Altered motoneuron properties and spinal serotonin contribute to motor deficits in a rabbit hypoxia ischemia model of cerebral palsy

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Abstract
Cerebral palsy (CP) is caused by a variety of factors attributed to early brain damage, resulting in permanently impaired motor control, marked by muscle stiffness and spasticity. To find out if altered physiology of spinal motoneurons could contribute to movement deficits, we performed whole cell patch clamp in neonatal rabbit spinal cord slices after developmental injury at 79% gestation. After preterm hypoxia-ischemia (HI) rabbits are born with muscle stiffness, motor deficits, and increased levels of serotonin (5HT) in the spinal cord. There is a range in severity, thus kits are classified as severely affected, mildly affected, or unaffected based on modified Ashworth scores and other behavioral tests. At postnatal day 0-5, we recorded electrophysiological parameters of 40 motoneurons and a subset of 12 were tested for sensitivity to 5HT. Using a multivariate analysis of neuronal parameters, we found significant differences between severe, mild, unaffected and sham control motoneurons. Severe HI motoneurons showed more sustained firing patterns, depolarized resting membrane potential, and increased instantaneous firing frequency. Interestingly changes in persistent inward currents (PICs) and morphology in severe HI motoneurons do not appear to contribute to excitability. However, severe HI motoneurons were more responsive to αmethyl5HT than sham controls. Since there are higher levels of spinal serotonin *in vivo*, this would further increase excitability of severe motoneurons and promote muscle stiffness. In summary, changes we observed in spinal motoneuron physiology likely contribute to severity of the phenotype, and therapeutic strategies for CP could target excitability of spinal motoneurons.

Introduction

Cerebral palsy is not well understood, despite its prevalence and seriousness. There exist only a few evidence-based treatments for cerebral palsy: the effectiveness of many currently used therapeutic strategies is unclear (49, 64). Part of the problem in treating CP may be the diversity of causes including neonatal stroke, placental insufficiency, preterm birth, inflammation, traumatic injury, difficulties during birth and many other contributing factors (42). Another problem could be that modeling the condition in animals is complicated, and a larger animal models are needed to study motor deficits (15).

Serotonin is generally thought of as a neurotransmitter and neuromodulator, but developmental disruption in 5HT is associated with neurological disorders including autism, Rett syndrome, Down's syndrome and, more recently, cerebral palsy (2, 5, 18, 21, 46, 62, 65, 68). In both rodents and rabbits, developmental HI injury results in increased spinal 5HT, which could have a direct impact on excitability and on development of spinal neurons (5, 21). Descending serotonergic tracts from the raphe are among the first to arrive in the spinal cord (at 9 weeks post conception in humans (54)), and during periods of activity the spinal cord is bathed in 5HT through volume transmission (12, 30). In addition to the developing raphe, other sources of 5HT during development include the maternal raphe and the placenta (7), thus ensuring exposure of the developing fetus to 5HT. Levels of 5HT progressively increase until reaching a developmental peak at 1-3 years of age (35, 38). Despite evidence 5HT is important for the fetal nervous system, the effects of altering the presence of 5HT during development are not known. Changes in endogenous 5HT neurotransmission also occur in adulthood after injury and disease and result in profound changes in neuronal activity. After spinal cord injury, muscle spasms are caused by a cascade of activity involving constitutive activity of 5HT receptors and increasing motoneuron (MN) excitability (9, 47, 48). In summary, altered spinal 5HT could have a profound impact on excitability in CP, and its effects on development could leave a permanent mark on MN excitability.

In order to assess changes in intrinsic properties of spinal motoneurons, we used the rabbit HI model of cerebral palsy (19). It's been shown in previous studies that HI injury during late gestation in rabbits can result in a variety of neurologic and muscular damage, including muscle stiffness (19), loss of neurons in cortical layers 3 and 5, white matter injury, thinning of the corticospinal tract (13), increased spinal 5HT (21), cell death in the spinal cord and decreased numbers of spinal MNs (20), increased sarcomere length, decreased muscle mass and hyperreflexia (56).

Changes in MN physiology are likely to contribute to motor deficits, yet this not been directly assessed in any model of cerebral palsy. In this study we assessed both excitability of the spinal MNs and their ability to respond to 5HT. Both of these factors could dramatically alter excitability in the spinal cord and be potential targets for treatment of cerebral palsy. Our hypothesis was that changes in MN properties would correspond to the severity of motor deficits and altered activity of spinal MNs could contribute to muscle stiffness.

Methods

All rabbits were used according to the University of Rhode Island's Animal Care and Use Committee guidelines. Pregnant New Zealand White rabbits (Charles River Laboratories, Inc, Wilmington MA), underwent HI procedures as described in (19). Briefly, at ~80% gestation (day 25 of gestation (E25) dams

were anesthetized, and the left femoral artery was isolated. A Fogarty balloon catheter was inserted into the femoral and advanced to the level of the descending aorta, above the uterine arteries and inflated for 40 minutes. Sham animals underwent the same procedures but without inflation of the catheter. After the procedure, the dam recovered and later gave birth to kits with HI injuries. Categorization of the severity of the phenotype was performed by a blinded observer, using a modified Ashworth scale, observation / tests for activity, locomotion, posture, righting reflex, muscle tone (as described in (19)).

