Research Paper

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Chronic pain susceptibility is associated with anhedonic behavior and alterations in the accumbal ubiquitin-proteasome system

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Abstract

It remains unknown why on similar acute/subacute painful conditions, pain persists in some individuals while in others it resolves. Genetic factors, mood, and functional alterations, particularly involving the mesolimbic network, seem to be key. To explore potential susceptibility or resistance factors, we screened a large population of rats with a peripheral neuropathy and we isolated a small subset (<15%) that presented high thresholds (HTs) to mechanical allodynia (reduced pain manifestation). The phenotype was sustained over 12 weeks and was associated with higher hedonic behavior when compared with low-threshold (LT) subjects. The nucleus accumbens of HT and LT animals were isolated for proteomic analysis by Sequential Window Acquisition of All Theoretical Mass Spectra. Two hundred seventy-nine proteins displayed different expression between LT and HT animals or subjects. Among several protein families, the proteasome pathway repeatedly emerged in gene ontology enrichment and KEGG analyses. Several alpha and beta 20S proteasome subunits were increased in LT animals when compared with HT animals (eg, PSMα1, PSMα2, and PSMβ5). On the contrary, UBA6, an upstream ubiquitin-activating enzyme, was decreased in LT animals. Altogether these observations are consistent with an overactivation of the accumbal proteasome pathway in animals that manifest pain and depressive-like behaviors after a neuropathic injury. All the proteomic data are available through ProteomeXchange with identifier PXD022478.

Keywords: Neuropathic pain, Allodynia, Proteomic screening, Nucleus accumbens, Resistance, Proteasome

1. Introduction

Chronic pain (CP) is a complex and incapacitating disorder with a significant impact on individuals and society.[18,49,75] It is commonly associated with sensory disturbances, morphofunctional brain reorganization,[13,36] and behavioral alterations—see for instances Refs. 30,58,60.

Mechanisms involved in the transition from acute to CP are still poorly understood.[7] In humans, the nucleus accumbens (NAc) seems to be key in this regard as its functional connectivity was shown to be predictive of pain trajectories[12]—see also Ref. 77. Data from preclinical studies demonstrate that this area is affected at multiple levels by CP—see for review Refs. 17,63,71—including decreased activity,[69] altered functional connectivity with other brain regions such as the prefrontal cortex and hippocampus,[11,16,23] increased number of accumbal newborn neurons,[39] and decreased dopamine and opioid receptor levels[10,23]—cf. with.[78] In addition, pain relief was associated with a transient increase in accumbal dopamine[52,79] (conflicting evidence exists regarding basal levels—see Ref. 29 and references within) and optogenetic activation of the NAc-ameriolated pain.[34] Finally, because of NAc's well-established role in motivated behavior and executive function, the morphofunctional alterations described can also provide a biological substrate for the emotional and cognitive alterations observed in CP[28,55–57]; see for comprehensive reviews.[30] For instance, it was demonstrated that decreased motivation in preclinical CP models required depression of excitatory synaptic transmission through galanin receptor 1 in the NAc medium spiny neurons.[70] However, contrary to clinical studies, in which pain trajectories are evaluated, in experimental CP studies, lesioned and nonlesioned (sham-operated) subjects are normally compared, which impedes to uncover pain resilience/susceptibility phenotypes.

We, therefore, took advantage of the fact that in Sprague Dawley (SD) rats a small subset of neuropathic rats does not develop hypersensitivity,[37,45] and we compared susceptible (painful) and resistant (painless) accumbal protein-level differences by Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH-MS).

2. Material and methods

2.1. Experimental subjects and neuropathic pain model

An initial population of seventy-two 2-month old males SD animals (Charles River Laboratories, Barcelona, Spain) was used. Animals were pair-housed in standard plastic cages with food and water

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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available ad libitum, in a room with controlled temperature (22 ± 1°C) and humidity (55%-60%), and in a 12-hour light/dark cycle (lights on at 8 AM). All procedures involving animals were approved by the respective local organizations, and the experiments were performed according to the European Community Council Directive 2010/63/EU guidelines. The spared nerve injury (SNI) model was used in all experiments.[31] Briefly, the right sciatic nerve was exposed, and an unilateral ligation and subsequent distal axotomy of the tibial and common peroneal nerves was performed. The sural nerve was spared. Surgical procedures were performed under deep anesthesia obtained with an intraperitoneal injection (i.p.) 1.5:1.0 mixture of ketamine (Imalgene, 100 mg·mL^{-1}; Merial, Lyon, France) and medetomidine (Domitor, 1 mg·mL^{-1}; Orion Pharma, Espoo, Finland) at a dose of 1 mg·kg^{-1}.[35]

2.2. Allodynia

Mechanical allodynia (a hallmark manifestation of neuropathic pain) was evaluated weekly until the end of the experiment by an experimenter blinded to the experiment. Before SNI, animals were habituated to the experimental setting—a small compartment on an elevated grid—for 5 minutes. In subsequent sessions, mechanical allodynia was assessed using the up-and-down method.[24] Briefly, the sural dermatome was probed with a series of von Frey (VF)-calibrated monofilaments: 15.0, 8.0, 6.0, 4.0, 2.0, 1.0, 0.6, and 0.4 g (North Coast Medical Inc). Starting with the 2.0 g filament, the test would advance upward if no response was elicited (=0) or downward if a brisk withdraw of the limb was produced (=X) until 6 measurements were obtained around the threshold point. If no response was obtained up to maximal force (15.0 g) or conversely, if all filaments elicited a response down to the minimal force (0.4 g), the values 15 and 0.25 were assumed as the 50% withdrawal threshold, respectively. Paw movements associated with locomotion or weight shifting were ignored. The 50% response threshold was then calculated using the following formula:

$$50\%\ \text{g Threshold} = \frac{\left(10^{Xf + k.\delta}\right)}{10000}$$

where Xf is the value (in log units) of the final VF monofilament, k is the tabular value corresponding to the pattern of positive and negative responses (X and 0 sequence), and δ is the mean difference (in log units) between stimuli (0.224). After 4 weeks, animals with the highest and lowest thresholds—high-threshold (HT; n=5) and low-threshold (LT; n = 5) groups, respectively—were selected for the subsequent analysis.

2.3. Anhedonia

Anhedonia was assessed by the sucrose preference test, as previously described.[2] At the end of the experiment, animals were food and water deprived for 12 hours. During the dark phase, 2 preweighed bottles containing water or a 2% (m/v) sucrose solution were presented to individually housed animals for 1 hour (test started at 8:30 PM). The anhedonia levels are negatively correlated with the percentage of sucrose intake (sucrose intake/[sucrose intake + water intake]).

2.4. Protein quantification by Sequential Window Acquisition of All Theoretical Mass Spectra

Animals were perfused transcardially with ice-cold sodium chloride 0.9% (NaCl) under deep sodium pentobarbital anesthesia (200 mg·kg^{-1} i.p.; Eutasil, Ceva Saúde Animal, Algés, Portugal). Brains were collected, and the left and right NAc macrodissected and immediately frozen (−80°C) until use. Samples from LT and HT animals were thawed and ultrasonicated (in 130 W Ultrasonic Processor with the following settings: 60% amplitude, 1 second on/off cycles, for 1-minute total sonication) in 200 μL of 50 mM of Tris-HCl, pH 7.4, with protease and phosphatase inhibitors. Samples were then centrifugated at 5000*g* for 5 minutes at 4°C, and supernatants were collected. To improve the extraction yield, pellets were resuspended in 100 μL of fresh buffer and subjected to an additional step of sonication. After centrifugation, the supernatants were collected. Samples were quantified using the 2-D Quant Kit (GE Healthcare, Madrid, Spain), and 100 μg of each sample were subjected to trypsin digestion using the Short-GeLC[4] for subsequent quantitative analysis by SWATH-MS.[5] Before electrophoresis, 1 mg of the recombinant protein maltose-binding protein - green fluorescent protein (MBP-GFP)—was added to each sample to account for sample processing variation,[6] and samples were denatured, reduced, and alkylated with acrylamide. In addition to the individual replicates, pooled samples (one per condition) were created to be used in information-dependent acquisition experiments to build a specific protein library for SWATH-MS analysis. These pooled samples were spiked with the recombinant protein and digested using the same condition of the individual replicates. Samples were analyzed on a TripleTOF 5600 System (AB Sciex) as previously described.[5] For more detailed information see supplementary information and supplementary table S1 (available at http://links.lww.com/PAIN/B262). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium[32] through the PRIDE[66,67] partner repository with the data set identifier PXD022478.

