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DNA methylation and behavioral changes induced by neonatal spinal transection

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ABSTRACT

Although the importance of epigenetic mechanisms in behavioral development has been gaining attention in recent years, research has largely focused on the brain. To our knowledge, no studies to date have investigated epigenetic changes in the developing spinal cord to determine the dynamic manner in which the spinal epigenome may respond to environmental input during behavioral development. Animal studies demonstrate that spinal cord plasticity is heightened during early development, is somewhat preserved following neonatal transection, and that spinal injured animals are responsive to sensory feedback. Because epigenetic alterations have been implicated in brain plasticity and are highly responsive to experience, these alterations are promising candidates for molecular substrates of spinal plasticity as well. Thus, the current study investigated behavioral changes in the development of weight-bearing locomotion and epigenetic modifications in the spinal cord of infant rats following a neonatal low-thoracic spinal transection or sham surgery on postnatal day (P)1. Specifically, global levels of methylation and methylation status of the *brain-derived neurotrophic factor* (*Bdnf*) gene, a neurotrophin heavily involved in both CNS and behavioral plasticity, particularly in development, were examined in lumbar tissue harvested on P10 from sham and spinal-transected subjects. Behavioral results demonstrate that compared to shams, spinal-transected subjects exhibit significantly reduced partial-weight bearing hindlimb activity. Molecular data demonstrate group differences in global lumbar methylation levels as well as exon-specific group differences in *Bdnf* methylation. This study represents an initial step toward understanding the relationship between epigenetic mechanisms and plasticity associated with spinal cord and locomotor development.

1. Introduction

A flurry of research in the past decade has made a strong case for epigenetic modifications as underlying mechanisms of plasticity in the central nervous system (CNS). One of these modifications, DNA methylation, is linked to both repression and activation of gene transcription (Chahrour et al., 2008; Nan et al., 1998) and is heavily associated with molecular and behavioral adaptation throughout the lifespan (Gluckman, Hanson, & Low, 2011; Joss-Moore, Metcalfe, Albertine, McKnight, & Lane, 2010; Renthal et al., 2007; Stankiewicz, Swiergiel, & Lisowski, 2013). However, work thus far has mainly been aimed at understanding these processes in the brain, leaving the spinal cord epigenome largely unexplored.

The spinal cord is known to adapt its behavioral (i.e. motor) output to environmental input, exhibiting learning and memory even

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when physically disconnected from the brain in both adulthood (Grau, 2014) and development (Brumley, Strain, Devine, & Bozeman, 2018; Robinson, 2015; Sharp, 2015; Strain & Brumley, 2014). Early spontaneous limb movements are controlled by the spinal cord and the pattern of those movements will change with environmental feedback (Brumley & Robinson, 2013), as well as when the spinal cord is disconnected from the brain (Robertson & Smotherman, 1990; Robinson, Blumberg, Lane, & Kreber, 2000). In rats, the ability of the spinal cord to exhibit such plasticity appears to be highest during the first two postnatal weeks. Motor function recovery following spinal cord transection earlier in development (before postnatal day 15) is considerably higher than recovery following transection in late development or adulthood – i.e. while early transection results in reduced/abnormal motor behavior, it does not result in total paralysis (Fayein & Viala, 1976; Viala, Viala, & Fayein, 1986; Weber & Stelzner, 1977; Yuan, Su, Chiu, Wu, & Lin, 2013).

This is referred to as the infant lesion effect (Goldberger, 1986;Robinson & Goldberger, 1986) a phenomenon of which the mechanisms are not fully understood, though they are thought to be due to plasticity intrinsic to the spinal cord and not due to regrowth of axons over the lesion site (Cummings & Stelzner, 1988; Tillakaratne et al., 2010; Weber & Stelzner, 1980).

