time](image)

Fig. 4. DHK does not alter voiding frequency. Control animals and animals subjected to WAS underwent intrathecal administration of vehicle or DHK before assessment of voiding frequency. While WAS treatment increased the average number of voids per 2-h period compared with control animals, DHK treatment did not alter the voiding frequency compared with similar animals treated with vehicle. Four animals per treatment group were examined. *P = 0.011. **P = 0.023.

![Graph showing percent response vs. force](image)

Fig. 5. CTX reverses induction of pain seen with WAS. A: while WAS was sufficient to significantly increase pain responses to suprapubic stimulation with von Frey filaments, administration of CTX during the stress period restored pain responses to baseline levels (WAS+CTX). CTX had no effect on animals who were not stressed (Control+CTX). B: decreases in withdrawal thresholds demonstrating tactile allodynia seen after WAS were abrogated by concurrent treatment with CTX during WAS. The key for B is the same as in A. Four animals per treatment group were examined. *P < 0.001.
CTX decreases pain responses via upregulation of Glt1. Cephalothin (CLT) is a β-lactam antibiotic with anti-inflammatory and antimicrobial effects similar to CTX that does not alter spinal Glt1 levels (13). We confirmed that CLT administration did not change spinal levels of Glt1, before or after 10 days of WAS (Fig. 7, P = 0.89). To confirm that the effect of CTX on pain responses resulted from its effect on Glt1 levels, and not from its antimicrobial or anti-inflammatory activities, we tested the ability of CLT to abrogate WAS-induced pain. While CTX significantly reversed the induction of pain by WAS (P < 0.001), animals treated with CLT at the same dose and administration schedule as CTX exhibited similar tactile allodynia and referred hyperalgesia as vehicle-treated animals (Fig. 8, P = 0.78), confirming that the effect of CTX on pain after chronic stress was independent of its action as an antibiotic.

These results suggest that CTX mediated its effect on pain by upregulating Glt1 levels in the spinal cord, an effect that should be reversible by direct Glt1 inhibition with DHK. We examined the ability of a continuous intrathecal administration of DHK throughout WAS to abrogate the CTX-mediated reversal of chronic pain development. An osmotic pump was attached to an implanted intrathecal catheter subcutaneously to deliver DHK continuously over a 14-day period. Three days after implantation, animals were subjected to WAS with daily CTX administration, which normally would prevent the induction of increased pain responses. Administration of DHK completely reversed the effects of CTX on pain after WAS, confirming the role of Glt1 downregulation in pain development (Fig. 9, P < 0.001).

CTX administration reverses previously established pain. As patients with IC/BPS present after the cycle of chronic pain is well established, we sought to determine the efficacy of Glt1 reduction in the reversal of previously established pain. We examined the therapeutic effect of CTX in animals after the establishment of visceral pain (Fig. 10). Animals were subjected to the WAS protocol, and the establishment of chronic pain was confirmed with behavioral testing (P < 0.001). Animals were then treated for 5 days with CTX or vehicle alone and retested for pain responses. Rats treated with vehicle demonstrated a persistence of the pain responses, while CTX-treated animals exhibited pain thresholds that were not significantly different from unstressed control animals (Fig. 10, P = 0.805). In addition, the increased voiding frequency seen in animals after WAS was also reversed by CTX administration (Fig. 2C, P = 0.003). To assess CTX as a potential therapeutic agent, we also tested its effect on animals with well-established pain, at time points distant from the inciting WAS stimulus. We allowed animals to recover for 60 days after WAS, periodically retesting for both tactile allodynia and visceral hyperalgesia to ensure persistence of the pain phenotype. As seen previously (12), pain responses persisted in stressed animals at this late time point (Fig. 11, P = 0.002). Administration of
CTX for 5 days even at this late time point was sufficient to return responses in both visceral hypersensitivity and tactile allodynia testing to normal thresholds ($P < 0.001$).