Patch Clamp Whole cell patch clamp is performed similar to previously published work (50). Briefly, horizontal spinal cord slices 350µm thick are obtained using a Leica 1000 vibratome. Slices are incubated for one hour at 30°C and perfused with oxygenated (95% O_2 and 5% CO_2) modified Ringer's solution containing (in mM): 111 NaCl, 3.09 KCl, 25.0 NaHCO3, 1.10 KH2PO4, 1.26 MgSO4, 2.52 CaCl2, and 11.1 glucose at 2 ml/min. Whole cell patch electrodes (1-3 MΩ) contain (in mM) 138 K-gluconate, 10 HEPES, 5 ATP-Mg, 0.3 GTP-Li and Texas Red dextran (150µM, 3000MW). PICs are measured in voltage clamp mode with holding potential of −90mV and depolarizing voltage ramps of both 22.5mV/s and 11.25 V/s bringing the cell to 0mV in 4s, and 8s respectively, and then back to the holding potential in the following 4 or 8 seconds. In current clamp, frequency – current measurements are obtained from current ramps and steps, as well as maximum firing rates and characteristics of action potentials and after-spike after hyperpolarization. Hyperpolarizing current steps are used to test Ih. After electrophysiological measurements are obtained, MNs are imaged to assess anatomical development, and photos are obtained of the electrode placement within the spinal cord slice, as shown in **Figure 1.** Images are acquired with a Nikon microscope fitted with a 40x water-dipping objective lens and two photon excitation fluorescence microscopy performed with a galvanometer-based Coherent Chameleon Ultra II laser. To optimize excitation of red/green fluorophores, the laser is tuned to 900nm. 3D reconstructions of motoneurons are created using Neurolucida 360° software.

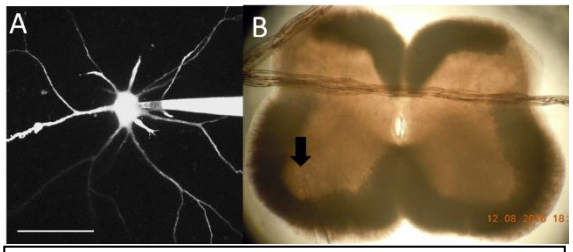

Figure 1 Patch clamp of MNs with dye-filling via patch electrode as in shown in **(A)**. Scale bar = 100µm. **(B)** Placement of patch electrode (at arrow) within the slice is captured with a photo.

Drug application Serotonergic drugs were bath applied and all electrophysiological parameters were recorded again. Either serotonin was applied at a concentration of 10µM or 0.3 µM α-methyl 5 hydroxy tryptamine in combination with 10 µM citalopram. Since perfusion rate was 2.5 ml/minute and dead space plus the bath volume was 10ml, all recordings were made 20 minutes after application, when the volume of the bath and tubing had been twice replaced.

Statistics A multivariate analysis using SPSS software was used for determining significance of parameters over groups. Injury classification (sham/control, HI unaffected, HI mildly affected, and HI severely affected), age of the kit, and spinal cord region (cervical, thoracic, lumbar or sacral) were all included as fixed factors. Paired T tests were used to determine significance of responses to serotonergic drugs.

Results

HI MNs show sustained firing

In rabbit kits severely injured by HI, MNs had some characteristics of hyperexcitability, including increased sustained firing. The frequency current (F-I) relationship was measured using current ramps, as shown in **Figure 2**. Depolarizing current ramps are used to evoke firing, and current at onset and offset of firing (I_{ON} and I_{OFF}) determines ΔI. In sham control MNs, ΔI was larger and always a positive value (191pA +/- 160pA), indicating firing ceased at a higher current amplitude than the current level that elicited firing on the ascending ramp (see figure 2A). Severe HI MNs had a smaller, and sometimes negative ΔI (84pA +/- 63pA), revealing increasingly sustained firing (see figure 2B). Figure 2 shows typical MNs from sham control and severe HI kits (black and red traces, respectively). Mean values for these parameters are shown in Figure 2C and D, with the intermediate classifications (HI unaffected and HI mildly affected) combined for brevity of the graphics only. To determine significance of the variables, a multivariate analysis was used that included 3 fixed factors: 1) condition (sham control, HI unaffected, HI mildly affected, and HI severely affected, 2) age (postnatal day 0-5), and 3) spinal region (cervical, thoracic, lumbar or sacral). Significance based on the multivariate analysis is indicated with asterisks (* $p < 0.05$). All data is included in table format in the supplementary data (Tables S1 – S15). Other significant differences between MNs are shown in **Figure 3**, including resting membrane potential (RMP) and instantaneous firing rates. Both parameters suggest increased excitability in HI-injured MNs: a depolarized RMP and faster rates of firing. Average resting membrane potential in sham control MNs was -60.3 +/-6.6mV, while in severe HI MNs it was -52.8 +/- 5.6mV ($p = 0.01$). Average instantaneous firing rate in sham control MNs was 98 Hz +/- 29 while severe HI MNs could fire at 129 Hz +/- 23 ($p = 0.02$). Sustained firing is also apparent in Figure 3, in the second current step which evokes a brief burst of action potentials followed by depolarization block in both MNs. The severe HI MN recovers and resumes firing while the sham control MN remains in depolarization block. Mean values for these parameters are shown in bar graphs (Figure 3D, E), with the intermediate classifications (HI unaffected and HI mildly affected) combined. Significance based on the multivariate analysis is indicated with asterisks (* $p < 0.05$). All data on the active and passive properties is included in the supplementary tables (Table S1 – Table S8).

