SCI-INDUCED MORPHINE TOLERANCE IS ASSOCIATED WITH DOPAMINE PATHWAY EXPRESSION

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I. Abstract

Opioids are commonly prescribed to relieve neuropathic pain after a spinal cord injury (SCI),[4] but often fail to be effective due to an injury-induced state that mimics opioid tolerance.[22] Previous studies have shown that the analgesic effects of morphine can be restored if morphine is administered in combination with a dopamine D3 receptor agonist or a dopamine D1 receptor antagonist, demonstrating that dopamine receptor activity modulates the response to opioids after SCI.[5] Therefore, it was hypothesized that SCI alters levels of dopamine and expression of its receptors in the brain and spinal cord and that these changes are associated with injury-induced morphine tolerance. Baseline nociceptive (pain) thresholds were measured in 8 uninjured and 16 spinal cord injured rats before and after injection of morphine (2mg/kg) or saline (control). Rats were then randomized to have thresholds re-assessed after injection of morphine + pramipexole (PPX, D3 agonist), morphine + SCH 39166 (SCH, D1 antagonist), pramipexole, or SCH. Lumbar spinal cord and striatal brain tissue were collected from each animal and processed for metabolomics, targeted mass spectrometry (MS) and Western blot to identify, quantify and compare levels of dopamine and its metabolites and receptors across groups. Morphine alone increased sensory thresholds in all uninjured but only 33% of injured rats. Based on this data, animals were categorized as morphine responders (n=5) or nonresponders (n=10). Morphine + PPX and morphine + SCH increased sensory thresholds in all injured animals, while PPX and SCH alone had no effect. Striatal dopamine levels in injured morphine nonresponders were significantly decreased compared to uninjured animals. Dopamine levels in injured morphine responders compared to injured morphine nonresponders are currently being analyzed further. Metabolomics principal component analysis (PCA) of lumbar cord identified three clusters that corresponded to injured morphine responders, injured morphine nonresponders, and uninjured animals. Preliminary pathway analysis points to differences in phenylalanine, tyrosine, and

tryptophan biosynthesis between these groups. Full pathway analysis is ongoing, but data suggests that differential dopaminergic pathway expression in the CNS following SCI is associated with morphine responsiveness. This provides early evidence that the dopamine system may provide a target for intervention in opioid resistant pain states.

II. Background and Introduction

Approximately 17,700 new cases of spinal cord injury are recorded in the United States each year.[19] Neuropathic pain presents in 53% of these cases, a prevalence rate greater than both musculoskeletal and visceral pain after SCI.[9] Quality of life is typically significantly reduced in patients experiencing chronic neuropathic pain,[3] especially when combined with SCI. When surveyed, these patients often report dysesthesia, allodynia, hyperalgesia, and other neuropathic symptoms that can persist for years after injury and mechanical recovery.[4,15] Neuropathic pain is also known to result in sleep disturbances and, consequently, heightened anxiety and depression.[20] One study found that as many as 43% of neuropathic pain patients suffered some form of unemployment as a result of their pain, often citing sleep deprivation as a major factor.[18] These physical, psychological, and sociological barriers demand a safe and effective treatment for neuropathic pain to restore quality of life for SCI patients.

Neuropathic pain remains one of the most difficult conditions to treat.[25] The potent analgesic property of opioids[14] has made them a common therapy for neuropathic pain after SCI,[12] but increased awareness of their negative side effects has assigned them clinical ambivalence in recent decades. While the increase in opioid prescriptions from 76 million in 1991 to 219 million in 2011[21] speaks to their availability and clinical relevance, the potential to develop tolerance and addiction to the substance over longer periods of use remains a primary

deterrent for opioid treatment under chronic pain conditions.[6] This tolerance from months of opioid treatment can lead to dangerously elevated doses, in some cases as high as three times the dosage of opioid-naïve patients[11], to achieve adequate analgesia while also increasing the likelihood of addiction from repeated exposure. Cases of dampened opioid analgesia in postoperative neuropathic pain also suggests that the injury itself can facilitate conditions for opioid tolerance without recent exposure to the drug.[5]

While hypotheses vary, it is clear that the development of a drug or drug combination that provides analgesia in response to neuropathic pain after SCI without increasing risk of tolerance is necessary to improving quality of life and overall patient outcomes. The relationship between μ-opioid receptors (MORs) and dopamine 3 receptors (D3Rs) and dopamine 1 receptors (D1Rs) provides a potential therapeutic mechanism. MORs, D3Rs, and D1Rs have all been shown to be present in the same neurons within the spinal dorsal horn,[1,10] an area involved in processing peripheral painful stimuli. MOR or D3R activation inhibits phosphorylation of the membrane, allowing these nociceptive neurons to remain sensitive to opioid binding. D1R activation offers an opposing action by stimulating opioid receptor phosphorylation and desensitizing the membrane to opioid binding.[16] Opioid tolerance has been shown to result in an increased ratio of D1Rs to D3Rs, creating an imbalanced net effect that decreases membrane MOR availability and prevents morphine from binding to receptors that provide analgesia (Figure 1).[7,20,23] D3R agonists and D1R antagonists function to reverse this effect.[8] In a preliminary study, 33% (n=5) of the rat population received analgesia from morphine treatment after spinal cord injury while 66% (n=10) did not, exhibiting a state of injury-induced morphine tolerance. When these morphine-tolerant rats were treated with a combination of morphine and pramipexole (D3 agonist) or a combination of morphine and SCH39166 (D1 antagonist), a significant increase in analgesia was observed. This demonstrates that dopamine plays an important role in post-SCI

tolerance modulation, but does not reveal the injury's direct effect on dopamine levels or if a change in dopamine levels is integral to the development of tolerance in the opioid receptor system.