2.5. Functional annotation

Gene ontology (GO) enrichment analysis, considering the biological process category,[9,21] and KEGG pathways of identified proteins, were performed using the combination of AmiGO and David Bioinformatics Resources[22,46,47] with the statistical Fisher exact test associated and a *P*-value of 0.05 as the cutoff (see supplementary tables S2 and S3, respectively, from the supplementary information, available at http://links.lww.com/PAIN/B262). Enrichment complexes analysis was performed at ConsensusPathDB Bioinformatics resource.[50] Complexes were considered whenever 20% of identified proteins were part of that complex at a cutoff *P* value of 0.05.

The heatmaps from the quantified proteins were performed in Morpheus (https://software.broadinstitute.org/morpheus/). Each row corresponds to a different protein, with their relative levels normalized to values between 0 and 1.

2.6. Statistical analysis

Statistical evaluation for protein quantifications was performed by the Mann–Whitney *U* test for single comparisons. For the analysis of repeated measures, a two-way analysis of variance with the Bonferroni test for multiple comparisons was used. Statistical analysis between 2 groups was made using the Student *t* test. The statistical analyses were performed in the SPSS statistic program (version 24; IBM Co). Data are presented as mean ± SEM. The significance value was set at $P < 0.05$.

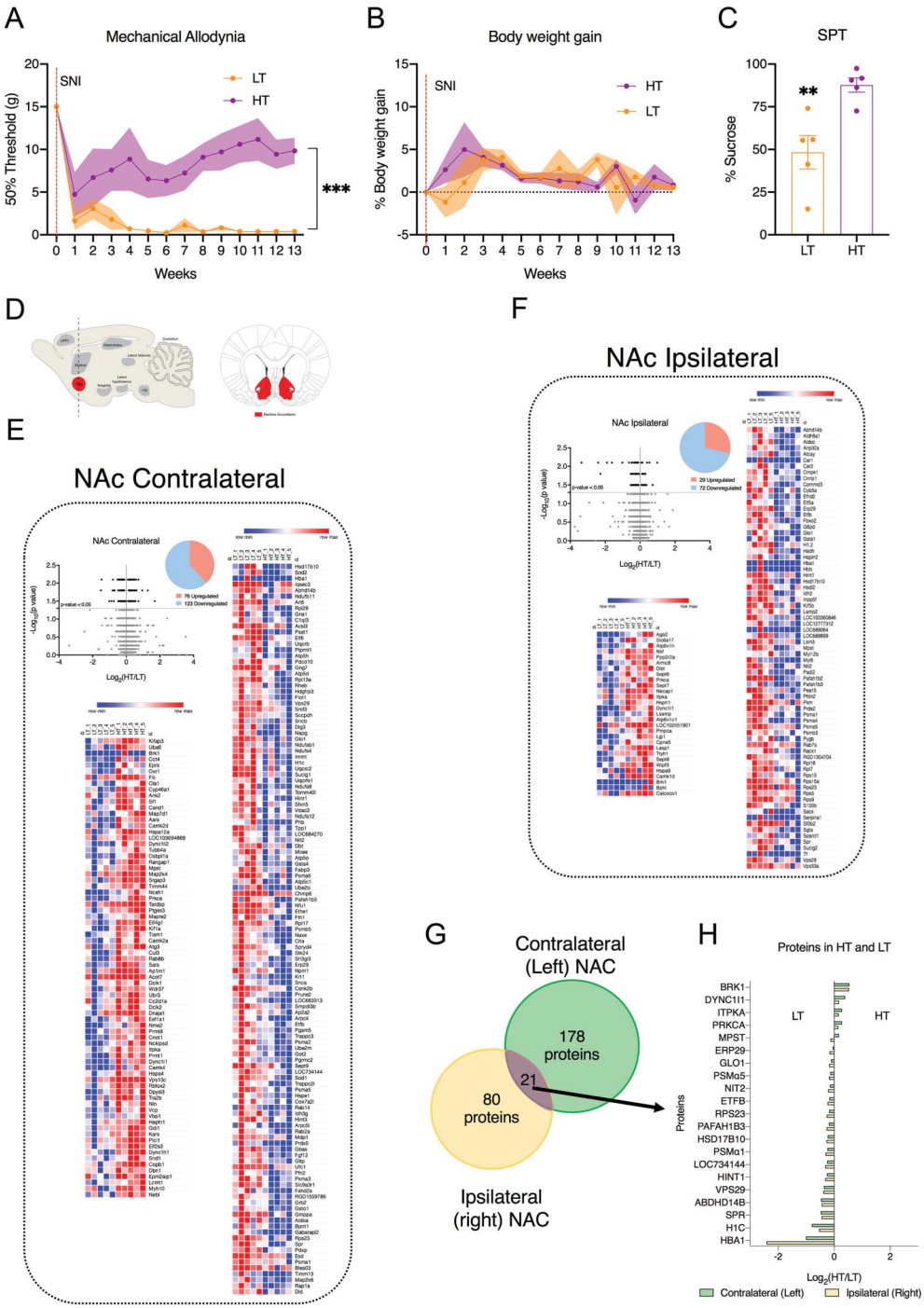

Figure 1. Characterization and quantitative proteomics profile of contralateral and ipsilateral NAc of LT and HT animals. Mechanical allodynia was assessed weekly after SNI installation in SD males. Animals with high thresholds (HTs) present reduced hypersensitivity to von Frey monofilaments than low-threshold (LT) animals (A). Although no differences were found in body weight gain (B) LT animals present an anhedonic-like phenotype in the sucrose preference test (SPT; C). Representative image of a sagittal and coronal section of NAc (D). Differential proteomics analysis (volcano plot and heatmap) of the contralateral NAc of HT and LT animals and the 2178 proteins quantified in the contralateral NAc and a heatmap representation of the 199 altered proteins (76 upregulated and 123 downregulated) in the Log_2 (HT/LT) comparison (E). The same statistical analysis was performed in the ipsilateral NAc, and we obtained 99 altered proteins (29 upregulated and 72 downregulated) in the HT/LT comparison (F). From all the significantly altered proteins, 21 proteins are altered in both contralateral and ipsilateral NAc (G). Their relative expression in both groups and brain side (H) reveals that most of them have the same fold-change tendency in both HT and LT animals. Data presented as mean± SEM; Two-way analysis of variance repeated measures were used in mechanical allodynia evolution, and the Mann–Witney *U* test and Student *t* test were used and statistical significance was considered for $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$; ABHD14B, abhydrolase domain containing 14b; BRK1, BRICK1 SCAR/WAVE actin nucleating complex subunit; DYNC1I1, dynein cytoplasmic 1 intermediate chain 1; ERP29, endoplasmic reticulum protein 29; ETFB, electron transfer flavoprotein beta subunit; GLO1, glyoxalase 1; H1C, similar to Histone H1.2 and Hba1 hemoglobin, alpha 1; Hint1, histidine triad nucleotide binding protein 1; HSD17B10, hydroxysteroid (17-beta) dehydrogenase; ITPKA, inositol-trisphosphate 3-kinase A; LOC734144, Uncharacterized protein; MPST, mercaptopyruvate sulfurtransferase; NAc, Nucleus Accumbens; NIT2, nitrilase family, member 2; oxidoreductase); PAFAH1B3, platelet-activating factor acetylhydrolase 1b catalytic subunit 3; PRKCA, protein kinase C alpha; PSMα1, proteasome subunit alpha 1; PSMα5, proteasome subunit alpha 5; RPS, ribosomal protein S23; SNI, spared nerve injury; SPR, sepiapterin reductase (7,8-dihydrobiopterin:NADP+; VPS29, retromer complex component.