Existing studies of epigenetic mechanisms in the spinal cord are sparse and mainly focus on pain (Jiang et al., 2017; Zhao et al., 2017), disease (Martin & Wong, 2013; Miranpuri et al., 2017), or injury (Mahar & Cavalli, 2018; Wang et al., 2011, 2016; Wang, Li, Li, Guan, & Deng, 2017; Weng et al., 2017, 2018) in adulthood. Given that these studies suggest a functional role for the spinal epigenome, and that there is some evidence of epigenetic regulation in the developing spinal cord (Akizu, Estarás, Guerrero, Martí, & Martínez-Balbás, 2010; Chestnut et al., 2011; Moyon et al., 2016; Ocklenburg et al., 2017; Ye et al., 2009), it is reasonable to hypothesize that the epigenome of the developing spinal cord contributes to its unique plasticity and behavioral outcomes.

No studies to our knowledge have examined locomotor outcomes in relation to epigenetic activity in the developing intact and transected spinal cord. The current study begins to address this gap by examining global and gene-specific methylation changes in lumbar tissue from developing rodents with either intact or complete transected spinal cords. Here we focus on brain-derived neurotrophic factor (*Bdnf*), a major player in CNS plasticity and particularly in developmental processes, including regulation of critical periods (Gianfranceschi et al., 2003; Hanover, Huang, Tonegawa, & Stryker, 1999; Huang et al., 1999). This gene is a known target of environmentally-induced epigenetic modifications in the brain (Blaze & Roth, 2017; Doherty, Forster, & Roth, 2016; Lubin, Roth, & Sweatt, 2008; Ma et al., 2009; Onishchenko, Karpova, Sabri, Castrén, & Ceccatelli, 2008; Roth, Lubin, Funk, & Sweatt, 2009; Ventskovska, Porkka-Heiskanen, & Karpova, 2015), and has been implicated in adult spinal learning (Gómez-Pinilla et al., 2007; Huie et al., 2012) and in locomotor improvement in response to training and exercise following transection (Joseph, Tillakaratne, & de Leon, 2012; Macias et al., 2009). In addition, overexpression of *Bdnf* alone has been associated with improved locomotor outcomes in spinal-transected animals (Boyce, Park, Gage, & Mendell, 2012; Ziemlińska et al., 2014). Taken together, these findings support the idea that epigenetic modification of the *Bdnf* gene is a prime focal point in the search for mechanisms of plasticity in the developing spinal cord.

Given our knowledge of epigenetic mechanisms underlying changes in brain and behavior throughout the lifespan and our knowledge of the unique behavioral outcomes following transection of the developing spinal cord (the mechanisms of which are largely unknown), bringing these two areas together is an exciting new direction for the field of developmental behavioral epigenetics. The mechanisms this work aims to elucidate may be particularly relevant to spinal cord dysfunction during infancy. Some examples include infant whiplash-shake injury syndrome, wherein spinal cord contusions are likely to contribute to symptomatology (Hadley, Sonntag, Rekate, & Murphy, 1989), and spinal cord damage occurring during the birth process (Reichard, 2008; Towbin, 1969). The results of this investigation and those to follow inform our understanding of neurobehavioral development as well as our understanding of the spinal cord as a responsive, dynamic part of the central nervous system. The current report of transection-induced methylation changes in the developing spinal cord coupled with locomotor outcomes is a novel contribution to developmental literature. Such knowledge may one day be exploited for the treatment and prevention of spinal cord abnormality and injury.

2. Methods

2.1. Subjects

Adult Sprague-Dawley rats were acquired from Simonsen laboratories, and time-mated to produce offspring for behavioral testing. Animals were socially housed, except that pregnant females were separated and singly housed one week before delivery. Pregnant/delivering females were monitored regularly throughout pregnancy and the birthing process.

Subjects were 16 male rat pups that underwent behavioral testing. On postnatal day 1 (P1; ~24 h after birth), subjects received a low-thoracic spinal cord transection (n = 8) or sham surgery (n = 8), and litters were culled to 8 pups per litter. All pups in a litter were treated with the same surgical manipulation (transection or sham surgery). Following surgery, pups were returned to the home cage with the dam. Subjects were then behaviorally tested on P10. Only one male rat pup per litter was tested in this study; other pups within the litter were allocated to other studies.