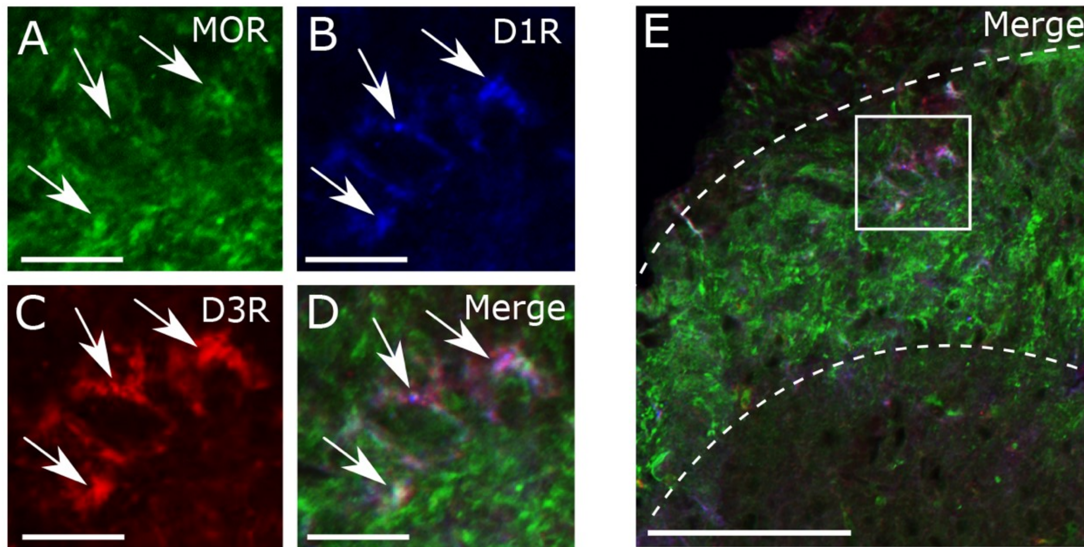


Figure 1. Immunohistochemistry of the spinal dorsal horn. Panels A-D are all represented by the white box in panel E, showing colocalization of μ-opioid receptors (A), D1Rs (B), and D3Rs (C).

Dopamine's intimate relationship with the opioid receptor system provides the opportunity for an indirect approach to restoring morphine efficacy, but few studies have examined how an injury such as SCI alters the quantity of available dopamine. A sharp decline in D1Rs in the periaqueductal gray (PAG) region of the brain was reported by Voulalas, et al,[26] but literature regarding cortical levels of dopamine following SCI is scarce. Dopamine and key enzymes involved in dopamine formation such as tyrosine hydroxylase also appear to decrease within the cord in response to SCI.[13] These data highlight the link between SCI-induced dopaminergic changes and the morphine tolerance that results from altered interaction with the opioid receptor system as described above. With the goal of improving drug efficacy and

decreasing risk of tolerance for opioidergic treatments, dopamine's connection between spinal cord injury and opioid tolerance must be examined in greater depth. The current study aims to addresses the hypothesis that animals responsive to morphine will exhibit levels of DA and its metabolites similar to uninjured animals while those unresponsive to morphine will show comparatively decreased DA levels. In addition, SCI responders, nonresponders, and uninjured animals will express independent metabolite clustering.

III. Methods

<u>Animals</u>

Female Long-Evans rats (N=24) weighing 200-225g were purchased from Charles River Laboratories (Wilmington, MA). Animals were maintained on a 12-hour light–dark cycle in a temperature-controlled environment with food and water available continuously. Rats were housed in pairs prior to surgery but individually afterward. All experimental procedures complied with the National Institute of Health (NIH) guidelines for animal care and were approved by the East Carolina University Institutional Animal Care and Use Committee.

<u>Lesion</u>

Animals were randomly assigned to uninjured (n=8) or SCI (n=16) groups prior to surgery. A contusive spinal cord injury was produced using a microprocessor-controlled PCI3000 Precision Impactor (Hatteras Instruments, Cary, NC) with a 1mm round, blunt impact tip. Rats were temporarily anesthetized via Isoflurane (1-3% to effect) inhalation and secured stereotactically (Stoelting Co, Wood Dale, IL). After dorsal midline incision and vertebral column exposure, a spinous process and vertebral lamina were removed between T12-L1. The

contusion injury (velocity of impact = 0.8m/sec, depth of impact = 1.5mm, dwell time = 85ms) was induced 0.5mm right of midline. Deep tissue and muscles were sutured and skin was stapled to close. Since the nature and location of this injury can lead to bladder dysfunction[24], animals showing bladder distention were externally manipulated to achieve adequate urination until function returned. One rat died during surgery.

Post-SCI Assessment

Hindlimb motor function was assessed weekly in injured animals using the Basso, Beattie, Bresnahan (BBB) Locomotor Scale until pain threshold testing. The BBB scale assesses hindlimb movements, weight-bearing coordination, toe clearance, and trunk stability. Only animals that scored 15 points (range=0-21) or higher underwent nociceptive testing to ensure that motor impairment was not contributing to changes in nociceptive thresholds. Animals were scored while experimenters observed them for 4 minute in an open environment.

Nociceptive Testing

A TF-1 unit (Columbus Instruments, Columbus OH) was used to measure tail flick response time as a measure of nociceptive threshold. Each rat was covered with a pouch while its tail was placed on the heat conduit 4cm from the tip. A 10s cutoff latency was employed as a precaution. Average latency was calculated over 3 trials. Tail flick testing occurred 30min after drug injection. Testing occurred as follows. Prior to surgery all animals were assessed for their baseline thermal thresholds. Uninjured rats were randomized to receive either morphine (2.0mg/kg) or saline (0.9%). Half of the rats in each group were then assigned either PPX (0.1mg/kg) or SCH (0.1mg/kg) while the other half were assigned either morphine (2.0mg/kg) + PPX (0.1mg/kg) or morphine (2.0mg/kg) + SCH (0.1mg/kg). After surgery and 3 weeks of BBB testing, SCI rats were randomized to receive morphine or saline as described previously. SCI rats

lacking an analgesic response following morphine injection were categorized as morphine nonresponders (n=10) while the others remained morphine responders (n=5). Using matched pairing for morphine responsiveness animals were randomly assigned either PPX and morphine + PPX or SCH and morphine +SCH. All drugs were administered with a subcutaneous injection with a 48hr window between each injection.