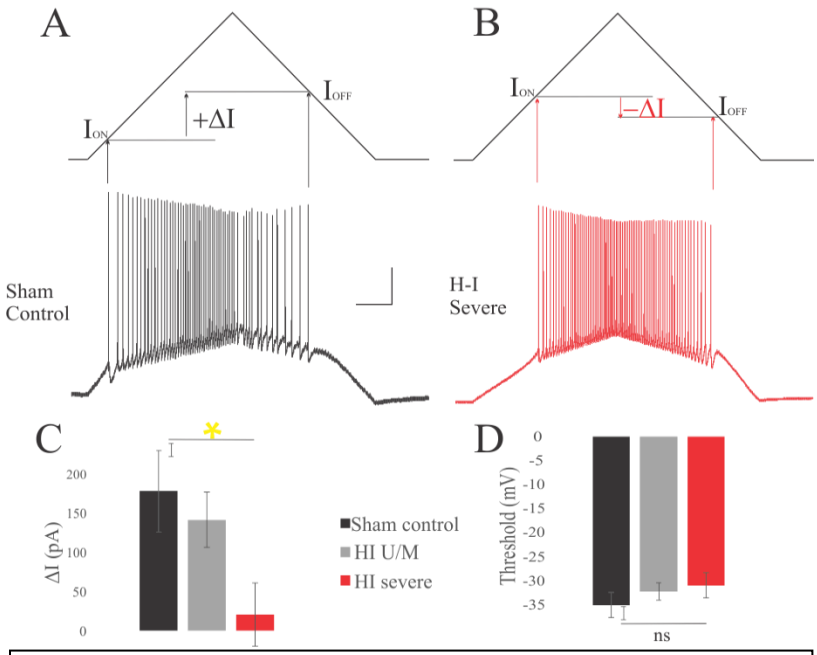

Figure 2: Severe HI motoneurons show more sustained firing than controls. Control motoneurons **(A)** have larger values for ΔI compared to severe HI **(B)**. Average ΔI and threshold are shown in **(C)** and **(D)** for sham control (n = 9), HI unaffected and mild (HI U/M; n = 20), and HI severe (n = 8). Error bars = SEM. Scale bars in A = 20mV (vertical) and 0.5s (horizontal) and applies to panels A and B.

Persistent inward currents suggest excitability is dampened after HI

PICs were significantly affected by hypoxia-ischemia, but instead of showing signs of hyperexcitability similar to the firing patterns, they revealed that intrinsic excitability may be dampened. Specifically, the voltage dependence was shifted to higher potentials so the maximum amplitude of the PIC in HI severe MNs occurred at a significantly more depolarized voltage compared to sham control MNs, as shown in **Figure 4**. This would suggest PICs would not be activated as easily in HI injured MNs as their control counterparts. Altered amplitude was not observed outright: the magnitude of the currents was similar (see figure 4D), however the normalized amplitude was significantly smaller in HI MNs (see figure 4E). Normalized amplitude is based on the whole cell capacitance of the MNs, which can be used as an indirect measurement of membrane area. In other words, the current generated per unit of membrane area in HI injured MNs was smaller than in control MNs, and it reached a peak at a higher voltage. These parameters are summarized in Figure 4B – E, with the intermediate classifications (HI unaffected and HI mildly affected) combined only for graphical presentation. Significance based on the multivariate analysis is indicated with asterisks (* $p < 0.05$; ** $p < 0.01$). All PIC data is included in supplementary tables S5 and S6, and all data on cell properties is included in supplementary tables S9 and S10.

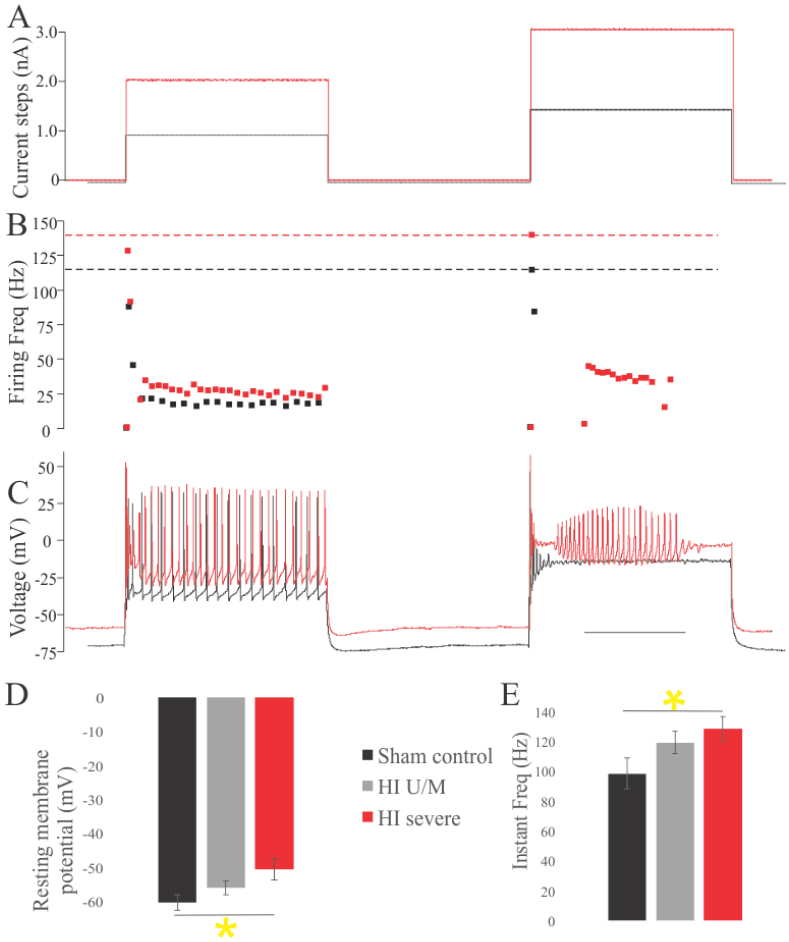

Figure 3: Altered RMP and firing frequency is present in HI-injured MNs. Depolarizing current steps **(A)** evoked action potentials **(C)** in a typical sham control (black trace) and severe HI (red trace) MN. **(B)** Dotted lines show peak (instantaneous) firing frequency of MNs in C. **(D)** Average resting membrane potential and **(E)** firing frequency are significantly different between groups (n = 8 sham control, 22 unaffected to mildly affected HI (HI U/M) and 8 severely affected HI MNs). Error bars = SEM. Scale bar in C = 0.5 sec and applies to panels A, B, and C.