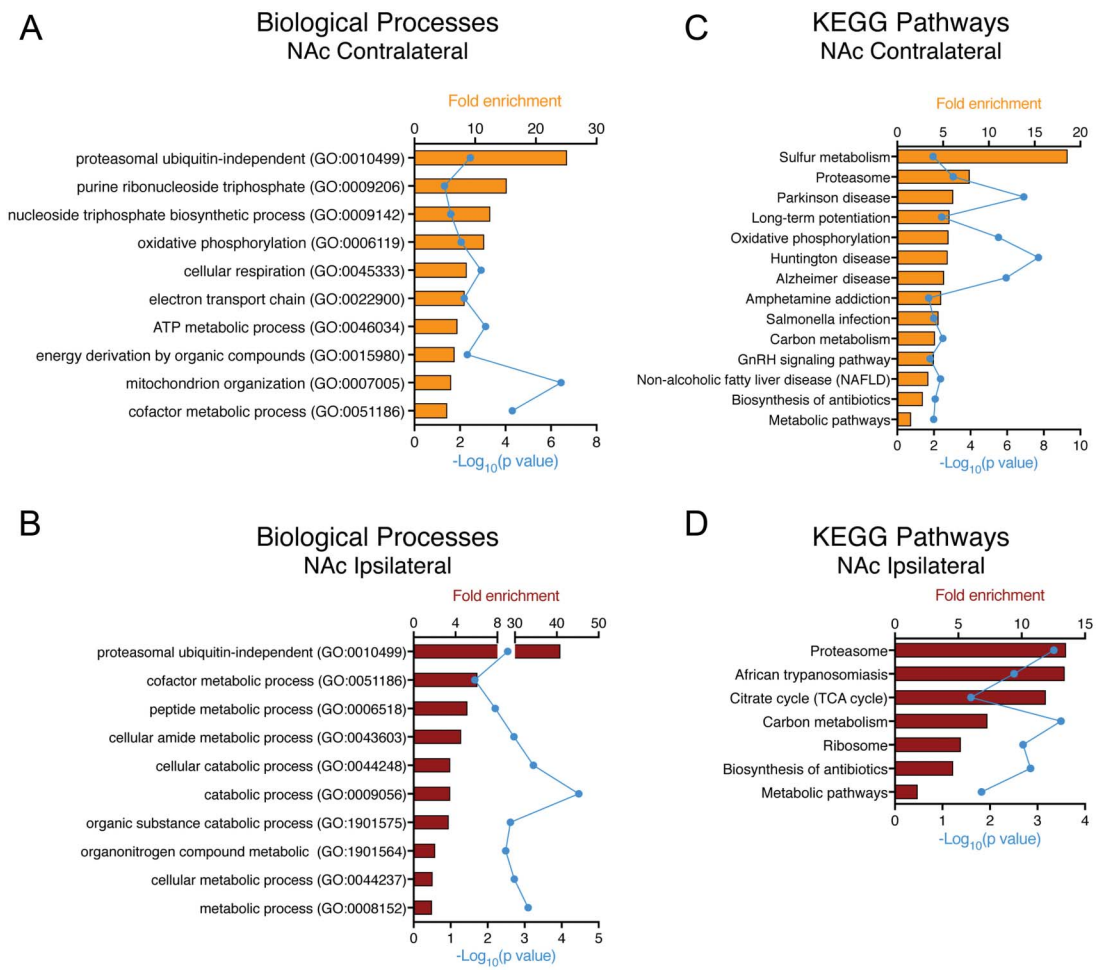

Figure 2. Gene ontology (GO) and KEGG pathways enrichment analysis of the proteins significantly altered in NAc. Biological Process enrichment analysis in the contralateral (A) and ipsilateral (B) NAc, and KEGG pathways enrichment analysis of the proteins significantly altered in the contralateral (C) and ipsilateral (D) NAc. All these analyses were performed against *Rattus norvegicus* reference list of 21465 proteins. Enrichment was confirmed by a statistical Fisher exact test and a *P* value of 0.05 as the cutoff. NAc, Nucleus Accumbens.

3. Results

3.1. Behavioral readouts

Thresholds to mechanical stimulation after SNI installation in SD rats were low and stable for most of the studied population. However, in a small number of animals (≈15%), after an initial drop, the mechanical threshold recovered and remained elevated until the end of the experiment. As expected, selected LT and HT animals presented significantly different mechanical allodynia thresholds, which were maintained throughout the experiment (**Fig. 1A**; weeks × threshold group: $F_{13,104} = 2.65$; $P = 0.0031$). Also, no major differences were observed regarding weight evolution (**Fig. 1B**). At the end of the experiment, a depressive-like phenotype (anhedonia) was observed in LT animals, as shown by a decreased preference for sucrose by these animals in the sucrose preference test (**Fig. 1C**; $t_8 = 3.698$, $P = 0.0061$).

3.2. Proteomics profile of nucleus accumbens from low-threshold and high-threshold animals

To understand which proteins play a role in the segregation of LT and HT animals, NAc from left (contralateral side) and right (ipsilateral side) hemispheres (**Fig. 1D**) were characterized through a nontargeted proteomics approach, the SWATH-MS. From the SWATH-MS analysis, 3616 proteins were identified, from which we were able to quantify 2178. A comparative analysis revealed that 76 and 123 proteins (199 in total) were significantly upregulated and downregulated in the contralateral NAc of HT animals in comparison with the contralateral NAc of LT animals (**Fig. 1E**). In the ipsilateral NAc, similar effects were found in 101 proteins, in which 72 and 29 were significantly down-regulated and upregulated, respectively, in the same HT vs LT comparison (**Fig. 1F**). Of these, 21 proteins were common to both contralateral and ipsilateral NAc (**Fig. 1G**), and for most cases, HT/LT change presented the same direction in the 2 hemispheres (**Fig. 1H**). The same strategy was used to compare contralateral and ipsilateral NAc within LT and HT animals (supplementary Fig. S1, available at http://links.lww.com/PAIN/B262).

3.3. Enriched biological processes and pathways in low-threshold and high-threshold animals

Gene ontology enrichment analysis was performed on the 199 proteins identified in the contralateral NAc that had significant differences between HT and LT animals (against *Rattus norvegicus* reference list of 21465 proteins).[42] The top 10 enriched biological processes (**Fig. 2A**; orange bars—fold enrichment; blue lines—log *P* value) encompassed, eg, proteasome catabolism, purine