All animals were kept on a 12-h light:dark cycle with access to food and water *ad libitum,* and maintained in accordance with the NIH, Institutes on Laboratory Animal Resources, and ISU Institutional Animal Care and Use Committee guidelines.

2.2. Spinal cord surgeries

Rat pups received a low-thoracic spinal cord transection or sham operation on P1. Aseptic spinal cord surgery followed previously published procedures (Strain and Brumley, 2014). First, subjects were anesthetized via hypothermia. Then a small incision was made

mid-back to expose the low-thoracic and lumbar spine, and a partial laminectomy between thoracic level 8–10 (T8–T10) was performed to expose the spinal cord. For subjects that received a spinal cord transection, the spinal cord and dorsal roots were cut using iridectomy scissors and a collagen matrix was inserted into the transection site. Sham subjects underwent all procedures as spinal subjects, except that the spinal cord and dorsal roots were not cut and no collagen was injected. The wound was then sutured. The surgery and suturing took about 2 min per subject.

Immediately after surgery, subjects were treated with a 50 µl subcutaneous injection of buprenorphine (.1 ml of .04 mg/kg solution) and .9% (wt/vol) saline, to help with pain management and fluid balance, respectively. Pups were then placed in a temperature-controlled incubator with littermates to recover. Once pups regained a pinkish skin color, were warm to the touch, exhibited normal breathing, and showed hindlimb kicking and twitching, they were returned to the dam. (This typically occurred within 30 min). Subjects remained with the dam and littermates in their home cage until day of testing.

2.3. Locomotion testing

On P10, rat pups were individually tested in an open field to evaluate spontaneous weight-bearing locomotion. The open-field environment (8" × 8" × 8" Plexiglas box) was placed inside a temperature-controlled infant incubator maintained at 30 °C. Subjects were placed in the center of the box for an acclimation period (30-min) inside the incubator and then immediately tested. Behavior inside the open field was recorded for a 20-min period from a lateral camera view. Following behavioral testing, subjects were euthanized via CO_2 inhalation.

2.4. Spinal cord extractions

Lumbar spinal tissue was extracted from separate animals than those that underwent behavioral testing, but otherwise were treated the same way (i.e., spinal or sham surgery). Following euthanasia, the lumbar spinal cord was extracted. An incision was made, similar to the one during surgery, to expose the spine and spinal cord. Using fine-tip forceps, the lumbar spinal cord was extracted, and the tissue was placed in a sterile Eppendorf tube. Immediately following extraction, samples were placed in a -80° freezer.

2.5. Behavioral scoring

Durations of behaviors in the open field were scored during later video playback in Datavyu (Version 1.3.4; Datavyu Team, 2014). Hindlimb weight-bearing behavior was categorized as non weight-bearing, partial weight-bearing, or full weight-bearing. Non weight-bearing behavior involved hindlimb movements with no additional weight put on the hindlimbs, and included hindlimb kicking, pivoting (which just involved the head and forelimbs), and crawling (in which the forelimbs and front part of the body dragged the hindquarters). Partial weight-bearing behavior involved hindlimb movements with some weight put on the hindlimbs and included partial rearing (one forelimb on the side of the box with the head and shoulders raised above the abdomen) and hindlimb-active crawling (both forelimbs and hindlimbs engaged with the abdomen touching/dragging the floor). Full weight-bearing behavior involved hindlimb movements with full weight put on the hindlimbs and included rearing (both forelimbs on the side of the box with the head and shoulders raised above the abdomen), walking (both forelimbs and hindlimbs engaged and the abdomen is not touching/dragging the floor), or standing (both forelimbs and hindlimbs engaged, the abdomen is not touching the floor, and the subject remains stationary). All behavior categories were summed over the 20-min testing period. Intra- and interrater reliability with a standard file was > 90%.