Morphology / membrane properties affected by HI injury

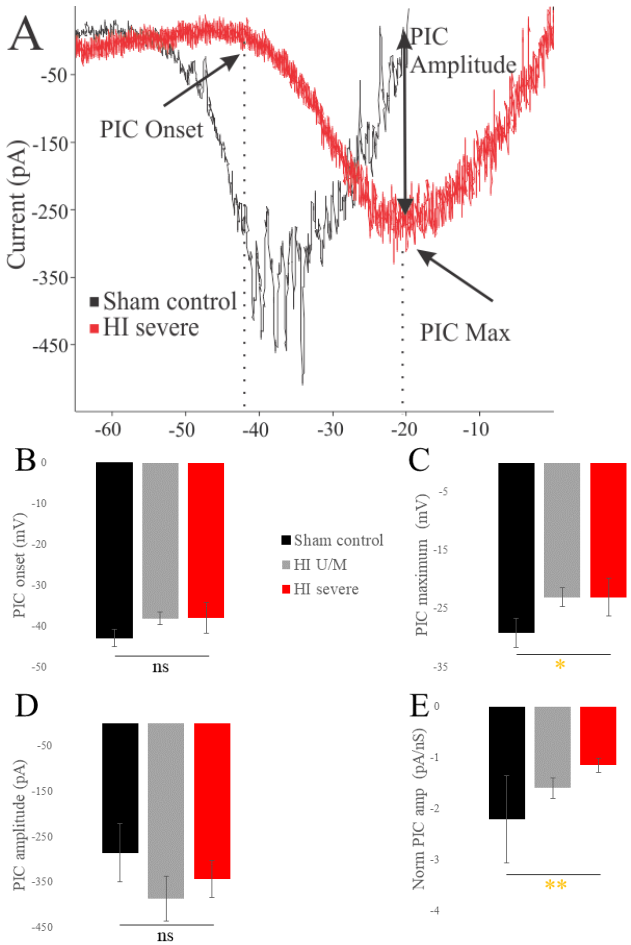

Figure 4: PICs are altered in HI injured motoneurons. **(A)** Typical PICs (leak subtracted) are shown from sham control (black) and HI severe (red) motoneurons. **(B)** PIC onset voltage is not significantly shifted after injury. **(C)** The voltage at which the PIC reached the maximum amplitude (PIC Max) was more depolarized while PIC amplitude **(D)** was not altered. **(E)** PIC amplitude normalized to capacitance was significantly smaller after HI injury. N = 9 sham control, 22 unaffected to mildly affected HI (HI U/M) and 8 severely affected HI MNs. Error bars = SEM.

As suggested by the normalized PIC amplitude, membrane properties are affected by HI injury, including a significantly larger capacitance (as shown in **Figure 5**). Whole cell capacitance is often used as an indirect measure of cell size (larger cells have larger capacitance), thus one might expect increased neuron size after HI injury. However the soma was unchanged in size. No significant changes were observed in soma largest cross-sectional area (Fig 5D), soma volume or soma surface area (data not shown). There was, however a significant increase in dendrite length which could account for the increased capacitance detected in electrophysiological recordings. No changes were measured in the number of dendrites, dendritic nodes

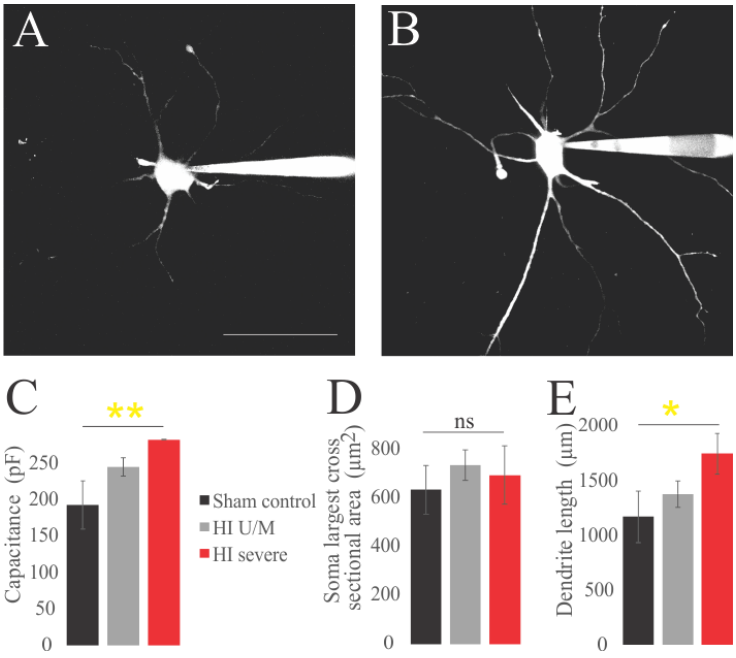

Figure 5: Morphology is affected by HI. Typical sham control **(A)** and HI severe **(B)** motoneurons filled with dye during patch clamp (electrodes visible on right). Average values of whole cell capacitance **(C)**, soma largest cross-sectional area **(D)** and dendrite length **(E)** are included for all neurons (n = 8 sham control; n = 21 unaffected and mild; n = 8 HI severe). Scale bar in A = 100µm, applies to A and B. Error bars = SEM.

(branching points), dendritic volume or dendritic surface area (data not shown). Significance based on the multivariate analysis is indicated with asterisks (* $p < 0.05$; ** $p < 0.01$). All data on neuron morphology is included in supplementary tables S11 – S14.

Motoneuron response to serotonin

Motoneurons were tested with application of serotonin or αmethyl5HT after their electrophysiological parameters had been measured. The response of MNs from kits that had been severely affected by HI was different from the response of MNs from sham animals. For example, PICs from sham control MNs showed a decrease in PIC amplitude while severe HI MNs showed a change in the voltage at onset and the voltage at maximum of the PIC, as shown in **Figure 6**. Modulation of action potential firing by 5HT also varied between groups. **Figure 7** highlights several key parameters that were differently altered,

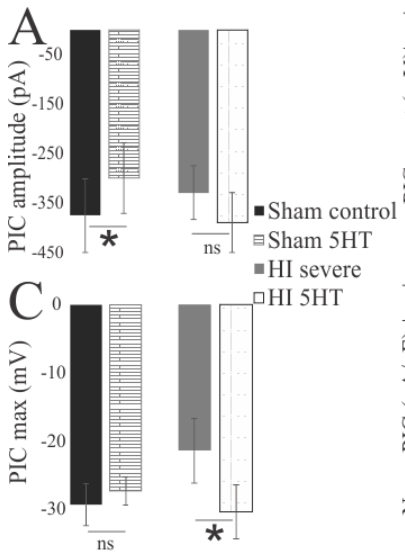

Figure 6: PICs were differently modulated in control and severe HI MNs by 5HT. PIC amplitude **(A)**, onset voltage **(B)** and maximum PIC voltage **(C)** were all significantly altered in the presence of 5HT in one group but not the other. N = 6 control MNs and 6 HI MNs (all before and after 5HT). Normalized PIC **(D)** was not significantly altered. N = 4 control and 5 HI MNs before and after 5HT. Error bars = SEM.