Tissue Collection

After testing was complete, animals were deeply anesthetized and decapitated. The striatum was dissected bilaterally for mass spectrometry and Western blot analysis. Three sections of spinal cord were dissected: the area directly surrounding the injury for lesion analysis and two sections below injury for mass spectrometry and Western blot analysis. Sections for lesion analysis were placed into 4% paraformaldehyde while sections for mass spectrometry and Western blot were flash frozen in liquid nitrogen until placed in temporary storage.

Lesion Analysis

Injured spinal cord sections were stored in 4% paraformaldehyde for 24 hours followed by 30% sucrose for 48 hours. 75µm longitudinal sections were cut using a Leica 2400 freezing microtome (#030G, Matsunami, Bellingham, WA). Sections were placed in PBS before mounting on TruBond 380 adhesive microscope slides. Slides were stained with cresyl violet and imaged using an Olympus BX51 microscope with an Olympus DP70 camera. For each animal, the section of cord with the largest lesion area was used to quantify the extent of gray matter damage. Using Image J (NIH) after setting the scale, the max width and length of the lesion was measured in millimeters(mm). Width and length were then multiplied together to calculate lesion area. The total number of sections displaying the lesion were also summed.

Western Blot

Tissues were homogenized using an Omni TH tissue homogenizer (#TH01 Omni International, Kennesaw, GA) at a ratio of 10µl buffer to 1mg tissue. Homogenization buffer was comprised of RIPA buffer (#R0278 Sigma-Aldrich, St. Lois, MO) with 10% 10X protease and 1% phosphatase inhibitors (#87786 Thermo Fisher Scientific, Waltham, MA and #P5726 Sigma-Aldrich, St. Lois, MO). Following homogenization, samples were centrifuged (13,000 rpm, 4°C 15 min) and supernatants were aliquoted and stored at -80°C. Protein concentrations were determined using a Pierce BCA Protein Assay kit (#23227 Thermo Fisher Scientific, Waltham, MA). The 96 well assay plate was read at 562 nm using an Infinite M200 microplate reader and i-control software (Tecan Trading AG, Mannedorf, Switzerland).

For Western blots, 20 µg of each lumbar spinal cord and striatum samples were denatured using 2 x Laemmli buffer (#1610737 Bio-Rad Hercules, CA) containing 5% β-mercaptoethanol and 1% SDS at 96°C for 10 min. Samples were loaded onto an Any kD Criterion TGX StainFree Protein Gel. (#5671125 Bio-Rad Hercules, CA) and allowed to run for40 minutes at 200V. The gel was then activated with UV light for 45 seconds using the BioRad ChemiDoc Imaging System (#12003153 Bio-Rad Hercules, CA) The proteins were transferred to a low fluorescent PVDF membrane using the Trans-Blot Turbo RTA Midi LF PVDF Transfer Kit (#170-4275, Bio-Rad, Hercules, CA) and the Trans-Blot Turbo (#1704150 Bio-Rad Hercules, CA) on the 1 midi gel turbo setting. An image was taken of the stain-free blot for calculation of total protein using the BioRad ChemiDoc. Membranes were then blocked using Odyssey Blocking Buffer in TBS (#927-50000 LI-COR Licoln, NE) and probed with primary and secondary antibodies. Fluorescence imaging was performed using the Odyssey Clx imaging system (#9140 LI-COR Lincoln, NE).

Antibodies

The primary antibodies used were anti-dopamine receptor D1 (Bioss bs-1007r 1:500 Woburn, MA) and anti-dopamine receptor D3 (Abcam ab155098 1:1000 Cambridge, MA). The secondary antibody used was IRDye 800CW got anti-rabbit (LI-COR Biosciences 926-32211, 1:30,000 and 1:15,000 Lincoln, NE).

Tissue Preparation

Below-level lumbar cord and striatum samples were transferred to bead tubes, 400µl of methanol, and 10µl of deuterated dopamine were added. Samples were homogenized using a bead mill homogenizer (Fisherbrand 15-340-163) for 10 seconds and then sonicated for 60 seconds. Tubes were centrifuged at 12,000 rpm for 10 mins at 4°C and supernatant was collected. Extracted samples were stored at -80°C until further processing. Prior to analysis, samples were dried down using a nitrogen dryer and resuspended in 200 µl of 50:50 methanol:water with 0.1% formic acid.

Untargeted Metabolomics LC/MS

An Eksigent 425 microLC/SCIEX 5600+ triple time-of-flight mass spectrometer was used to conduct untargeted analysis. A Halo C18 0.5 x 50 mm 2.7 µm column was used for separation of the analytes on the micro LC. The flow rate was 10 µl/min and 5 µL of sample was injected. Mobile phase A: water with 0.1% formic acid and mobile phase B: acetonitrile. A linear gradient was utilized with a flow rate of 10 µL/min where the gradient started with 10% B for 2 min, increased to 90% B for 15 min, held for 5 min, dropped to 10%B over 2 min and equilibrated for 10 min for a total run time of 30 min.