The effect of CTX to abrogate the increased pain seen after chronic WAS, however, did not endure after discontinuation of CTX (Washout, Fig. 10). Thus, while useful therapeutically at reversing the chronic pain resulting from WAS in this model of IC/BPS, treatment with CTX was not curative and required continuous administration for persistent efficacy.

**DISCUSSION**

Previously, we demonstrated that chronic WAS, in the absence of a chemical stimulus, produces a chronic pain syndrome in susceptible animals characterized by tactile allodynia, visceral hyperalgesia, increased colonic motility, anxiety behaviors, and increased voiding frequency (12, 26), a phenotype similar to human IC/BPS. In this study, we demonstrate that these symptoms inversely correlate with alterations in spinal Glt1 expression; lower pain thresholds are associated with lower Glt1 protein levels. Exogenous inhibition of Glt1 via DHK treatment without psychological stress reproduces both bladder hyperalgesia and tactile allodynia, suggesting Glt1 inhibition is sufficient to establish chronic visceral pain. The relationship between glutamate processing and hyperalgesia is implicated in several other models of visceral pain (30), but this is the first report demonstrating a role for Glt1 in referred bladder pain and voiding dysfunction in an IC/BPS animal model generated without noxious stimuli.

CTX administration upregulates Glt1 expression and functional glutamate uptake in the spinal cord (8, 13). CTX treatment in the WAS model inhibits the visceral pain induced by chronic psychological stress, demonstrating Glt1 downregulation is necessary for bladder hyperalgesia. This inhibition of chronic pain resulted from CTX-mediated downregulation of

![Graph](image-url)
spinal Glt1, not an anti-inflammatory or antibiotic effect, as equimolar doses of CLT, a β-lactam antibiotic that does not affect spinal Glt1, had no effect on pain following chronic stress. In addition, continuous intrathecal infusion during WAS of DHK, a specific inhibitor of Glt1 activity, abrogated the effect of CTX on visceral pain. These results verify that CTX’s ability to enhance glutamate transport via Glt1 upregulation is responsible for its clinical activity in pain prevention.

Glt1 is emerging as a central mediator in multiple neurological diseases. In addition to chronic pain, glutamate modulation via Glt1 is implicated in anoxic and traumatic brain injury (19), addiction (21), neurodegenerative diseases (25), episodic neurologic illnesses such as epilepsy and migraine (1), and even psychiatric illness such as depression and schizophrenia (9). As Glt1 downregulation is both necessary and sufficient for the establishment of chronic pain in our model, Glt1 is a promising target for therapeutic intervention in syndromes of bladder hyperalgesia such as IC/BPS as well. As CTX has been used clinically for years with minimal side effects and is safe at high doses for long periods (3), selective Glt1 inhibition would likely have few deleterious effects in humans.

In the WAS animal model, CTX is also able to attenuate chronic stress-induced visceral pain in a therapeutic manner after the inciting stress. To be clinically efficacious, therapy for patients with IC/BPS must be able to reverse previously established chronic visceral pain. Even after months of established visceral pain, CTX treatment returned pain thresholds to pre-stress baseline levels, although the visceral hyperalgesia returned upon discontinuation of CTX therapy.