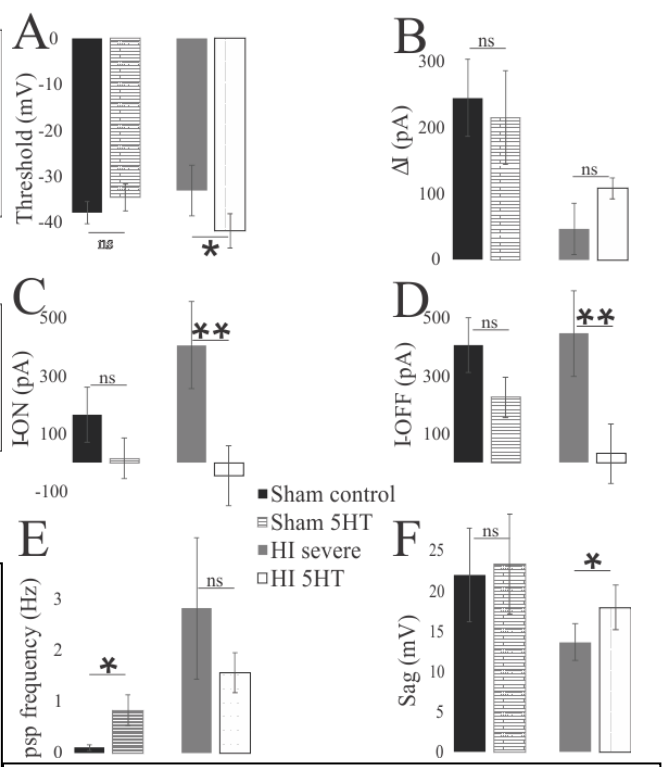

Figure 7: Action potential firing is differently modulated in control and severe HI MNs by 5HT. Threshold voltage **(A)** was hyperpolarized in HI MNs but not control. **(B)** ΔI was not significantly altered in either group. I-ON and I-OFF **(C-D)** were significantly altered in HI MNs with 5HT but not in controls. **(E)** The frequency of postsynaptic potentials was significantly different in control MNs before and after 5HT application, but not HI MNs. **(F)** Hyperpolarization activated sag currents were also differentially altered by 5HT in control and HI MNs. N = 6 control MNs and 6 HI MNs (all before and after 5HT). Error bars = SEM.

including the current at firing onset (I-ON), the current at firing offset (I-OFF), threshold voltage and the frequency of post synaptic potentials (psps). Significance based on paired T tests (before and after 5HT) is indicated with asterisks (* $p < 0.05$; ** $p < 0.01$). All data on changes in active and passive properties before and after 5HT is included in Supplementary Table S15.

Discussion

Summary

Electrophysiological properties of spinal MNs are altered by developmental HI injury, and the magnitude of changes are correlated to severity of motor deficits. This work also found altered responsiveness of MNs to serotonergic agonist αmethyl5HT. Interestingly, severely affected HI MNs showed a robust increase in excitability in the presence of αmethyl5HT, while sham control MNs had only a weak response to αmethyl5HT. Since traditional views of CP largely view motor dysfunction as a result of the damaged motor cortex improperly signaling to spinal neurons, our new evidence suggests this is only part of the problem. Spinal MNs are not developing the same after HI and show an overall change in excitability, including elevated responses to 5HT compared to sham control MNs. Whether these changes are directly due to the HI insult or indirectly due to downstream effects must be determined by future work.

Contribution of serotonin to motoneuron excitability

Serotonin is a potent neuromodulator of MN activity. Starting early in development, 5HT increases MN excitability, enhances PICs, regulates muscle tone and reflexes, and amplifies synaptic inputs (17, 29, 31, 33, 39, 59, 60, 69). Depolarization of the resting membrane potential, increased action potential firing through hyperpolarization of the voltage threshold and enhanced PIC, increased action potential height and reduction of high-voltage activated Ca^{2+} entry are all associated with 5HT receptor activation in MNs (3, 22, 25, 32–34, 37, 39, 41). There are seven families of 5HT receptors ($5HT_1$ – $5HT_7$) containing 14 mammalian receptors. Motoneurons express receptors from each family with the apparent exception only of $5HT_6$ receptors (14, 16, 45, 55, 67, 69). The most well-defined roles in MN excitability are for $5HT_1$, and $5HT_2$ receptors. Previous studies have shown that activation of $5HT_{2A/2B/2C}$ receptors upregulate both Na^+ and Ca^{2+} PICs and increase intrinsic excitability (27, 28, 36). In the present study we activated the MNs with α methyl 5HT. This is an agonist for $5HT_{2/1C}$ receptors, though its effects on MNs are commonly attributed to the more well-established effects of $5HT_2$ receptors. Generally $5HT_2$ receptors excite MNs while $5HT_1$ receptors are inhibitory (36). Changing activity patterns in adults can also alter expression serotonin receptors: after exercise, $5HT_{1A}$ receptor mRNA decreased in motoneurons (66), while 5HT immunoreactivity increased (4). Thus the serotonergic system is highly plastic, both during development and in adulthood, in health and after injury.