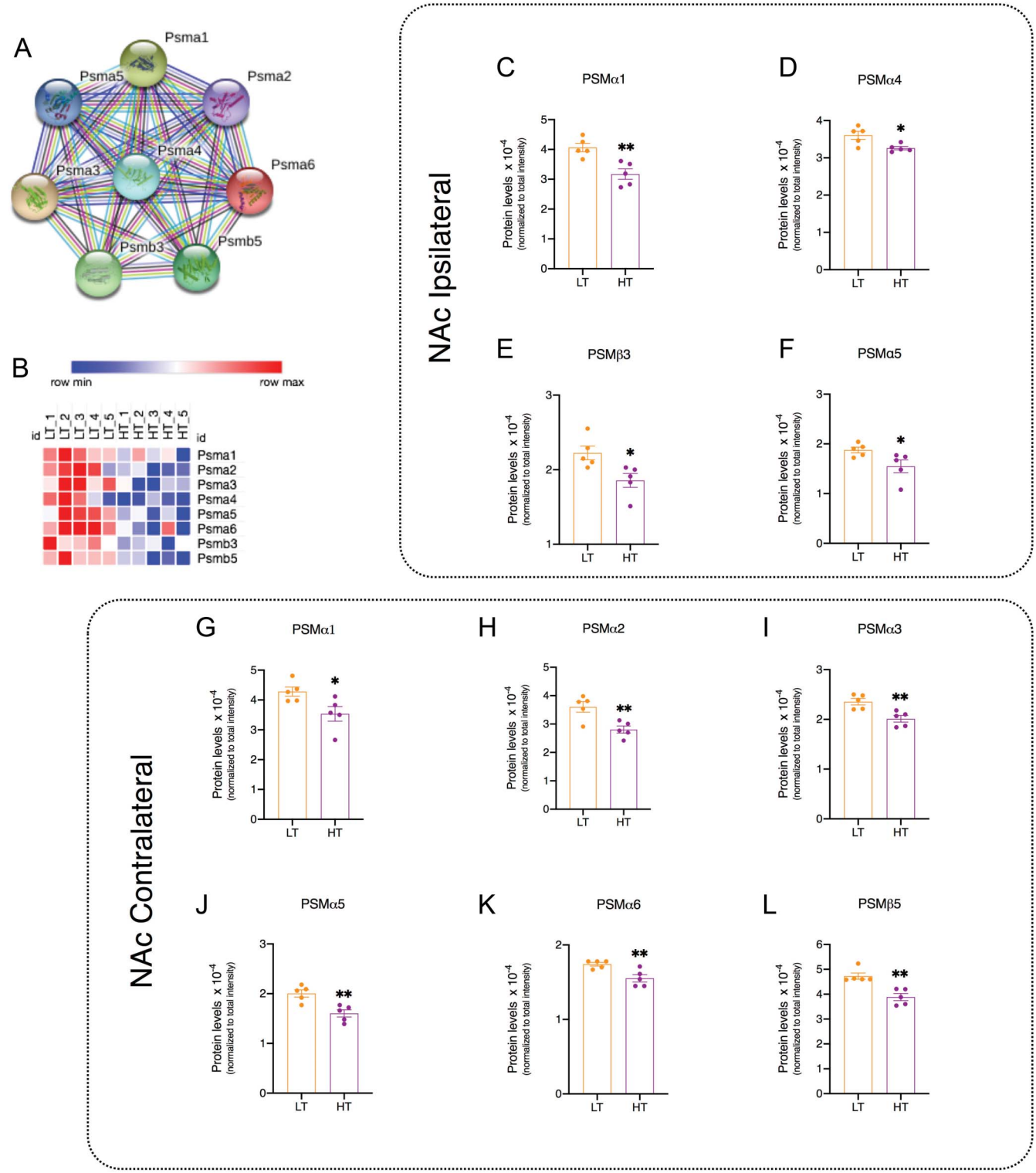

Figure 3. Graphical and quantitative representation of the ubiquitin and proteasome-related proteins altered in the NAc between LT and HT animals. Proteasome-related proteins enriched in our protein database (A). The heatmap demonstrates that all the proteins are upregulated in LT animals in comparison with HT animals (B). Specifically, PSMα1 (C), PSMα4 (D), PSMα5 (F) and Psmβ3 (E) are reduced in the ipsilateral NAc of HT animals; and PSMα1 (G), PSMα2 (H), PSMα3 (I), PSMα5 (J), PSMα6 (K), and Psmβ5 (L) are reduced in the ipsilateral NAc of HT animals. Bar graphs presented as mean± SEM; Mann–Witney *U* test and statistical significance was considered for $P < 0.05$. NAc, nucleus accumbens; HT, high threshold; LT, low threshold; proteasome subunit alpha 1, 2, 3, 4, and 5 (PSMα1, PSMα2, PSMα3, PSMα4, PSMα5, and PSMα6) and proteasome subunit beta 3 and 5 (PSMβ3 and PSMβ5).

ribonucleoside triphosphate biosynthesis, adenosine diphosphate phosphorylation, and mitochondrial morphogenesis and distribution (supplementary table S2 for full list, available at http://links.lww.com/PAIN/B262). On the ipsilateral NAc, 101 proteins were identified as having differences between HT and LT animals. As in the contralateral side, enrichment was found in proteasome catabolism, although all other identified processes were different from the contralateral analysis (**Fig. 2B**).

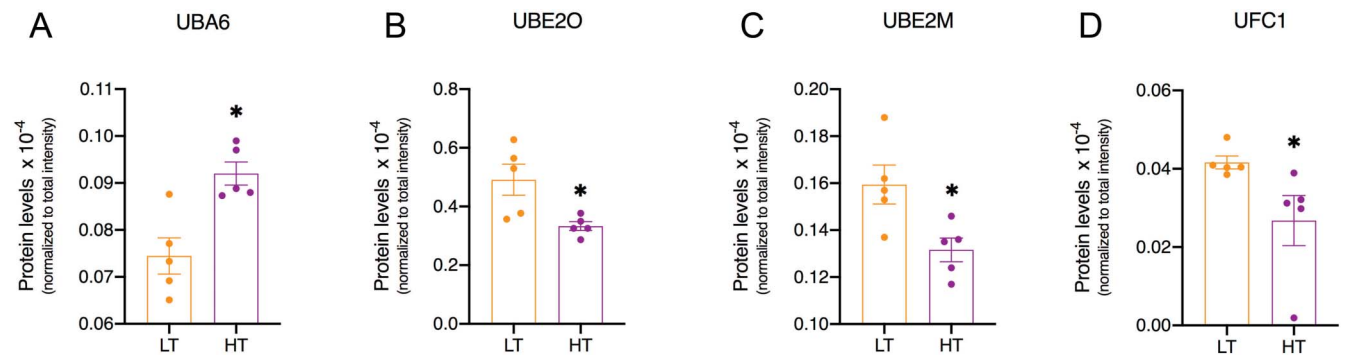

Figure 4. Ubiquitin pathway in low-threshold and high-threshold animals. E1-like enzyme (UBA6) was found to be upregulated (A) while E2-like enzymes, UBE2O (B), UBE2M (C), and UFC1 (D) were found to be downregulated in contralateral NAc HT/LT comparisons. Bar graphs presented as mean ± SEM; Mann–Witney *U* test and statistical significance was considered for $P < 0.05$. HT, high threshold; LT, low threshold.

The same proteins were analyzed in terms of enriched KEGG pathways. Again, proteasome-related processes were highlighted as being altered in contralateral and ipsilateral NAc (**Figs. 2C and D**). Furthermore, the contralateral NAc presented a large enrichment score in proteins associated with neurodegenerative disorders such as Alzheimer disease, Parkinson disease, and Huntington disease, as well as oxidative stress, long-term potentiation, and protein degradation (**Fig. 2C**). On the other hand, within the ipsilateral NAc, we found alterations in pathways related to protein degradation, protein synthesis, and energy production (**Fig. 2D**) (supplementary table S3—full details available at http://links.lww.com/PAIN/B262).

From all analyses, we verified that protein degradation and protein clearance pathways seem to be a key differentiating factor between LT and HT animals. An additional protein complex-based gene sets analysis was performed using the ConsensusPathDB Bioinformatics resource, and the 20S proteasome was the complex with more identified proteins, namely proteasome subunit alpha 1, 2, 3, 4, and 5 (PSMα1, PSMα2, PSMα3, PSMα4, PSMα5, and PSMα6) and proteasome subunit beta 3 and 5 (PSMβ3 and PSMβ5) (**Fig. 3A**). Then, we looked to the relative expression of each protein (**Fig. 3B**), and all were increased in the ipsilateral ([PSMα1; **Fig. 3C**]; [PSMα4; **Fig. 3D**]; [PSMα5; **Fig. 3F**]; and [PSMβ3; **Fig. 3E**]) and contralateral NAc of LT animals ([PSMα1; **Fig. 3G**]; [PSMα2; **Fig. 3H**]; [PSMα3; **Fig. 3I**]; [PSMα5; **Fig. 3J**]; [PSMα6; **Fig. 3K**]; and [PSMβ5; **Fig. 3L**]) in comparison with HT animals. Moreover, an upstream analysis in the ubiquitin-proteasome system (UPS) demonstrated that E1-like enzyme