Data was acquired for MS and MS/MS analysis using independent data acquisition for the topmost abundant 20 ions in positive ionization mode. The scan range for MS was 80-1250 Da. Principal component analysis (PCA) and t-tests were conducted using MarkerView 1.3.1. Putative metabolite/pathway identification was performed by using HMDB.ca database (molecular weight tolerance ± 0.05 DA, Adduct type: M+H, and M+Na) to identify peaks. MetaboAnalyst.ca was utilized to conduct pathway analysis (Fisher's Exact Test and relativebetweenness centrality, rattus norvegicus pathway library).

Targeted LC/MS

A ThermoSci hypersil gold 50 x 3 mm column was used for separation of the analytes on an Exion HPLC. The column temperature was maintained at 32°C. A gradient was used to separate the compounds using mobile phase A: 95:5 water with 0.1% formic acid:acetonitrile and mobile phase B: acetonitrile. A linear gradient was performed as follows: 0% B for 2 min, 90% B for 9 min, 90% B for 1 min, 0% B for 1 min, hold at 0% B for 5 min for a total run time of 18 min. The flow rate was 0.3 mL/min and 5 µL of sample was injected. MS-MS analysis was conducted using an AB SCIEX 3200 triple quadrupole mass spectrometer. The mass spectrometer was in positive ionization mode and analysis was conducted using multiple reaction monitoring (MRM). The source parameters were set to a curtain gas 50 psi; heater gas 50 psi, ion spray voltage 5500 V; and source temperature 500 °C. The instrument parameters were optimized using direct infusion of each analyte using a split tee injection with the LC flow. Each targeted parent ion and two fragment ions are listed along with the corresponding retention time.

SCIEX Analyst software was used for instrument control. Confirmation analysis was performed using MultiQuant where the calibrators and quality controls were carried through the same

processes as the specimens being tested. Least squared regression with 1/x weighing was used to evaluate the linearity with adequate compensation for heteroscedasticity during all experiments.

Statistical Analysis

Metabolomics and mass spec analysis and software are described above. Tail flick results are presented as percent analgesia, which is calculated as maximum possible effect (MPE) using the following formula MPE = [(test latency − baseline latency)/(cutoff latency − baseline latency)]*100. A one-way ANOVA was used to assess effect of drug condition. Holm-Sidak multiple comparison test was used to compare morphine alone to each drug condition. Lesion data was analyzed using unpaired t-tests. Western blot data was normalized to total protein and normalized signal intensity was analyzed using an unpaired t-test to examine differences between morphine responsive and nonresponsive rats. Data are presented as means ± SEM. Differences at $p<0.05$ were considered significant. Analyses were done using Prism 7 v. 7.02 (GraphPad Software, Inc, LaJolla, CA).

IV. Results

Morphine alone provided analgesia in only 33% (n=5) of rats after SCI. Morphine + PPX and morphine + SCH both significantly restored opioidergic pain relief in the other 66% (n=10) of rats previously not responsive to morphine. Isolated administration of morphine, PPX, and SCH did not restore analgesia in injured nonresponders (Figure 3). Morphine, morphine + PPX, and morphine + SCH all facilitated significantly increased sensory thresholds above controls in uninjured rats (Figure 2). Saline did not provide any remarkable analgesia in any group.

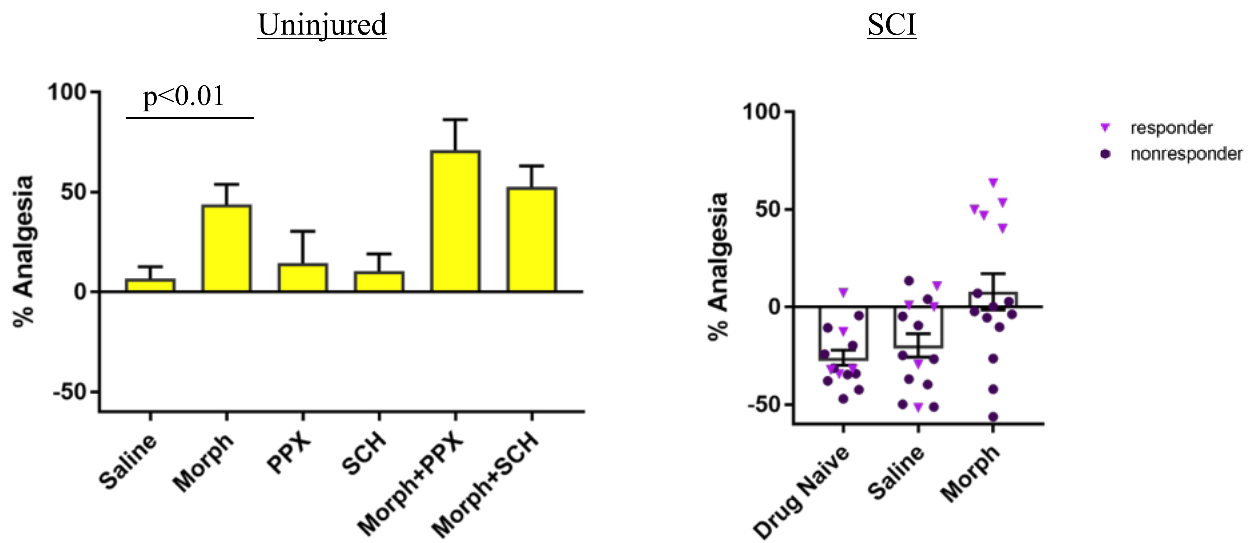


Figure 2. Yellow bar graph depicts nociceptive thresholds for uninjured rats (n=8) in each given drug condition. Morphine provided significantly improved analgesia compared to saline control. SCI graph shows analgesia for all injured rats (n=15) in each given drug condition. Only 5 rats achieved analgesia with morphine alone.