Developmental role of 5HT

The evolutionary importance of serotoninergic signaling during development is suggested from the existence of several mechanisms which prevent its loss. During gestation in humans, fetal exposure to serotonin arises from maternal sources, from the developing fetal brainstem and from the placenta (6, 7). Even when the CNS is isolated from an embryonic mouse, removal of the raphe nucleus is not sufficient to eliminate serotonin: in the absence of serotonergic projections, aromatic acid decarboxylase cells in the developing spinal cord can synthesize 5HT (11), a mechanism that could play a role in serotonergic signaling in the adult spinal cord after descending serotonergic fibers are lost in spinal cord injury (63). In stark contrast to most brainstem-spinal projecting neurons (including rubro- reticulo- and vestibulospinal projecting neurons) which are only capable of axon regeneration at early developmental time points, serotonergic raphe-spinal explants from chick show robust neurite outgrowth and maintained expression

of 5-hydroxytryptamine even when obtained well after the permissive period and grown on growth factor-free media (8).

The effects of 5HT on motor circuits are numerous, and dynamic throughout development and adulthood. In the spinal cord, neurons begin expressing 5HT receptors early in embryonic development whether or not 5HT is present (24, 69). When 5HT transmission is blocked during development, it alters maturation of inhibitory synapses (1, 11). After birth, mouse MNs become much more responsive to 5HT (69), including increasing responsiveness of the PIC to neuromodulation by serotonin (and other neuromodulators) with increasing postnatal age (51). Serotonin also changes neuron outgrowth: $5HT_{1A}$ and $5HT_{2A}$ receptor activation increased neurite outgrowth, dendritic branching, spine formation and cell survival (10, 23, 44). In the present study, our finding that dendritic length was increased in HI severe motoneurons is likely the effect of exposure to increased spinal serotonin after HI. In addition to having effects on development, serotonin receptors are themselves affected by development. During postnatal maturation hypoglossal MNs increase expression of $5HT_{2A}$ and $5HT_{2C}$ receptors, while $5HT_{1B}$ receptor expression transiently falls around 2 weeks of age and then increases again (58). After HI injury in rabbits, mRNA for $5HT_2$ receptors was reduced in the spinal cord and expression of mRNA for SERT transporters increased (21). It is not yet known if expression of other 5HT receptor subtypes is altered either during normal development in rabbits or after HI injury. Future studies are needed to further investigate these changes.

A role for 5HT in etiology of motor dysfunction

Several conditions including stroke, dorsal rhizotomy, spinal cord injury and traumatic brain injury alter serotonergic signaling, and augmenting serotonin therapeutically could improve recovery (17, 26, 40, 47, 48). Acute intermittent hypoxia, which has been found to be therapeutic for some of the above conditions, acts through serotonin receptors (57, 61), at least in phrenic motoneurons. Thus, there could be specific effects of both hypoxia and injury that promote upregulation of serotonergic signaling.

Contribution of spinal motoneurons to dysfunction in cerebral palsy

In patients with cerebral palsy, there have been case reports of increased spasticity after starting selective serotonin reuptake inhibitors for treatment of depression (52), and reports of successful treatment of baclofen withdrawal symptoms with serotonergic antagonists (43, 53). However exploiting serotonergic signaling pathways for treatment of CP has not been under-explored. Since 5HT is known to largely increase excitability of MNs, its contribution to muscle stiffness and spasticity in cerebral palsy is central to our study. Our results confirm that serotonergic signaling robustly increases MN activity. Previous work that explored intrathecal delivery of serotonergic antagonist methysergide showed that muscle stiffness in rabbits affected by HI was alleviated by blockade of 5HT receptors (21). Here we show that MNs are directly responsive to serotonin, in fact even more so after HI injury. This data supports further exploration of serotonergic modulators, and therapies that are effective at restored balance of serotonergic function for alleviation of spasticity.

Conclusion

Changes in MN physiology after developmental injury are consistent with motor deficits in rabbits. This suggests not only brain injuries but also changes in the spinal cord contribute to impaired function in

cerebral palsy. Exploring both altered maturation of spinal neurons and loss of descending connectivity should be pursued to improve outcomes for individuals with cerebral palsy.

References

1. **Allain A-E**, **Ségu L**, **Meyrand P**, **Branchereau P**. Serotonin controls the maturation of the GABA phenotype in the ventral spinal cord via 5-HT1B receptors. *Ann N Y Acad Sci* 1198: 208–219, 2010.

2. **Bar-Peled O**, **Gross-Isseroff R**, **Ben-Hur H**, **Hoskins I**, **Groner Y**, **Biegon A**. Fetal human brain exhibits a prenatal peak in the density of serotonin 5-HT1A receptors. *Neurosci Lett* 127: 173–176, 1991.

3. **Bayliss DA**, **Umemiya M**, **Berger AJ**. Inhibition of N- and P-type calcium currents and the after-hyperpolarization in rat motoneurones by serotonin. *J Physiol* 485: 635–647, 1995.

4. **Behan M**, **Moeser AE**, **Thomas CF**, **Russell JA**, **Wang H**, **Leverson GE**, **Connor NP**. The effect of tongue exercise on serotonergic input to the hypoglossal nucleus in young and old rats. *J Speech, Lang Hear Res* 55: 919–929, 2012.

5. **Bellot B**, **Peyronnet-Roux J**, **Gire C**, **Simeoni U**, **Vinay L**, **Viemari JC**. Deficits of brainstem and spinal cord functions after neonatal hypoxia-ischemia in mice. *Pediatr Res* 75: 723–730, 2014.

6. **Bonnin A**, **Goeden N**, **Chen K**, **Wilson ML**, **King J**, **Shih JC**, **Blakely RD**, **Deneris ES**, **Levitt P**. A transient placental source of serotonin for the fetal forebrain. *Nature* 472: 347–350, 2011.

7. **Bonnin A**, **Levitt P**. FETAL , MATERNAL , AND PLACENTAL SOURCES OF SEROTONIN AND NEW IMPLICATIONS FOR DEVELOPMENTAL PROGRAMMING OF THE BRAIN. *Neuroscience* 197: 1–7, 2011.

8. **Borisoff JF**, **Pataky DM**, **Mcbride CB**, **Steeves JD**. Raphe – Spinal Neurons Display an Age-Dependent Differential Capacity for Neurite Outgrowth Compared to Other Brainstem – Spinal Populations. 28: 16–28, 2000.