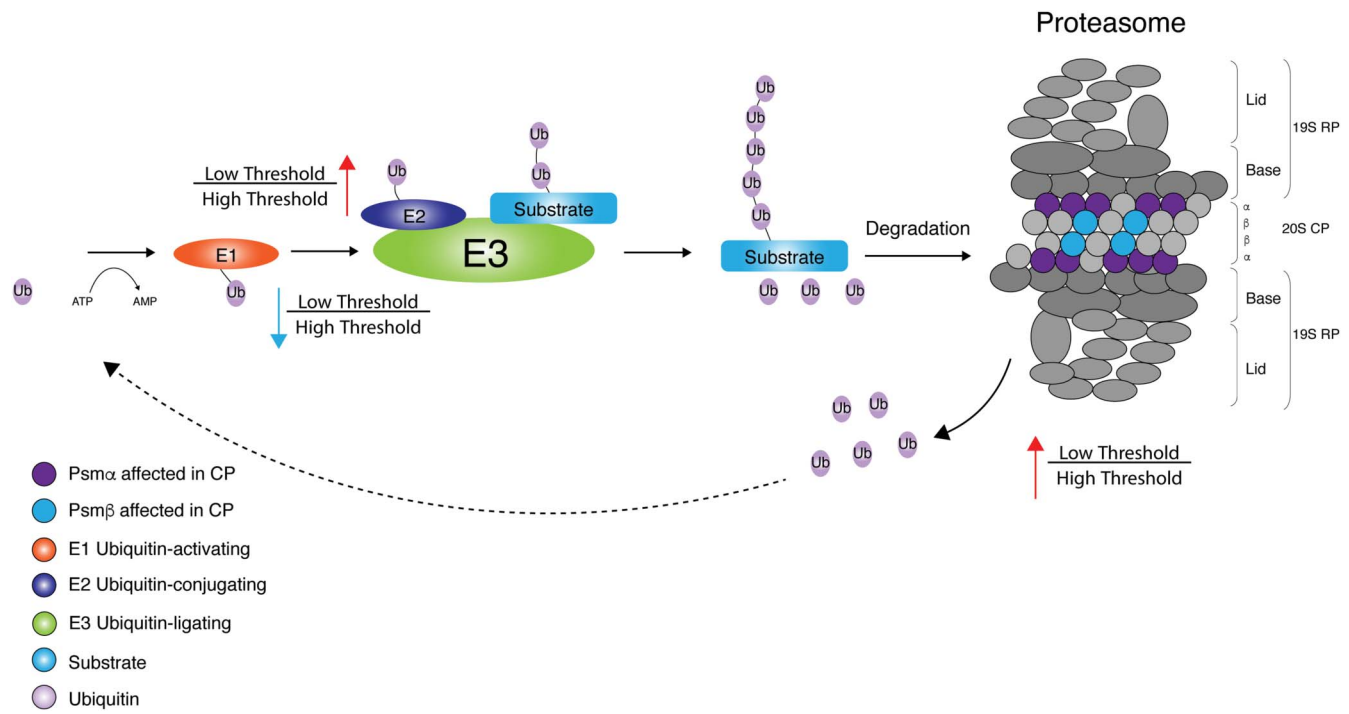

Figure 5. Schematic representation of the ubiquitin-proteasome system in low-threshold and high-threshold animals. The 26S proteosome degrades mostly proteins tagged with polyubiquitin chains. Pain-susceptible LT animals presented increased levels of ubiquitin-conjugating enzymes (E2 enzymes), particularly UBE2M (ubiquitin-conjugating enzyme E2M), UBE2O (ubiquitin-conjugating enzyme E2O), and UFC1 (ubiquitin-fold modifier conjugating enzyme 1) in comparison to HT animals. A similar bias was observed for both PSMα and PSMβ (proteasome subunits α and β, respectively). Upstream in the pathway, ubiquitin-like modifier activating enzyme 6 (UBA6), an E1-activating enzyme, was found to be reduced in low threshold/high threshold comparisons. CP, catalytic complex; RP, regulatory complex.

[ubiquitin-like modifier activating enzyme 6 (UBA6), **Fig. 4A**] was downregulated in the contralateral NAc of LT animals (no ipsilateral differences, data not shown). On the other hand, E2-like enzymes [ubiquitin-conjugating enzyme E2O (UBE2O) (**Fig. 4B**); ubiquitin-conjugating enzyme E2M (UBE2M) (**Fig. 4C**); and ubiquitin-fold modifier conjugating enzyme 1 (UFC1) (**Fig. 4D**)] were upregulated in contralateral NAc.

4. Discussion

To understand the molecular factors associated with the manifestation of pain and depression in pain susceptible or resistant rats, we performed a proteomic analysis of NAc-macrodissected tissue obtained from animals that evolved into these distinct conditions after SNI. Pain-resistant animals represented a small subset of SNI animals with no evident signs of allodynia. Such is aligned with previous results in SD rats.[37,45] Also, HT SNI animals presented higher hedonic behavior when compared with LT SNI rats, further indicating that the HT phenotype reflected reduced pain and pain negative affect. Importantly, because LT and HT are both SNI, the potential confounding effect of the peripheral lesion on subsequent analysis could be excluded. In addition, although generalization to pain models other than traumatic neuropathies was not evaluated, our approach on spontaneously resistant/susceptible animals is closer to that observed in clinical settings. For instance, in a well-known study that analyzed pain trajectories in subjects with a subacute low back pain episode, susceptible and resistant subjects presented at baseline similar pain scores, although the former scored worst in mood-related parameters.[12] Interestingly, stronger functional connectivity between the NAc and the prefrontal cortex predicted the evolution to CP.[12]

In our analysis of the NAc's proteomic landscape from resistant and susceptible rats, both biological process enrichment and KEGG pathway enrichment analyses strongly pointed to differences in proteasome-related mechanisms. The proteasome is a large protein complex responsible for protein degradation and, therefore, essential for homeostasis. The most studied form of the proteasome is the 26S (apparent sedimentation coefficient[82]); it is constituted by 2 complexes, a 20S catalytic core and 1 or 2 terminal 19S regulatory complexes that unfold and inject client proteins into the former—see for review.[14,19,26,27,62,74] The 20S is a cylinder-like structure formed by 2 α-rings and 2 β-rings each made of 7 structurally similar α or β subunits, respectively (**Fig. 5**); the former has a gate function, and the latter contains the proteolytically active sites (references above). In our study, several α and β 20S subunits were present in relatively higher amounts in LT animals. Another set of evidence in our data comes from the analysis of ubiquitin and related players. The ubiquitination of target proteins through multilayered and reversible enzymatic reactions acts as a tag for proteasomal degradation.[68] Intriguingly, although ubiquitin-conjugating enzymes (E2), which are critical to ubiquitin/protein attachment,[72] were also augmented in LT/HT comparisons, specifically UBE2O,[76] UBE2M, and UFC1,[83] and the ubiquitin-activating enzyme (E1) UBA6, an essential enzyme to activate ubiquitin,[15,40,44] was reduced in LT/HT comparisons (**Fig. 5**), which might reflect an UPS upstream regulation mechanism.

It is not clear if such molecular landscape reflects pain susceptibility/resistance or is an adaptation to ongoing pain. However, previous proteomic studies in CP models found no or limited evidence of alterations in UPS players—[33,65,73] dorsal root ganglia,[54] sciatic nerve,[48] brainstem,[3] and amygdala[51]—in neuropathic pain models. Also, a systematic review of proteomic studies (muscle, blood, saliva, and cerebrospinal fluid) on several human chronic pain conditions showed a similar picture.[41] Surprisingly, proteasome inhibitors, such as α,β-epoxy-ketone tetrapeptide epoxomicin (intrathecal),[64] lactacystin (intrathecal),[61] and MG-132 (intrathecal and subcutaneous),[1,80,81] have systematically shown to attenuate allodynic and hyperalgesic responses in neuropathic pain—see for review.[20] In addition, the proteasome was associated with the manifestation of depressive-like behaviors. Transgenic mice with a deletion of the proteasome α3 subunit (α3ΔN) N-terminal tail—the open-gate mutant[25]—contrary to wild-type controls, presented a reduced depressive-like phenotype in the forced-swimming test after chronic stress.[53] Indeed, proteasomal *PSMA7*, *PSMD9,* and *PSMD13* genes have also been associated with depression and antidepressant response in humans.[43,59] Finally, pharmacological interventions with upstream UPS inhibitors, such as UBC9 protein (E2 SUMO-conjugating enzyme) inhibitors, ameliorated neuropathic pain symptoms and decreased $Na_V1.7$-associated currents.[38]

Altogether, evidence indicates that protein clearance processes are enhanced particularly in the NAc of LT animals, probably because of increased protein aggregation and/or cellular stress. Indeed, susceptible and resistant animals also differed in pathways related to neurodegenerative disorders—such as Alzheimer, Parkinson, and Huntington diseases—see Ref. 8 for a review on shared pathophysiological mechanisms. More importantly, by placing our attention on a small subset of neuropathic but pain resistant rats, we were able to associate (un) favorable pain trajectories and the NAc's UPS.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgments

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Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/B262.