SCI
Nonresponders

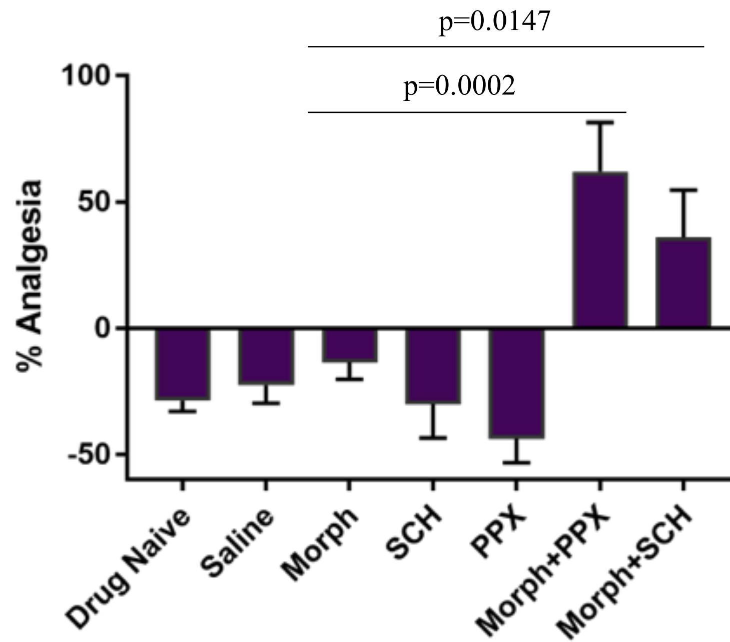


Figure 3. Graph of only injured morphine nonresponders. Morphine + PPX and morphine + SCH restored analgesia after failure to respond to morphine alone (see Figure 2).

Principle Component Analysis (PCA) of lumbar cord samples displayed independent clustering across SCI responders, nonresponders, and uninjured groups with the exception of one responder sample (Figure 4). PCA of striatum tissue showed tight clustering of samples except for one nonresponder sample which clustered with uninjured samples (Figure 5). Significant metabolites were identified between groups with a t-test ($p<0.05$) and tentatively identified. Comparing groups, 83 metabolites were present in differing quantities between uninjured and injured lumbar cord samples. Tentative identities included enkephalin and homovanillic acid (HVA). A total of 39 metabolites varied between responders and nonresponders in the lumbar cord. Tentative identities included HVA and 3-methoxytyramine (3-MT) (Table 1). Comparing uninjured and injured striatum samples revealed 135 metabolites that varied between the two

groups. Tentative identities included dopamine and prostaglandins. A total of 27 metabolites varied between striatal responders and nonresponders. 3-Methoxytyramine remains a tentative identity for one of the metabolites (Table 2).

Lumbar Cord

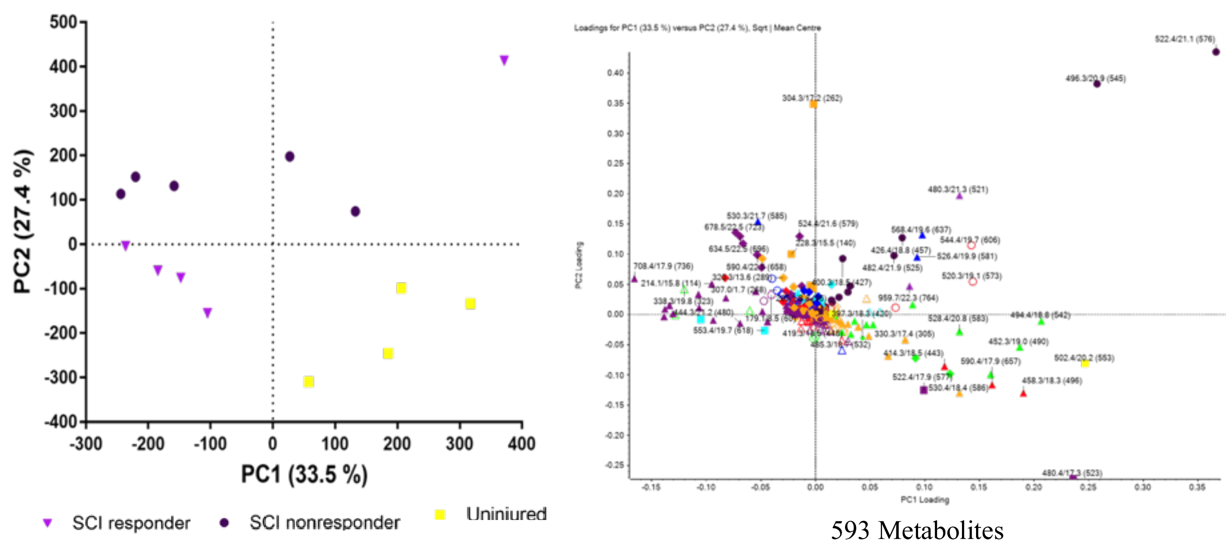
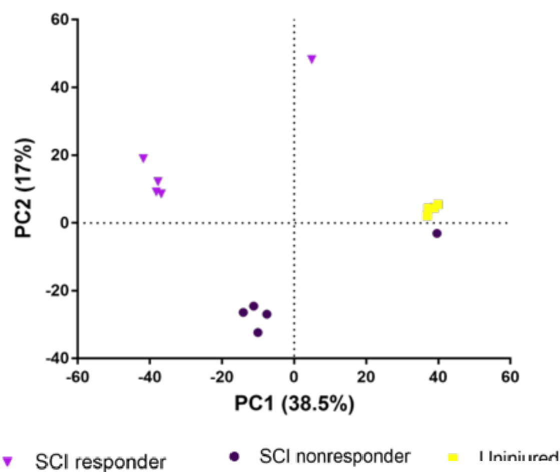