9. **Bos R**, **Sadlaoud K**, **Boulenguez P**, **Buttigieg D**, **Liabeuf S**, **Brocard C**, **Haase G**, **Bras H**, **Vinay L**. Activation of 5-HT2A receptors upregulates the function of the neuronal K-Cl cotransporter KCC2. 110, 2013.

10. **Bou-Flores C**, **Lajard A**, **Monteau R**, **De Maeyer E**, **Seif I**, **Lanoir J**, **Hilaire G**. Abnormal Phrenic Motoneuron Activity and Morphology in Neonatal Monoamine Oxidase A-Deficient Transgenic Mice : Possible Role of a Serotonin Excess. 20: 4646–4656, 2000.

11. **Branchereau P**, **Chapron J**, **Meyrand P**. Descending 5-Hydroxytryptamine Raphe Inputs Repress the Expression of Serotonergic Neurons and Slow the Maturation of Inhibitory Systems in Mouse Embryonic Spinal Cord. *J Neurosci* 22: 2598–2606, 2002.

12. **Bunin MA**, **Mark Wightman R**. Paracrine neurotransmission in the CNS: Involvement of 5-HT. *Trends Neurosci* 22: 377–382, 1999.

13. **Buser JR**, **Segovia KN**, **Dean JM**, **Nelson K**, **Beardsley D**, **Gong X**, **Luo NL**, **Ren J**, **Wan Y**, **Riddle A**, **McClure MM**, **Ji X**, **Derrick M**, **Hohimer AR**, **Back SA**, **Tan S**. Timing of appearance of late oligodendrocyte progenitors coincides with enhanced susceptibility of preterm rabbit cerebral white matter to hypoxia-ischemia. *J Cereb Blood Flow Metab* 30: 1053–1065, 2010.

14. **Cabaj AM**, **Majczyński H**, **Couto E**, **Gardiner PF**, **Stecina K**, **Sławińska U**, **Jordan LM**. Serotonin controls initiation of locomotion and afferent modulation of coordination via 5-HT7 receptors in adult rats. *J Physiol* 595: 301–320, 2017.

15. **Cavarsan CF**, **Gorassini MA**, **Quinlan KA**. Animal models of developmental motor disorders: parallels to human motor dysfunction in cerebral palsy. *J Neurophysiol* 122: 1238–1253, 2019.

16. **Cotel F**, **Exley R**, **Cragg SJ**, **Perrier JF**. Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. *Proc Natl Acad Sci U S A* 110: 4774–4779, 2013.

17. **D'Amico JM**, **Li Y**, **Bennett DJ**, **Gorassini MA**. Reduction of spinal sensory transmission by facilitation of 5-HT 1B/D receptors in noninjured and spinal cord-injured humans. *J Neurophysiol* 109: 1485–1493, 2013.

18. **De Filippis B**, **Chiodi V**, **Adriani W**, **Lacivita E**, **Mallozzi C**, **Leopoldo M**, **Domenici MR**, **Fuso A**, **Laviola G**. Long-lasting beneficial effects of central serotonin receptor 7 stimulation in female mice modeling Rett syndrome. *Front Behav Neurosci* 9: 1–11, 2015.

19. **Derrick M**, **Luo NL**, **Bregman JC**, **Jilling T**, **Ji X**, **Fisher K**, **Gladson CL**, **Beardsley DJ**, **Murdoch G**, **Back SA**, **Tan S**. Preterm Fetal Hypoxia-Ischemia Causes Hypertonia and Motor Deficits in the Neonatal Rabbit: A Model for Human Cerebral Palsy? *J Neurosci* 24: 24–34, 2004.

20. **Drobyshevsky A**, **Quinlan KA**. Spinal cord injury in hypertonic newborns after antenatal hypoxia-ischemia in a rabbit model of cerebral palsy. *Exp Neurol* 293: 13–26, 2017.

21. **Drobyshevsky A**, **Takada SH**, **Luo K**, **Derrick M**, **Yu L**, **Quinlan KA**, **Vasquez-Vivar J**, **Nogueira MI**, **Tan S**. Elevated spinal monoamine neurotransmitters after antenatal hypoxia-ischemia in rabbit cerebral palsy model. *J Neurochem* 132: 394–402, 2015.

22. **Elliot P**, **Wallis DI**. Serotonin and L-Norepinephrine as Mediators of Altered Excitability in Neonatal Rat Motoneurons Studied In Vitro. *Neuroscience* 47: 533–544, 1992.

23. **Fricker AD**, **Rios C**, **Devi LA**, **Gomes I**. Serotonin receptor activation leads to neurite outgrowth and neuronal survival. *Mol Brain Res* 138: 228–235, 2005.

24. **Gao B**, **Ziskind-Conhaim L**. Development of chemosensitivity in serotonin deficient spinal cords of rat embryos. *Dev Biol* 158: 79–89, 1993.

25. **Gilmore J**, **Fedirchuk B**. The excitability of lumbar motoneurones in the neonatal rat is increased by a hyperpolarization of their voltage threshold for activation by descending serotonergic fibres. *J Physiol* 558: 213–224, 2004.

26. **Gu S**, **Wang C**. Early Selective Serotonin Reuptake Inhibitors for Recovery after Stroke : A Meta-Analysis and Trial Sequential Analysis. *J Stroke Cerebrovasc Dis* 27: 1178–1189, 2018.

27. **Harvey PJ**, **Li X**, **Li Y**, **Bennett DJ**. Endogenous monoamine receptor activation is essential for enabling persistent sodium currents and repetitive firing in rat spinal motoneurons. *J Neurophysiol* 96: 1171–1186, 2006.

28. **Harvey PJ**, **Li X**, **Li Y**, **Bennett DJ**. 5-HT 2 receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury. *J Neurophysiol* 96: 1158–1170, 2006.

29. **Heckman CJ**, **Mottram C**, **Quinlan K**, **Theiss R**, **Schuster J**. Motoneuron excitability: The importance of neuromodulatory inputs. *Clin Neurophysiol* 120: 2040–2054, 2009.