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References

[1] Ahmed AS, Ahmed M, Li J, Gu HF, Bakalkin G, Stark AA, Harris HE. Proteasome inhibitor MG132 modulates inflammatory pain by central mechanisms in adjuvant arthritis. Int J Rheum Dis 2017;20:25–32.
[2] Alves ND, Correia JS, Patrício P, Mateus-Pinheiro A, Machado-Santos AR, Loureiro-Campos E, Morais M, Bessa JM, Sousa N, Pinto L. Adult hippocampal neuroplasticity triggers susceptibility to recurrent depression. Transl Psychiatry 2017;7.
[3] Alzate O, Hussain SRA, Goettl VM, Tewari AK, Madiai F, Stephens RL, Hackshaw KV. Proteomic identification of brainstem cytosolic proteins in a neuropathic pain model. Mol Brain Res 2004;128:193–200.
[4] Anjo SI, Santa C, Manadas B. Short GeLC-SWATH: a fast and reliable quantitative approach for proteomic screenings. Proteomics 2015;15: 757–62.
[5] Anjo SI, Santa C, Manadas B. SWATH-MS as a tool for biomarker discovery: from basic research to clinical applications. Proteomics 2017; 17:3–4.
[6] Anjo SI, Simões I, Castanheira P, Grãos M, Manadas B. A generic normalization method for proper quantification in untargeted proteomics screening. bioRxiv 2018.
[7] Apkarian AV, Baliki MN, Farmer MA. Predicting transition to chronic pain. Curr Opin Neurol 2013;26:360–7.
[8] Apkarian AV, Scholz J. Shared mechanisms between chronic pain and neurodegenerative disease. Drug Discov Today Dis Mech 2006;3.
[9] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene Ontology: tool for the unification of biology. Nat Genet 2000;25:35–29.
[10] Austin PJ, Beyer K, Bembrick AL, Keay KA. Peripheral nerve injury differentially regulates dopaminergic pathways in the nucleus accumbens of rats with either "pain alone" or "pain and disability. Neuroscience 2010; 171:329–43.
[11] Baliki MN, Chang PC, Baria AT, Centeno MV, Apkarian AV. Resting-sate functional reorganization of the rat limbic system following neuropathic injury. Sci Rep 2014;4:6186.
[12] Baliki MN, Petre B, Torbey S, Herrmann KM, Huang L, Schnitzer TJ, Fields HL, Apkarian AV. Corticostriatal functional connectivity predicts transition to chronic back pain. Nat Neurosci 2012;15:1117–19.
[13] Baliki MN, Schnitzer TJ, Bauer WR, Apkarian AV. Brain morphological signatures for chronic pain. PLoS One 2011;6:e26010.
[14] Bard JAM, Goodall EA, Greene ER, Jonsson E, Dong KC, Martin A. Structure and function of the 26S proteasome. Annu Rev Biochem 2018; 87:697–724.
[15] Bialas J, Groettrup M, Aichem A. Conjugation of the ubiquitin activating enzyme UBE1 with the ubiquitin-like modifier FAT10 targets it for proteasomal degradation. PLoS One 2015;10:e0120329.
[16] Bilbao A, Falfan-Melgoza C, Leixner S, Becker R, Singaravelu SK, Sack M, Sartorius A, Spanagel R, Weber-Fahr W, Falfán-Melgoza C, Leixner S, Becker R, Singaravelu SK, Sack M, Sartorius A, Spanagel R, Weber-Fahr W, Falfan-Melgoza C, Leixner S, Becker R, Singaravelu SK, Sack M, Sartorius A, Spanagel R, Weber-Fahr W, Falfán-Melgoza C, Leixner S, Becker R, Singaravelu SK, Sack M, Sartorius A, Spanagel R, Weber-Fahr W, Falfan-Melgoza C, Leixner S, Becker R, Singaravelu SK, Sack M, Sartorius A, Spanagel R, Weber-Fahr W. Longitudinal structural and functional brain network alterations in a mouse model of neuropathic pain. Neuroscience 2018;387:104–15.
[17] Borsook D, Linnman C, Faria V, Strassman AM, Becerra L, Elman I. Reward deficiency and anti-reward in pain chronification. Neurosci Biobehav Rev 2016;68:282–97.
[18] Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur J Pain 2006;10:287–333.
[19] Budenholzer L, Cheng CL, Li Y, Hochstrasser M. Proteasome structure and assembly. J Mol Biol 2017;429:3500–24.
[20] Caputi FF, Rullo L, Stamatakos S, Candeletti S, Romualdi P. Interplay between the endogenous opioid system and proteasome complex: beyond signaling. Int J Mol Sci 2019;20.
[21] Carbon S, Douglass E, Dunn N, Good B, Harris NL, Lewis SE, Mungall CJ, Basu S, Chisholm RL, Dodson RJ, Hartline E, Fey P, Thomas PD, Albou LP, Ebert D, Kesling MJ, Mi H, Muruganujan A, Huang X, Poudel S, Mushayahama T, Hu JC, LaBonte SA, Siegele DA, Antonazzo G, Attrill H, Brown NH, Fexova S, Garapati P, Jones TEM, Marygold SJ, Millburn GH, Rey AJ, Trovisco V, Dos Santos G, Emmert DB, Falls K, Zhou P, Goodman JL, Strelets VB, Thurmond J, Courtot M, Osumi DS, Parkinson H, Roncaglia P, Acencio ML, Kuiper M, Lreid A, Logie C, Lovering RC, Huntley RP, Denny P, Campbell NH, Kramarz B, Acquaah V, Ahmad SH, Chen H, Rawson JH, Chibucos MC, Giglio M, Nadendla S, Tauber R, Duesbury MJ, Del NT, Meldal BHM, Perfetto L, Porras P, Orchard S, Shrivastava A, Xie Z, Chang HY, Finn RD, Mitchell AL, Rawlings ND, Richardson L, Sangrador-Vegas A, Blake JA, Christie KR, Dolan ME, Drabkin HJ, Hill DP, Ni L, Sitnikov D, Harris MA, Oliver SG, Rutherford K, Wood V, Hayles J, Bahler J, Lock A, Bolton ER, De Pons J, Dwinell M, Hayman GT, Laulederkind SJF, Shimoyama M, Tutaj M, Wang SJ, D'Eustachio P, Matthews L, Balhoff JP, Aleksander SA, Binkley G, Dunn BL, Cherry JM, Engel SR, Gondwe F, Karra K, MacPherson KA, Miyasato SR, Nash RS, Ng PC, Sheppard TK, Shrivatsav Vp A, Simison M, Skrzypek MS, Weng S, Wong ED, Feuermann M, Gaudet P, Bakker E, Berardini TZ, Reiser L, Subramaniam S, Huala E, Arighi C, Auchincloss A, Axelsen K, Argoud GP, Bateman A, Bely B, Blatter MC, Boutet E, Breuza L, Bridge A, Britto R, Bye-A-Jee H, Casals-Casas C, Coudert E, Estreicher A, Famiglietti L, Garmiri P, Georghiou G, Gos A, Gruaz-Gumowski N, Hatton-Ellis E, Hinz U, Hulo C, Ignatchenko A, Jungo F, Keller G, Laiho K, Lemercier P, Lieberherr D, Lussi Y, Mac-Dougall A, Magrane M, Martin MJ, Masson P, Natale DA, Hyka NN, Pedruzzi I, Pichler K, Poux S, Rivoire C, Rodriguez-Lopez M, Sawford T, Speretta E, Shypitsyna A, Stutz A, Sundaram S, Tognolli M, Tyagi N, Warner K, Zaru R, Wu C, Chan J, Cho J, Gao S, Grove C, Harrison MC, Howe K, Lee R, Mendel J, Muller HM, Raciti D, Van Auken K, Berriman M, Stein L, Sternberg PW, Howe D, Toro S, Westerfield M. The gene ontology resource: 20 years and still GOing strong. Nucleic Acids Res 2019;47:D330–8.
[22] Carbon S, Ireland A, Mungall CJ, Shu S, Marshall B, Lewis S, Lomax J, Mungall C, Hitz B, Balakrishnan R, Dolan M, Wood V, Hong E, Gaudet P. AmiGO: online access to ontology and annotation data. Bioinformatics 2009;25:288–9.
[23] Chang PC, Pollema-Mays SL, Centeno MV, Procissi D, Contini M, Baria AT, Martina M, Apkarian AV. Role of nucleus accumbens in neuropathic pain: linked multi- scale evidence in the rat transitioning to neuropathic pain. PAIN 2014;155:1128–39.
[24] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53:55–63.
[25] Choi WH, De Poot SAH, Lee JH, Kim JH, Han DH, Kim YK, Finley D, Lee MJ. Open-gate mutants of the mammalian proteasome show enhanced ubiquitin-conjugate degradation. Nat Commun 2016;7:1–12.
[26] Collins GA, Goldberg AL. The logic of the 26S proteasome. Cell 2017; 169:792–806.
[27] Coux O, Zieba BA, Meiners S. The proteasome system in Health and disease. Adv Exp Med Biol 2020;1233:55–100.
[28] Cunha AM, Esteves M, Pereira-Mendes J, Guimarães MR, Almeida A, Leite-Almeida H. High trait impulsivity potentiates the effects of chronic pain on impulsive behavior. Neurobiol Pain 2019:100042.
[29] Cunha AM, Guimarães MR, Kokras N, Sotiropoulos I, Sousa N, Almeida A, Dalla C, Leite-Almeida H. Mesocorticolimbic monoamines in a rodent model of chronic neuropathic pain. Neurosci Lett 2020:737.
[30] Cunha AM, Pereira-Mendes J, Almeida A, Guimarães MR, Leite-Almeida H. Chronic pain impact on rodents' behavioral repertoire. Neurosci Biobehav Rev 2020;119:101–27.
[31] Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. PAIN 2000;87:149–58.
[32] Deutsch EW, Bandeira N, Sharma V, Perez-Riverol Y, Carver JJ, Kundu DJ, García-Seisdedos D, Jarnuczak AF, Hewapathirana S, Pullman BS, Wertz J, Sun Z, Kawano S, Okuda S, Watanabe Y, Hermjakob H, MacLean B, MacCoss MJ, Zhu Y, Ishihama Y, Vizcaíno JA. The ProteomeXchange consortium in 2020: enabling "big data" approaches in proteomics. Nucleic Acids Res 2020;48:D1145–52.
[33] Ding Q, Wu Z, Quo Y, Zhao C, Jia Y, Kong F, Chen B, Wang H, Xiong S, Que H, Jing S, Liu S. Proteome analysis of up-regulated proteins in the rat spinal cord induced by transection injury. Proteomics 2006;6:505–18.
[34] Elina K, Moon HC, Soochong K, Kim HK, Won SY, Hyun S-H, Park YS. Optical modulation on the nucleus accumbens core in the alleviation of neuropathic pain in chronic dorsal root ganglion compression rat model. Neuromodulation 2020;23:167–76.
[35] Esteves M, Almeida AM, Silva J, Silva Moreira P, Carvalho E, Pêgo JM, Almeida A, Sotiropoulos I, Sousa N, Leite-Almeida H. MORPhA Scale: behavioral and electroencephalographic validation of a rodent anesthesia scale. J Neurosci Methods 2019;324:108304.