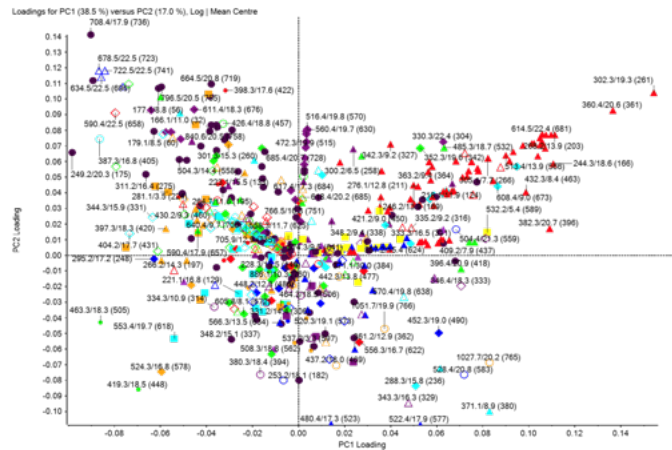
Figure 4. PCA (left) shows independent clustering of lumbar cord samples across groups. Outlier in upper right quadrant showed abnormally small lesion size. Total metabolite distribution (right) shows metabolic variability. Metabolites closer to the crosshair intersection were more commonly expressed across groups. Lumbar cord samples showed less metabollic variability than striatal samples.

	Uninjured vs SCI	SCI responder vs SCI nonresponder
Metabolites	83	39
Tentative ID’s	Enkephalin Homovanillic acid*	Homovanillic acid* 3 -Methoxytyramine*

Table 1. Notable metabolites tentatively identified in lumbar cord samples. Asterisks indicate metabolites closely related to dopamine (see Figure 10).

Striatum





578 Metabolites

Figure 5. PCA (left) shows independent clustering of striatum samples across groups. Outliers in upper and lower right quadrants showed abnormally large lesions. Total metabolite distribution (right) shows metabolic variability. Metabolites closer to the crosshair intersection were more commonly expressed across groups. Striatum samples showed greater metabolic variability than lumbar cord samples.

	Uninjured vs SCI	SCI responder vs SCI nonresponder
Metabolites	135	27
Tentative ID's	Dopamine* Prostaglandins	3-Methoxytyramine*

Table 2. Notable metabolites tentatively identified in striatum samples. Asterisks indicate metabolites closely related to dopamine or dopamine itself (see Figure 10).

Mass spectrometry showed dopamine quantity was significantly decreased in morphine nonresponders in the striatum, but DOPAC remained constant across groups (Figure 6). Dopamine in the lumbar spinal cord was significantly decreased in both responders and nonresponders (Figure 7).

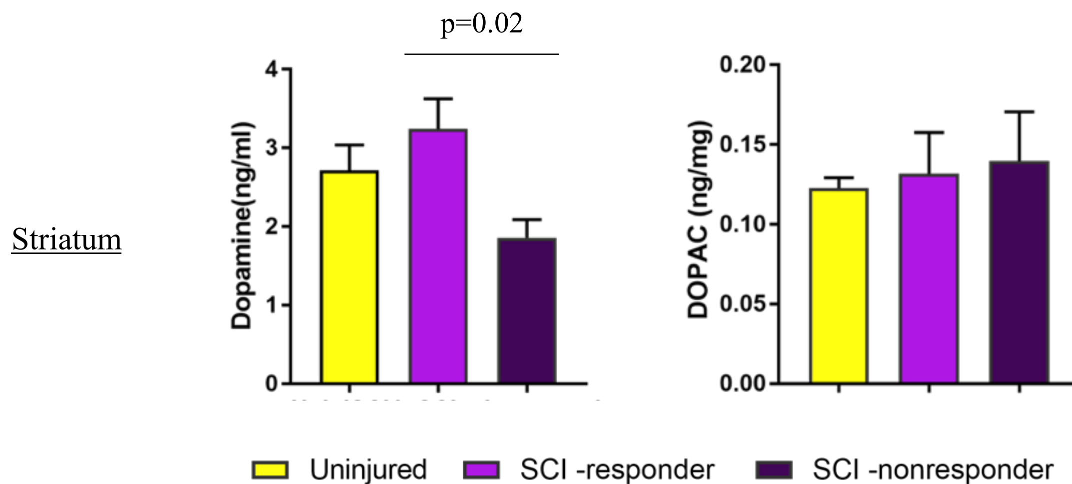


Figure 6. Targeted mass spectrometry for dopamine (left) and DOPAC (right) quantities in the striatum. Dopamine was decreased in nonresponders compared to responders. DOPAC remained consistent across groups.

Lumbar Cord

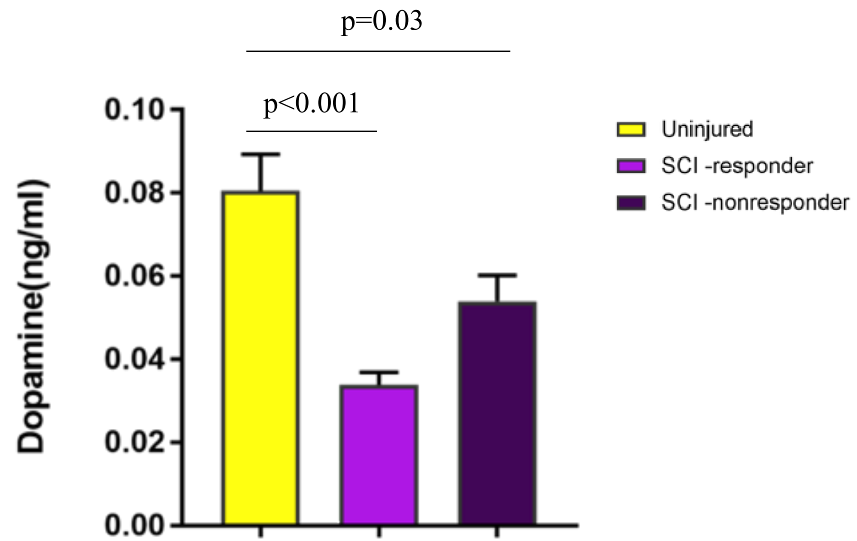


Figure 7. Targeted mass spectrometry for dopamine quantity in the lumbar cord. Dopamine was decreased in both responders and nonresponders compared to uninjured animals.