We hypothesize that chronic stress in anxiety-prone animals induces a cascade of events that results in the decreased pain thresholds, voiding and defecatory dysfunction, and anxiety behaviors. The rapid return of pain upon removal of CTX suggests Glt1 downregulation is maintained by other upstream cellular processes. Initial signals generated as a consequence of WAS likely result in sustained global changes in nociceptive pathways that are able to reestablish Glt1 inhibition once CTX is removed. These global changes, however, likely impact more than just glutamate metabolism. The inability of DHK to induce increased voiding frequency similar to that seen after WAS suggests that changes in glial Glt1 expression are not sufficient to generate the entire clinical phenotype seen in this model, suggesting there are Glt1-independent mechanisms that...
govern these changes in voiding. In contrast, CTX blocked
the development of and reversed previously established voiding
dysfunction, suggesting that CTX may impact these upstream
global changes that control Glt1 expression as well as voiding
behaviors. While the mechanism of action is not well charac-
terized, CTX has been demonstrated to promote the nuclear
translocation of NF-κB (11), a signal transduction pathway
with myriad downstream targets involved in cellular activation.
Thus stress likely activates a cascade of events inducing an
IC/BPS-like phenotype; however, Glt1 downregulation is just
one of the downstream effects. The establishment of voiding
frequency may require DHK-independent pathways different
from those inducing visceral pain. As CTX can influence these
pathways, this treatment may have more profound effects on
nociception than measured in this study as Glt1 inhibition
alone. Identification of the upstream events influenced by CTX,
but independent of DHK, will be critical in understanding the
effects of stress on bladder sensitivity in susceptible individu-
als and determining treatment modalities effective against the
entire constellation of symptoms.

Research attempting to identify stress-induced mediators
that establish chronic pain has implicated several pathways,
stress-associated catecholamine production and β2-adrenergic
receptor activation (18), increased mast cell activation and
histamine release, or local inflammation promoting cytokine,
growth factor, and chemokine release, all of which can pro-
duce changes in neural activity. Genetic alterations in glutam-
ate transport may also predispose to chronic pain conditions.
For example, a genome-wide analysis discovered an allelic
variant in a regulatory gene associated with idiopathic migraine
that results in Glt1 downregulation (1).

While the exact interplay between environmental stresses,
intrinsic inflammation, and neuronal dysregulation in the
development and maintenance of chronic pain remains unclear,
a model is emerging in which inciting stress in a genetically
susceptible individual results in alterations in immune and glial
cell function that leads to the enhancement of neuronal sensi-
tivity and nociceptor activity. This dysfunctional neural pro-
cessing of painful stimuli may result in visceral hyperalgesia in
a subpopulation of stress-susceptible individuals, which may
manifest in the urinary tract as a clinical phenotype of
IC/BPS. A better understanding of these underlying molecu-
lar mechanisms and the identification of specific targets
critical in establishing bladder hyperalgesia will hopefully
lead to the development of targeted therapies capable of
providing much-wanted relief to a marginalized, debilitated
population, allowing these affected individuals to improve
their quality of life. As we gain additional insight into the
mechanisms that govern IC/BPS, we may find that many of
the results obtained in this model of bladder hyperalgesia
will be applicable to other chronic functional pain syn-
dromes.

Thus psychological stress plays a critical role in functional
pain disorders like IC/BPS. In this study, we utilize a novel
animal model in which chronic psychological stress induces
visceral pain and urinary frequency in the absence of direct
bladder irritants or inflammatory agents with high construct
validity to the clinical manifestation of IC/BPS. The establish-
ment of chronic pain correlates with alterations in glutamate
neurotransmission attributable to the downregulation of glial
Glt1 receptors. Exogenous upregulation of Glt1 via CTX
treatment had a profound therapeutic effect; CTX both inhib-
ited the development of and reversed established pain and
voiding dysfunction. The efficacy of Glt1 manipulation over a
wide range of neurological diseases points to glutamate me-
tabolism as a critical convergence of pathologies for these
varied diseases. Gaining greater insight into the molecular
mechanisms controlling Glt1 expression and function is central
in both understanding the pathophysiology of chronic pain and
in identifying novel therapeutic approaches to these costly,
treatment-refractory diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: A.L.A., S.B., and L.V.R. provided conception and
design of research; A.L.A., F.C.J., U.J.L., and S.B. performed experiments;
of experiments; A.L.A. prepared figures; A.L.A. drafted manuscript; A.L.A.,
U.J.L., and L.V.R. edited and revised manuscript; A.L.A., U.J.L., S.B., and
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