[36] Farmer MA, Baliki MN, Apkarian AV. A dynamic network perspective of chronic pain. Neurosci Lett 2013;520:197–203.
[37] De Felice M, Sanoja R, Wang R, Vera-Portocarrero L, Oyarzo J, King T, Ossipov MH, Vanderah TW, Lai J, Dussor GO, Fields HL, Price TJ, Porreca F. Engagement of descending inhibition from the rostral ventromedial medulla protects against chronic neuropathic pain. PAIN 2011;152:2701–9.
[38] Francois-Moutal L, Dustrude ET, Wang Y, Brustovetsky T, Dorame A, Ju W, Moutal A, Perez-Miller S, Brustovetsky N, Gokhale V, Khanna M, Khanna R. Inhibition of the Ubc9 E2 SUMO-conjugating enzyme-CRMP2 interaction decreases NaV1.7 currents and reverses experimental neuropathic pain. PAIN 2018;159:2115–27.
[39] García-González D, Dumitru I, Zuccotti A, Yen T-Y, Herranz-Pérez V, Tan LL, Neitz A, García-Verdugo JM, Kuner R, Alfonso J, Monyer H. Neurogenesis of medium spiny neurons in the nucleus accumbens continues into adulthood and is enhanced by pathological pain. Mol Psychiatry 2020:1–17.
[40] Gavin JM, Chen JJ, Liao H, Rollins N, Yang X, Xu Q, Ma J, Loke H-K, Lingaraj T, Brownell JE, Mallender WD, Gould AE, Amidon BS, Dick LR. Mechanistic studies on activation of ubiquitin and di-ubiquitin-like protein, FAT10, by ubiquitin-like modifier activating enzyme 6, Uba6. J Biol Chem 2012;287:15512–22.
[41] Gerdle B, Ghafouri B. Proteomic studies of common chronic pain conditions - a systematic review and associated network analyses. Expert Rev Proteomics 2020;17:483–506.
[42] Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, Scott G, Steffen D, Worley KC, Burch PE, Okwuonu G, Hines S, Lewis L, DeRamo C, Delgado O, Dugan-Rocha S, Miner G, Morgan M, Hawes A, Gill R, Celera, Holt RA, Adams MD, Amanatides PG, Baden-Tillson H, Barnstead M, Chin S, Evans CA, Ferriera S, Fosler C, Glodek A, Gu Z, Jennings D, Kraft CL, Nguyen T, Pfannkoch CM, Sitter C, Sutton GG, Venter JC, Woodage T, Smith D, Lee H-M, Gustafson E, Cahill P, Kana A, Doucette-Stamm L, Weinstock K, Fechtel K, Weiss RB, Dunn DM, Green ED, Blakesley RW, Bouffard GG, De Jong PJ, Osoegawa K, Zhu B, Marra M, Schein J, Bosdet I, Fjell C, Jones S, Krzywinski M, Mathewson C, Siddiqui A, Wye N, McPherson J, Zhao S, Fraser CM, Shetty J, Shatsman S, Geer K, Chen Y, Abramzon S, Nierman WC, Havlak PH, Chen R, Durbin KJ, Egan A, Ren Y, Song XZ, Li B, Liu Y, Qin X, Cawley S, Worley KC, Cooney AJ, D'Souza LM, Martin K, Wu JQ, Gonzalez-Garay ML, Jackson AR, Kalafus KJ, McLeod MP, Milosavljevic A, Virk D, Volkov A, Wheeler DA, Zhang Z, Bailey JA, Eichler EE, Tuzun E, Birney E, Mongin E, Ureta-Vidal A, Woodwark C, Zdobnov E, Bork P, Suyama M, Torrents D, Alexandersson M, Trask BJ, Young JM, Huang H, Wang H, Xing H, Daniels S, Gietzen D, Schmidt J, Stevens K, Vitt U, Wingrove J, Camara F, Mar Alba M, Abril JF, Guigo R, Smit A, Dubchak I, Rubin EM, Couronne O, Poliakov A, Hubner N, Ganten D, Goesele C, Hummel O, Kreitler T, Lee Y-A, Monti J, Schulz H, Zimdahl H, Himmelbauer H, Lehrach H, Jacob HJ, Bromberg S, Gullings-Handley J, Jensen-Seaman MI, Kwitek AE, Lazar J, Pasko D, Tonellato PJ, Twigger S, Ponting CP, Duarte JM, Rice S, Goodstadt L, Beatson SA, Emes RD, Winter EE, Webber C, Brandt P, Nyakatura G, Adetobi M, Chiaromonte F, Elnitski L, Eswara P, Hardison RC, Hou M, Kolbe D, Makova K, Miller W, Nekrutenko A, Riemer C, Schwartz S, Taylor J, Yang S, Zhang Y, Lindpaintner K, Andrews TD, Caccamo M, Clamp M, Clarke L, Curwen V, Durbin R, Eyras E, Searle SM, Cooper GM, Batzoglou S, Brudno M, Sidow A, Stone EA, Venter JC, Payseur BA, Bourque G, Lopez-Otin C, Puente XS, Chakrabarti K, Chatterji S, Dewey C, Pachter L, Bray N, Yap VB, Caspi A, Tesler G, Pevzner PA, Haussler D, Roskin KM, Baertsch R, Clawson H, Furey TS, Hinrichs AS, Karolchik D, Kent WJ, Rosenbloom KR, Trumbower H, Weirauch M, Cooper DN, Stenson PD, Ma B, Brent M, Arumugam M, Shteynberg D, Copley RR, Taylor MS, Riethman H, Mudunuri U, Peterson J, Guyer M, Felsenfeld A, Old S, Mockrin S, Collins F. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature 2004;428:493–521.
[43] Gragnoli C. Proteasome modulator 9 and depression in type 2 diabetes. Curr Med Chem 2012;19:5178–80.
[44] Groettrup M, Pelzer C, Schmidtke G, Hofmann K. Activating the ubiquitin family: UBA6 challenges the field. Trends Biochem Sci 2008;33:230–7.
[45] Guimarães MR, Soares AR, Cunha AM, Esteves M, Borges S, Magalhães R, Moreira PS, Rodrigues AJ, Sousa N, Almeida A, Leite-Almeida H. Evidence for lack of direct causality between pain and affective disturbances in a rat peripheral neuropathy model. Genes, Brain Behav 2019;18:e12542.
[46] Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1–13.
[47] Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57.
[48] Jiménez CR, Stam FJ, Li KW, Gouwenberg Y, Hornshaw MP, De Winter F, Verhaagen J, Smit AB, Jimenez CR, Stam FJ, Li KW, Gouwenberg Y, Hornshaw MP, De Winter F, Verhaagen J, Smit AB. Proteomics of the injured rat sciatic nerve reveals protein expression dynamics during regeneration. Mol Cell Proteomics 2005;4:120–32.
[49] Johannes CB, Le TK, Zhou X, Johnston JA, Dworkin RH. The prevalence of chronic pain in United States adults: results of an Internet-based survey. J Pain 2010;11:1230–9.
[50] Kamburov A, Stelzl U, Lehrach H, Herwig R. The ConsensusPathDB interaction database: 2013 Update. Nucleic Acids Res 2013;41: 793–800.
[51] Karu K, Swanwick RS, Novejarque-Gadea A, Antunes-Martins A, Thomas B, Yoshimi E, Foster W, Fang M, McMahon SB, Bennett DLH, Rice ASC, Okuse K. Quantitative proteomic analysis of the central amygdala in neuropathic pain model rats. J Proteome Res 2020;19: 1592–619.
[52] Kato T, Ide S, Minami M. Pain relief induces dopamine release in the rat nucleus accumbens during the early but not late phase of neuropathic pain. Neurosci Lett 2016;629:73–8.
[53] Kim JH, Kim A, Yun Y, Park S, Lee JH, Lee YS, Lee MJ. Reduced chronic restraint stress in mice overexpressing hyperactive proteasomes in the forebrain. Mol Brain 2020;13:1–4.
[54] Komori N, Takemori N, Hee KK, Singh A, Hwang SH, Foreman RD, Chung K, Jin MC, Matsumoto H. Proteomics study of neuropathic and nonneuropathic dorsal root ganglia: altered protein regulation following segmental spinal nerve ligation injury. Physiol Genomics 2007;29: 215–30.
[55] Leite-Almeida H, Almeida-Torres L, Mesquita AR, Pertovaara A, Sousa N, Cerqueira JJ, Almeida A. The impact of age on emotional and cognitive behaviours triggered by experimental neuropathy in rats. PAIN 2009;144: 57–65.
[56] Leite-Almeida H, Cerqueira JJ, Wei H, Ribeiro-Costa N, Anjos-Martins H, Sousa N, Pertovaara A, Almeida A. Differential effects of left/right neuropathy on rats' anxiety and cognitive behavior. PAIN 2012;153: 2218–25.
[57] Leite-Almeida H, Guimarães MR, Cerqueira JJ, Ribeiro-Costa N, Anjos-Martins H, Sousa N, Almeida A. Asymmetric c-Fos expression in the ventral orbital cortex is associated with impaired reversal learning in a right-sided neuropathy. Mol Pain 2014;10:1744–8069–10–41.
[58] Leite-Almeida H, Pinto-Ribeiro F, Almeida A. Animal models for the study of comorbid pain and psychiatric disorders. Mod Trends Pharmacopsychiatry 2015;30:1–21.
[59] Minelli A, Magri C, Barbon A, Bonvicini C, Segala M, Congiu C, Bignotti S, Milanesi E, Trabucchi L, Cattane N, Bortolomasi M, Gennarelli M. Proteasome system dysregulation and treatment resistance mechanisms in major depressive disorder. Transl Psychiatry 2015;5:e687.
[60] Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. Prog Neurobiol 2011;93: 385–404.
[61] Moss A, Blackburn-Munro G, Garry EM, Blakemore JA, Dickinson T, Rosie R, Mitchell R, Fleetwood-Walker SM. A role of the ubiquitin-proteasome system in neuropathic pain. J Neurosci 2002;22:1363–72.
[62] Murata S, Yashiroda H, Tanaka K. Molecular mechanisms of proteasome assembly. Nat Rev Mol Cell Biol 2009;10:104–15.
[63] Navratilova E, Atcherley C, Porreca F. Brain circuits encoding reward from pain relief. Trends Neurosci 2015;38:741–50.
[64] Ossipov MH, Bazov I, Gardell LR, Kowal J, Yakovleva T, Usynin I, Ekstrom TJ, Porreca F, Bakalkin G, Ekström TJ, Porreca F, Bakalkin G. Control of chronic pain by the ubiquitin-proteasome system in the spinal cord. J Neurosci 2007;27:8226–37.
[65] Park E, Ahn J, Jeon S, Cho H, Chung K, Cho J, Youn D. Proteomic analysis of the dorsal spinal cord in the mouse model of spared nerve injury-induced neuropathic pain. J Biomed Res 2017;0:1–9.
[66] Perez-Riverol Y, Csordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ, Inuganti A, Griss J, Mayer G, Eisenacher M, Pérez E, Uszkoreit J, Pfeuffer J, Sachsenberg T, Yilmaz S, Tiwary S, Cox J, Audain E, Walzer M, Jarnuczak AF, Ternent T, Brazma A, Vizcaíno JA. The PRIDE database and related tools and resources in 2019: improving support for quantification data. Nucleic Acids Res 2019;47: D442–50.
[67] Perez-Riverol Y, Xu QW, Wang R, Uszkoreit J, Griss J, Sanchez A, Reisinger F, Csordas A, Ternent T, Del-Toro N, Dianes JA, Eisenacher M, Hermjakob H, Vizcaíno JA. PRIDE inspector toolsuite: moving toward a universal visualization tool for proteomics data standard Formats and quality assessment of ProteomeXchange datasets. Mol Cell Proteomics 2016;15:305–17.
[68] Pohl C, Dikic I. Cellular quality control by the ubiquitin-proteasome system and autophagy. Science 2019;366:818–22.