2.6. Global DNA methylation (5-mC) & hydroxymethylation (5-hmC)

Lumbar spinal cord tissue was thawed following storage at -80° and then homogenized for nucleic acid extraction according to the manufacturer's instructions (Qiagen AllPrep DNA/RNA kit). MethylFlash™ Methylated DNA Quantification Kits were then used to quantify levels of genome-wide methylation (5-mC) and hydroxymethylation (5-hmC) according to the manufacturer's instructions (Epigentek, Brooklyn, NY), with the only deviation being the added step of mechanically shaking the plate (using a plate reader with this function) immediately after each step where a new component has been added. Using this kit, capture and detection antibodies are employed to detect either methylated or hydroxymethylated DNA which is then colorimetrically quantified (via measurement of light absorption) against a standard curve formed with control DNA provided with the kit. Absorbance was measured using the Infinite ® F50 microplate reader (Tecan, Männedorf, Switzerland) with the amount of 5-mC or 5-hmC DNA being proportional to the intensity of the optical density. Samples were run in vertical duplicates at a strict concentration of 100 ng/well with total volume added per well not ranging outside of 2–5 µl per well.

2.7. Locus-specific DNA methylation

The same DNA used for global methylation assays was used to assess locus-specific methylation levels in each group. DNA was bisulfite modified (Qiagen Inc.) and direct bisulfite sequencing (BSP) was then performed as previously described (Parrish, Day, & Lubin, 2012; Roth et al., 2009), using primer sets targeting DNA associated with *Bdnf* exons I and IV, encompassing transcription factor binding and start sites. The ratio between peak values of G and A [G/G + A] was determined using Chromas software, allowing