Western blot data shows D1R expression did not vary significantly across groups in the striatum and lumbar cord, but expression was slightly elevated in morphine responders in the lumbar cord (Figures 8 and 9).

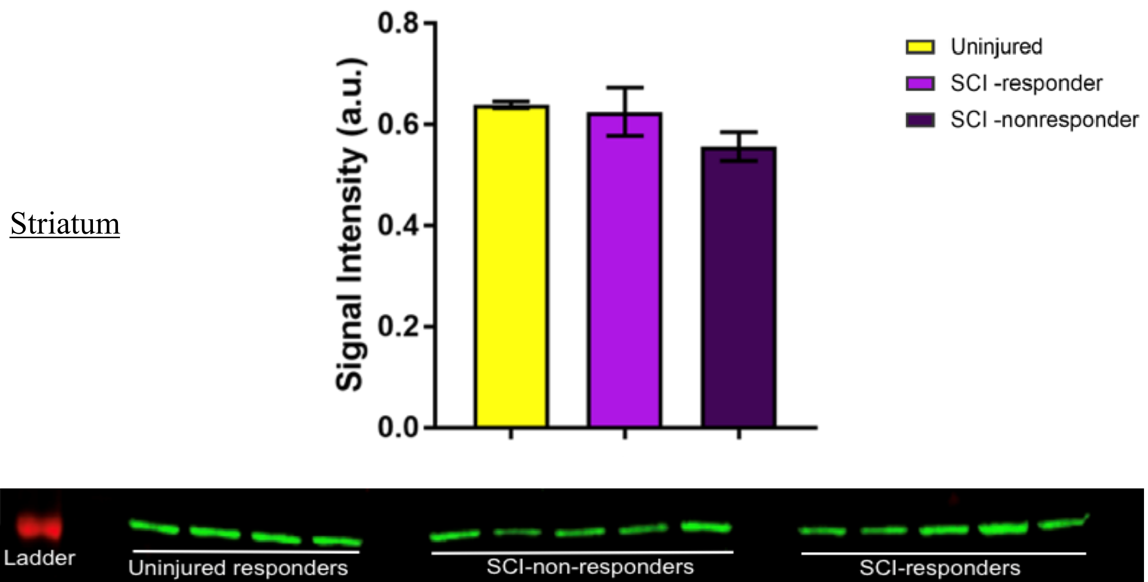


Figure 8. Western Blot signal intensities for D1R expression in striatum tissue. No significant variation in receptor expression was seen across groups.

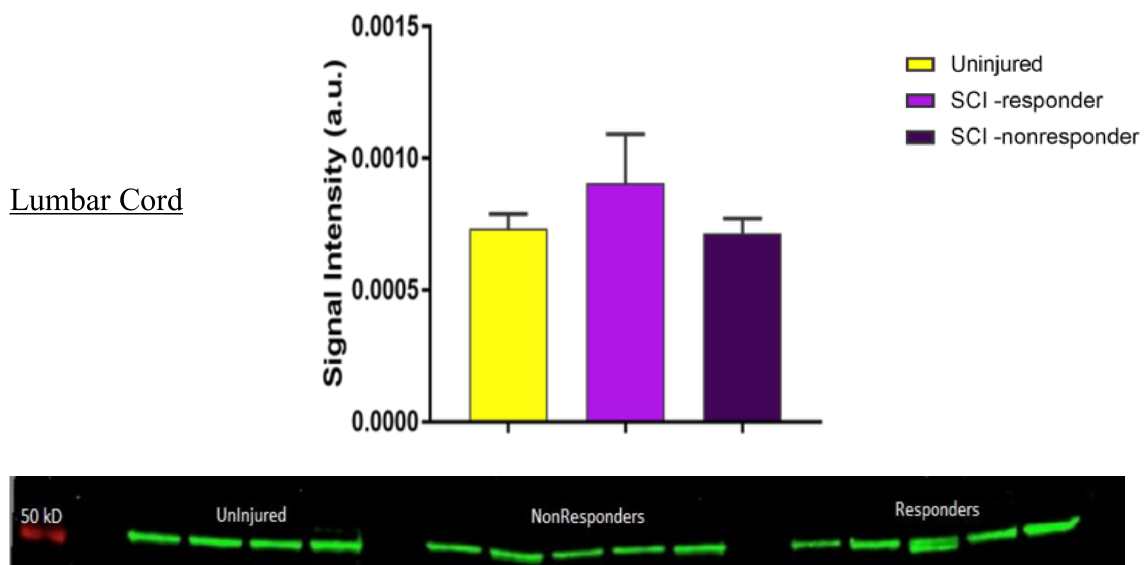


Figure 9. Western Blot signal intensities for D1R expression in lumbar cord tissue. No significant variation in receptor expression was seen across groups.

V. Discussion

Tail flick testing supported the position that certain populations are unresponsive to morphine post SCI. In this study, 66% (n=10) of injured rats fell into this nonresponder category. Morphine + PPX and morphine + SCH administration in subsequent SCI nonresponder testing were shown to reverse this trend, significantly restoring analgesia ($p=0.002$, $p=0.0147$) to animals who previously did not experience pain relief from morphine alone.