[69] Pollema-Mays SL, Centeno MV, Chang Z, Apkarian AV, Martina M. Reduced ΔFosB expression in the rat nucleus accumbens has causal role in the neuropathic pain phenotype. Neurosci Lett 2018:0–1.
[70] Schwartz N, Temkin P, Jurado S, Lim BK, Heifets BD, Polepalli JS, Malenka RC. Chronic pain. Decreased motivation during chronic pain requires long-term depression in the nucleus accumbens. Science 2014;345:535–42.
[71] Serafini RA, Pryce KD, Zachariou V. The mesolimbic dopamine system in chronic pain and associated affective comorbidities. Biol Psychiatry 2020;87:64–73.
[72] Stewart MD, Ritterhoff T, Klevit RE, Brzovic PS. E2 enzymes: more than just middle men. Cell Res 2016;26:423–40.
[73] Sui P, Watanabe H, Ossipov MH, Bakalkin G, Artemenko K, Bergquist J. Proteomics of neuropathic Pain : proteins and signaling pathways affected in a rat model. J Proteome Res 2014;13:3957–65.
[74] Tanaka K. The proteasome: overview of structure and functions. Proc Jpn Acad Ser B Phys Biol Sci 2009;85:12–36.
[75] Tsang A, Von Korff M, Lee S, Alonso J, Karam E, Angermeyer MC, Borges GLG, Bromet EJ, de Girolamo G, de Graaf R, Gureje O, Lepine JP, Haro JM, Levinson D, Oakley Browne MA, Posada-Villa J, Seedat S, Watanabe M. Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders. J Pain 2008;9:883–91.
[76] Ullah K, Zubia E, Narayan M, Yang J, Xu G. Diverse roles of the E2/E3 hybrid enzyme UBE2O in the regulation of protein ubiquitination, cellular functions, and disease onset. FEBS J 2019;286:2018–34.
[77] Vachon-Presseau E, Centeno MV, Ren W, Berger SE, Tetreault P, Ghantous M, Baria A, Farmer M, Baliki MN, Schnitzer TJ, Apkarian AV. The emotional brain as a predictor and amplifier of chronic pain. J Dent Res 2016;95:605–12.
[78] Wawrzczak-Bargieła A, Ziółkowska B, Piotrowska A, Starnowska-Sokół J, Rojewska E, Mika J, Przewłocka B, Przewłocki R. Neuropathic pain dysregulates gene expression of the forebrain opioid and dopamine systems. Neurotox Res 2020;37:800–14.
[79] Xie JY, Qu C, Patwardhan A, Ossipov MH, Navratilova E, Becerra L, Borsook D, Porreca F. Activation of mesocorticolimbic reward circuits for assessment of relief of ongoing pain: a potential biomarker of efficacy. PAIN 2014;155:1659–66.
[80] Yang L, Wang S, Lim G, Sung B, Zeng Q, Mao J. Inhibition of the ubiquitin-proteasome activity prevents glutamate transporter degradation and morphine tolerance. PAIN 2008;140:472–8.
[81] Yang L, Wang S, Sung B, Lim G, Mao J. Morphine induces ubiquitin-proteasome activity and glutamate transporter degradation. J Biol Chem 2008;283:21703–13.
[82] Yoshimura T, Kameyama K, Takagi T, Ikai A, Tokunaga F, Koide T, Tanahashi N, Tamura T, Cejka Z, Baumeister W, Tanaka K, Ichihara A. Molecular characterization of the "26s" proteasome complex from rat liver. J Struct Biol 1993;111:200–11.
[83] Zhou W, Xu J, Tan M, Li H, Li H, Wei W, Sun Y. UBE2M is a stress-inducible dual E2 for Neddylation and ubiquitylation that promotes targeted degradation of UBE2F. Mol Cell 2018;70:1008–24.e6.