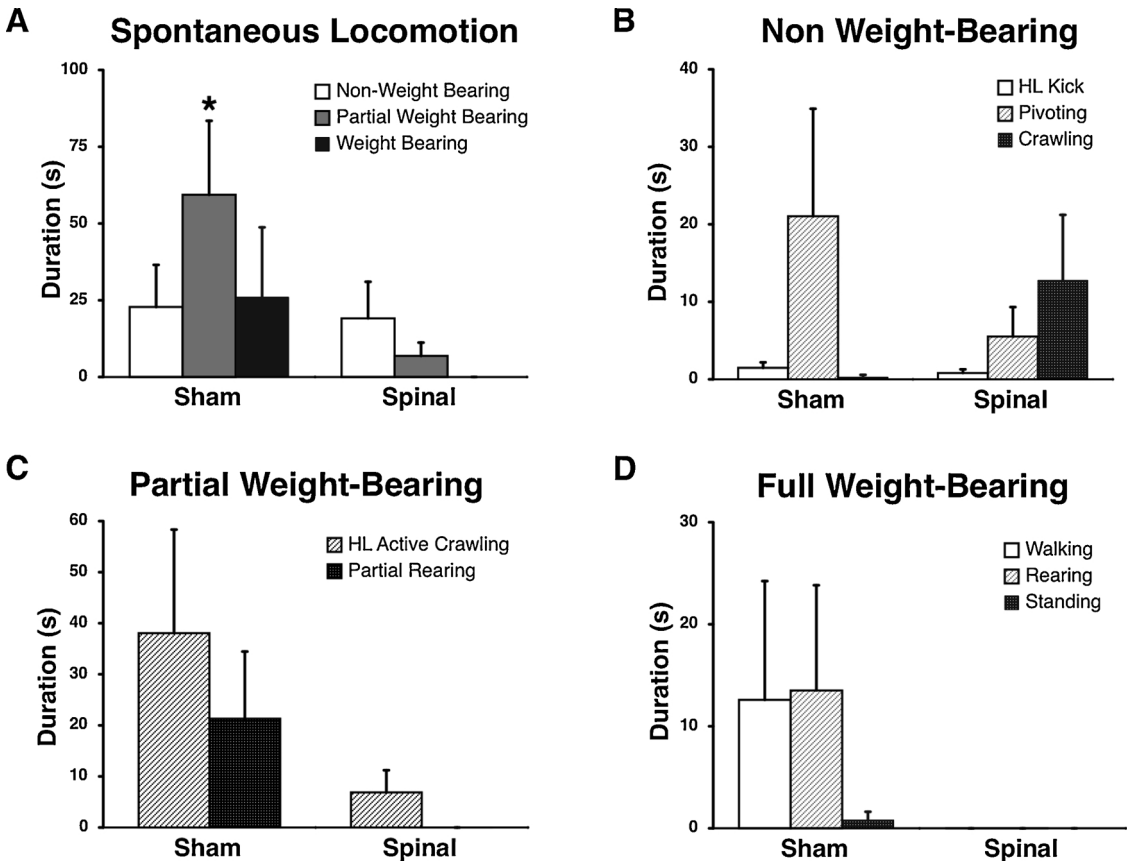

Fig. 1. Duration of open-field weight-bearing locomotor activity by sham (n = 8) and spinal-transected subjects (n = 8). Overall, shams showed significantly more partial weight-bearing locomotion (gray bars) than spinal-transected subjects (**A**). Specific categories of non weight-bearing (**B**), partial weight-bearing (**C**), and full weight-bearing behavior (**D**) were not significantly different between sham and transect subjects; though note that transect subjects did not show any partial rearing or full weight-bearing behavior at all. Bars show mean durations; lines depict SEM. *p < .05.

for estimation of methylation levels at each CG cite within the target regions. Additionally, verification of BSP results was performed using methylation-specific real-time PCR (MSP; Bio-Rad CFX96 system). Bisulfite-modified DNA was amplified using methylated and unmethylated primer sets associated with exons I and IV (Blaze, Scheuing, & Roth, 2013; Roth, Matt, Chen, & Blaze, 2014). The relative fold change of spinal-transected animals versus sham animals was obtained using the comparative C_t method (Livak & Schmittgen, 2001) and the methylation index was then obtained by dividing the fold change value for the methylated primer set by the fold change value for the unmethylated primer set (Blaze et al., 2013; Doherty et al., 2016; Roth et al., 2014). Product specificity was confirmed via melt curve analysis and gel electrophoresis.

2.8. Statistical analyses

A series of unpaired *t*-tests were used to determine statistical significance between spinal-transected and sham subjects for durations of locomotor behavior. The independent variable was surgery condition, and dependent variables were durations of spontaneous non-, partial-, and full weight-bearing locomotion. Group differences in global 5mC and 5hmC, as well as gene-specific methylation, were analyzed using unpaired *t*-tests (spinal-transected vs sham animals). As is standard in the field, differences were considered to be statistically significant for $p < .05$.

3. Results

3.1. Locomotor behavior

Fig. 1 shows durations of non, partial, and full weight-bearing behavior during spontaneous locomotion in the open field for sham and spinal cord transected subjects. There was no significant difference in overall non weight-bearing behavior between sham and spinal subjects [$t_{14} = .204$, $p = .84$]. As shown in Fig. 1A, spinal-transected subjects showed significantly less overall partial weight-bearing [$t_{14} = 2.14$, $p = .05$] behavior compared to sham subjects. There was no difference between groups for full weight-bearing

[t_{14} = 1.14, p = .27] behavior; however, as can be seen in Fig. 1A, full weight-bearing behavior was only expressed by sham subjects, and not at all by spinal subjects. Full weight-bearing behavior was shown by 3 (out of 8) sham subjects.

Fig. 1B shows durations of the different non weight-bearing locomotor activities. There were no significant differences in hindlimb kicking [t_{14} = −.786, p = .44], pivoting [t_{14} = −1.08, p = .30], or crawling [t_{14} = 1.48, p = .16] between spinal-transected subjects and shams. Fig. 1C shows durations of the different partial weight-bearing locomotor activities. There were no significant differences in hindlimb-active crawling [t_{14} = −1.50, p = .16] or partial rearing [t_{14} = 1.62, p = .13] between spinal and sham groups. However, note that spinal subjects did not show any partial rearing at all. Full weight-bearing behavior categories are shown in Fig. 1D. Although differences between spinal and sham subjects were not significant for any category (walking [t_{14} = 1.08, p = .30], standing [t_{14} = 1.0, p = .33], and rearing [t_{14} = 1.31, p = .21]), spinal subjects did not show any full-weight bearing behavior, as noted above.