PCA data supported the hypothesis that SCI responders, nonresponders, and uninjured animals would express independent metabolite clustering. While this trend was shown throughout all groups in both the lumbar cord and striatum, outliers were seen. Spinal microtomy and imaging revealed that the SCI responder sample in the upper right quadrant of Figure 3 displayed a lesion size of $0.4mm^2$ compared to the average size of $2.1mm^2$. The responder and nonresponder outliers in Figure 4 showed abnormally large lesions of $6.4mm^2$ and $3.2mm^2$ compared to their respective group averages of $2.1mm^2$ and $1.7mm^2$. More data is required to establish a reliable connection between lesion size and PCA readings, but severity of injury offers an explanation for inconsistent metabolite expression. Overall metabolite distribution graphs will offer more meaningful data when individual data points are identified, but greater metabolite density in the lumbar cord distribution suggests that less variability in metabolite expression can be expected in this tissue after SCI compared to the striatum. This provides evidence of the need for future studies to identify specific changes in cortical dopaminergic biochemistry after SCI, as this data suggests these changes to be copious. Tentative metabolite identification continues to progress, but initial data showing variability in enkephalin, HVA, 3MT, prostaglandins, and dopamine is promising. Differences between responders and nonresponders in metabolites such as HVA and 3-MT that are directly involved in dopamine

metabolism further supports the idea that dopamine plays a significant role in the development of SCI-induced tolerance.

Dopamine Pathway

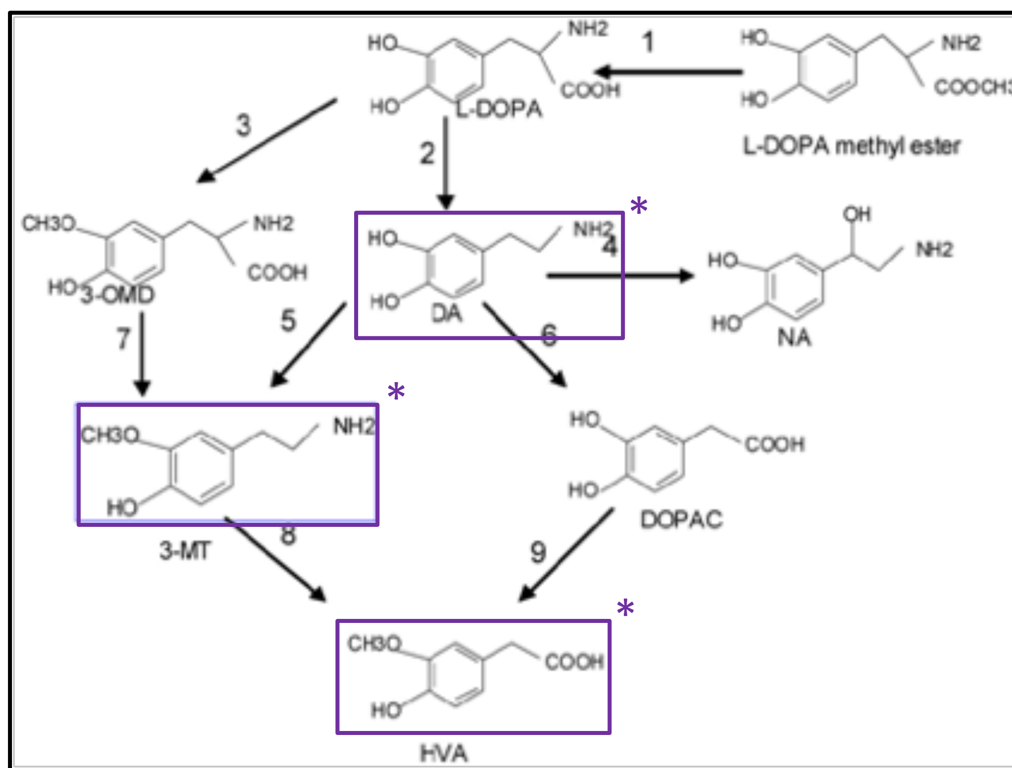


Figure 10. Dopamine metabolic pathway.[2] Boxed and starred metabolites indicate tentatively identified molecules that differ across uninjured, SCI responders, and SCI nonresponders in the lumbar cord and striatum (see Tables 1 and 2).

The similarity in dopamine quanitity in the striatum for uninjured and SCI responders supports the hypothesis. This relative decrease of striatal dopamine levels in nonresponders provides evidence that a decrease in dopamine quantity could contribute to morphine tolerance. The data showing both responders and nonresponders to experience a decrease in dopamine levels in the lumbar cord does not support the hypothesis and suggests that the corresponding dopamine decline in the spinal cord is due to the injury itself while cortical processes are more

independent. It is still unknown if the decrease in spinal dopamine is due to the inability of dopamine to travel from the cortex to below the injury or if dopaminergic spinal neurons simply decrease their production after SCI. DOPAC quantity was anticipated to differ among groups due to its metabolic relationship to dopamine, but future mass spectrometry trials targeting other dopaminergic metabolites should be conducted to isolate specific changes among groups and tissues types.

Constant D1R expression across groups and tissue types supports the idea that changes in dopamine production and metabolism are the main contributors to a state of SCI-induced morphine tolerance rather than receptor presence. This result does not parallel the expected upregulation of D1R in a state of morphine tolerance that was described earlier. Future Western blots examining D3R expression levels as well as comparing relative D1R and D3R concentration are needed to gain a better understanding of dopamine receptor dynamics within the context of morphine tolerance.

Medical intervention remains a major impetus for studying dopamine's role in SCIinduced morphine tolerance. Increased opioid abuse, lowered life expectancies, and difficulty achieving analgesia in both acute and chronic pain patients certainly warrants an eventual pivot from animal models to human participants. If patient populations display similar resistance to opioid intervention as seen in this study, then the ability to categorize individuals as a morphine responder or nonresponder has the potential to reduce both healthcare costs and risk of opioid tolerance and addiction. Future studies will utilize targeted metabolomics to confirm metabolite identities and explore other effectors of the opioid system, providing an avenue through which morphine responsiveness categorization and expedited analgesic intervention can occur.

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