3.2. Global 5-mC and 5-hmC

Global 5-mC levels in lumbar spinal tissue (see Fig. 2A) were significantly elevated in spinal-transected compared to sham animals (t_{21} = 4.224, p = .0004). In contrast, global 5-hmC levels did not significantly differ between groups (t_{15} = 1.724, p = .1052; data not shown). Overall, 5-hmC levels were particularly difficult to pick up at all in these subjects, suggesting that this modification was at low levels in developing spinal cord tissue.

3.3. Locus-specific DNA methylation

Bisulfite sequencing PCR (BSP) was employed to examine group differences in *Bdnf* methylation at exons I (Fig. 2B) and IV (Fig. 2C) in lumbar spinal tissue. Spinal-transected subjects, in comparison to shams, exhibited significantly higher levels of methylated *Bdnf* exon I DNA (t_{15} = 2.502, p = .0244) but significantly lower levels of methylated *Bdnf* exon IV DNA (t_{24} = 2.901, p = .0078).

Additionally, methylation specific real-time PCR (MSP) was used to verify our locus-specific data. Using this method, increased methylation in transected subjects was detected at exon I (t_{17} = 2.562, p = .0202) but group differences in methylation were not detected at exon IV (t_{16} = .1098, p = .6874). Because changes in exon IV methylation in this tissue appear to be driven by CG sites toward the middle of the targeted region of DNA, it is not surprising that this change was not detected by MSP, which is more sensitive to changes at either end of the DNA region being amplified.

4. Discussion

The current study is among the first to examine the epigenome of the developing spinal cord and the first to examine how that relates to environmentally-induced motor outcomes. The data reported here support the hypothesis that epigenetic alterations are associated with transection of the developing spinal cord and the resulting changes in development of motor behavior. As expected, motor behavior was partially preserved in spinal-transected subjects. Specifically, rat pups that underwent a complete spinal cord transection on P1 exhibited some non- and partial weight bearing locomotion (e.g., pivoting, crawling, and hindlimb active crawling) on P10. This result likely would not be the case if the spinal transection and behavioral testing had occurred in adulthood. Spinal-transected rats also exhibited significantly higher total levels of methylated cytosines (i.e. global methylation) in lumbar tissue when compared to the sham rats, suggesting sweeping epigenetic changes in response to the developmental perturbation. Global measurements of hydroxymethylation were attempted but were largely undetectable in this tissue with the chosen method. Our investigation of a gene-specific response to spinal transection, however, revealed significant group differences in *Bdnf* methylation that were exon-dependent.

Though well described, the exact mechanisms underlying the infant lesion effect remain to be fully elucidated. Given the explosion of research on epigenetic mechanisms of brain plasticity and behavioral outcomes in both developing and mature organisms, epigenetic alterations are prime candidates in the search for mechanisms underlying spinal cord development and associated behavioral trajectories. The results of the current study support this idea by linking changes in methylation to the altered pattern of motor development seen in subjects that underwent early spinal cord transection.

Though unexamined in the developing spinal cord until now, global methylation levels have been shown to rise in the adult spinal cord following injury (Wang et al., 2011). Additionally, administration of the globally-acting epigenetic agent 5-azacytidine inhibited this injury-induced rise, a change accompanied by attenuation of pain outcomes, suggesting a functional role for global changes in the spinal epigenome. The global changes reported in the current study align with adult literature and are suggestive of large increases in spinal methylation in response to developmental spinal cord injury. Given that the global measure used here does not provide any gene-specific information, it is difficult to surmise what the reported increase may mean in the larger pictures of development and injury outcome. However, it does provide a springboard for future investigation of the epigenetic state of several genes implicated in these areas. For example, it would be pertinent to investigate genes such as *Ctip2*, known to be involved in the development of corticospinal motor neurons (Arlotta et al., 2005), as well as immediate early genes that display rapid responses to spinal injury (Di Giovanni et al., 2003).

Given some evidence for a functional role of hydroxymethylation in the spinal cord (Pan et al., 2016), we also sought to investigate group differences in global levels of this mark. Hydroxymethylated cytosines are oxidized methylcytosines that may be a step toward demethylation (Ito et al., 2011) or a stable influencer of transcriptional states in the CNS (Szulwach et al., 2011).

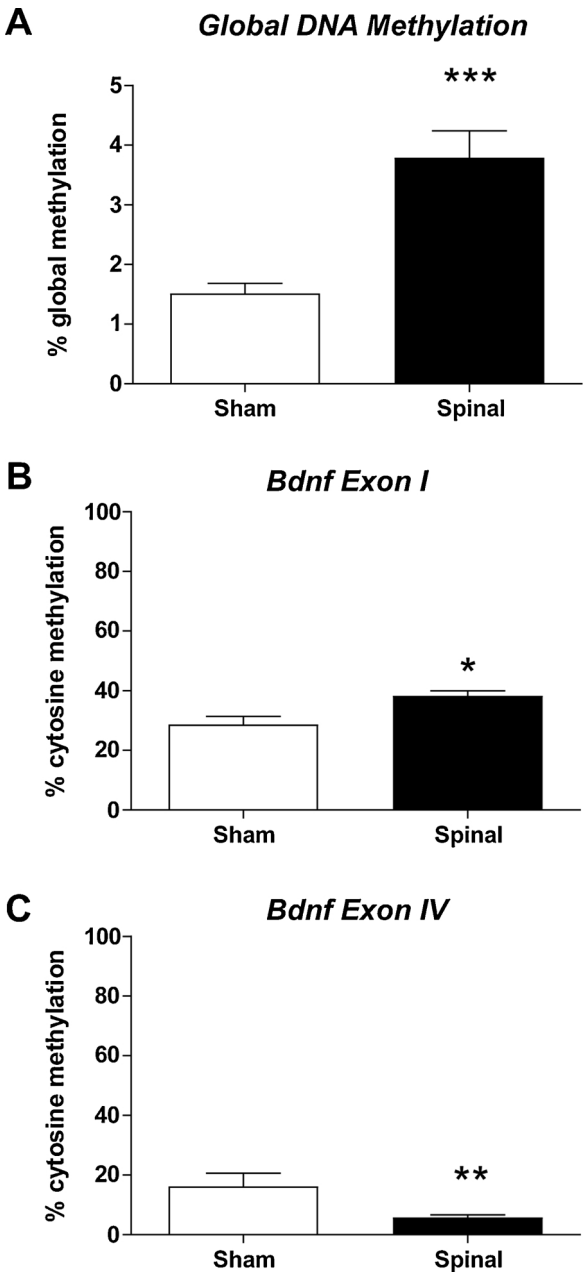

Fig. 2. Percent global methylation levels in lumbar tissue of sham and transect subjects (**A**). N = 10–13/group; ***p < .001 vs. shams; *Bdnf* methylation at exon I (**B**) and exon IV (**C**) in lumbar tissue of sham and transect subjects as determined by bisulfite sequencing PCR (BSP). N = 6–11/group (exon I), 10–16/group (exon IV); *p < .05 (exon I), ** < .01 (exon IV) vs. shams; error bars represent SEM.

However, hydroxymethylation levels were not detectable within the parameters of the current study. Because this mark has been successfully measured in the developing brain (Doherty et al., 2016; Wang et al., 2012) and is known to be linked to behavior and early experience (Massart et al., 2014; Pan et al., 2016), future work may consider a more sensitive assay to characterize it in the developing spinal cord.

Though functionally significant and experience-dependent epigenetic regulation of *Bdnf* has been established in the brain and in some peripheral tissue (Doherty et al., 2016; Fuchikami et al., 2011; Kim et al., 2013; Onishchenko et al., 2008; Perroud et al., 2013; Roth et al., 2009; Unternaehrer et al., 2012, 2015), this is the first report to our knowledge that examines the relationship between *Bdnf* methylation in the developing spinal cord and motor behavior. The data reported here are in support of epigenetic regulation of *Bdnf* in the developing spinal cord and as a potential mechanism for altered behavioral outcomes in response to changes in environmental input that occur following injury. It will be important work for future investigations to correlate spinal levels of *Bdnf* methylation with motor outcomes in the same individuals, to further confirm the relationship between locomotor performance and

methylation suggested by the current findings. Work underway in our labs is currently examining this issue, including in older animals when weight-bearing activity is more fully developed.

The dynamic nature of experience-dependent *Bdnf* methylation has been established in previous studies (for review see (Doherty & Roth, 2016)). Methylation of this gene is known to vary by many factors, including which exon is being examined. *Bdnf* has nine noncoding exons and one common coding exon (Aid, Kazantseva, Piirsoo, Palm, & Timmusk, 2007). Of these exons, data exist that demonstrate the environmental sensitivity of select exons, including those examined in this study (see (Doherty & Roth, 2016)). It is thus not surprising that the direction of methylation in response to transection differs between exons in the current investigation. Decoding the exact impact of these differences on transection-induced behavioral alterations will depend on several future investigations. Though an increase in methylation is generally thought to result in decreased gene expression, this is not always the case (Chahrour et al., 2008) and the direction of the relationship can be affected by several factors (Jones, 2012; Massart et al., 2014). Thus, work currently underway in our labs intends to examine gene expression in sham and spinal-transected animals to determine the precise effects of these methylation changes, a necessary step forward in understanding their potential role in motor behavior trajectories. Given that spinal transection limits behavioral (motor) plasticity, it is reasonable to hypothesize that *Bdnf* expression would be lower in transected versus sham animals. This would also be consistent with the regulatory role that *Bdnf* plays in defining experience-dependent critical periods in other systems. In the developing visual system, for example, lack of environmental input (i.e. dark-rearing) results in a reduction of *Bdnf* and a prolonged critical period for visual plasticity (Huang et al., 1999). Based on findings such as these, a reduction in *Bdnf* expression in response to reduced stimulation via transection would be expected. The same study reported that acceleration of *Bdnf* expression in the postnatal period resulted in rapid maturation of visual acuity and a truncated critical period (Huang et al., 1999). A critical period for motor development has been reported to occur in rats between postnatal days 8 and 13 during which hindlimb loading and unloading appears to be especially important (Walton, Lieberman, Llinás, Begin, & Llinás, 1992). It is an interesting hypothesis that environmentally-induced (i.e. transection-induced) epigenetic downregulation of *Bdnf* would occur during this time, possibly prolonging the critical period in a compensatory response to transection. To determine the validity of this hypothesis and to better elucidate the role of methylation in the locomotor realms examined here, measurement of protein levels will also be necessary, and eventually, manipulation of methylation levels in sham and transected animals.

Taken together, the current results support the hypothesis that epigenetic modifications in the developing spinal cord are related to the dynamic manner in which it responds to environmental input, thereby providing a new avenue of exploration in the pursuit to understand mechanisms of behavioral development. In humans, it recently has been suggested that epigenetic regulation of genes in the spinal cord may underlie the initiation of hemispheric asymmetries in the brain and lateralization of function (i.e., handedness; Ocklenburg et al., 2017; Schmitz, Kumsta, Moser, Gunturkun, & Ocklenburg, 2018). We predict that better understanding of epigenetic regulation of genes in the spinal cord will lead to increased understanding of typical neurobehavioral processes during development, including those that contribute to neural plasticity and to the production of developmental outcomes.

Declaration of Competing Interest

None.

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