30. **Hentall ID**, **Pinzon A**, **Noga BR**. Spatial and temporal patterns of serotonin release in the rat's lumbar spinal cord following electrical stimulation of the nucleus raphe magnus. *Neuroscience* 142: 893–903, 2006.

31. **Hounsgaard J**, **Hultborn H**, **Jespersen B**, **Kiehn O**. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J Physiol* 405: 345–367, 1988.

32. **Hsiao CF**, **Del Negro CA**, **Trueblood PR**, **Chandler SH**. Ionic basis for serotonin-induced bistable membrane properties in guinea pig trigeminal motoneurons. *J Neurophysiol* 79: 2847–2855, 1998.

33. **Hsiao CF**, **Trueblood PR**, **Levine MS**, **Chandler SH**. Multiple effects of serotonin on membrane properties of trigeminal motoneurons in vitro. *J Neurophysiol* 77: 2910–2924, 1997.

34. **Inoue T**, **Itoh S**, **Kobayashi M**, **Kang Y**, **Matsuo R**, **Wakisaka S**, **Morimoto T**. Serotonergic modulation of the hyperpolarizing spike afterpotential in rat jaw-closing motoneurons by PKA and PKC. *J Neurophysiol* 82: 626–637, 1999.

35. **Lackovic Z**, **Jakupcevic M**, **Bunarevic A**, **Damjanov I**, **Relja M**, **Kostovic I**. Serotonin and norepinephrine in the spinal cord of man. *Brain Res* 443: 199–203, 1988.

36. **Ladewig T**, **Lalley PM**, **Keller BU**. Serotonergic modulation of intracellular calcium dynamics in neonatal hypoglossal motoneurons from mouse. *Brain Res* 1001: 1–12, 2004.

37. **Larkman PM**, **Kelly JS**. Ionic Mechanisms Mediating 5-Hydroxytryptamine- and Noradrenaline-Evoked Depolarization of Adult Rat Facial Motoneurones. *J Physiol* 456: 473–490, 1992.

38. **Lewinsohn R**, **Glover V**, **Sandler M**. Development of benzylamine oxidase and monoamine oxidase A and B in man. *Biochem Pharmacol* 29: 1221–1230, 1980.

39. **Li X**, **Murray K**, **Harvey PJ**, **Ballou EW**, **Bennett DJ**. Serotonin facilitates a persistent calcium current in motoneurons of rats with and without chronic spinal cord injury. *J Neurophysiol* 97: 1236–1246, 2007.

40. **Liepert J**. Update on pharmacotherapy for stroke and traumatic brain injury recovery during rehabilitation. *Curr Opin Neurol* 29: 700–705, 2016.

41. **Lindsay AD**, **Feldman JL**. Modulation of respiratory activity of neonatal rat phrenic motoneurones by serotonin. *J Physiol* 461: 213–233, 1993.

42. **MacLennon A**, **International Cerebral Palsy Task Force**. A template for definint a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *Br Med J* 319: 1054–1059, 1999.

43. **Meythaler JM**, **Roper JF**, **Brunner RC**. Cyproheptadine for intrathecal baclofen withdrawal. *Arch Phys Med Rehabil* 84: 638–642, 2003.

44. **Mogha A**, **Guariglia SR**, **Debata PR**, **Wen GY**, **Banerjee P**. Serotonin 1A receptor-mediated signaling through ERK and PKCα is essential for normal synaptogenesis in neonatal mouse hippocampus. *Transl Psychiatry* 2: 1–12, 2012.

45. **Morales M**, **Battenberg E**, **Bloom FE**. Erratum: Distribution of neurons expressing immunoreactivity for the 5HT3 receptor subtype in the rat brain and spinal cord (Journal of Comparative Neurology (1998) 402 (385-401)). *J Comp Neurol* 406: 141, 1999.

46. **Muller CL**, **Anacker AMJ**, **Veenstra-VanderWeele J**. The serotonin system in autism spectrum disorder: From biomarker to animal models. *Neuroscience* 321: 24–41, 2016.

47. **Murray KC**, **Nakae A**, **Stephens MJ**, **Rank M**, **D'Amico J**, **Harvey PJ**, **Li X**, **Harris RLW**, **Ballou EW**, **Anelli R**, **Heckman CJ**, **Mashimo T**, **Vavrek R**, **Sanelli L**, **Gorassini MA**, **Bennett DJ**, **Fouad K**. Recovery of motoneuron and locomotor function after spinal cord injury depends on constitutive activity in 5-HT 2C receptors. *Nat Med* 16: 694–700, 2010.

48. **Murray KC**, **Stephens MJ**, **Ballou EW**, **Heckman CJ**, **Bennett DJ**. Motoneuron excitability and muscle spasms are regulated by 5-HT2B and 5-HT2C receptor activity. *J Neurophysiol* 105: 731–748, 2011.

49. **Novak I**, **Mcintyre S**, **Morgan C**, **Campbell L**, **Dark L**, **Morton N**, **Stumbles E**, **Wilson SA**, **Goldsmith S**. A systematic review of interventions for children with cerebral palsy: State of the evidence. *Dev Med Child Neurol* 55: 885–910, 2013.

50. **Quinlan KA**, **Schuster JE**, **Fu R**, **Siddique T**, **Heckman CJ**. Altered postnatal maturation of electrical properties in spinal motoneurons in a mouse model of amyotrophic lateral sclerosis. *J Physiol* 589: 2245–2260, 2011.

51. **Revill AL**, **Chu NY**, **Ma L**, **LeBlancq MJ**, **Dickson CT**, **Funk GD**. Postnatal development of persistent inward currents in rat XII motoneurons and their modulation by serotonin, muscarine and noradrenaline. *J Physiol* 597: 3183–3201, 2019.

52. **Rone LA**, **Ferrando SJ**. Serotonin reuptake inhibitor-related extrapyramidal side effects in two patients with cerebral palsy. *Psychosomatics* 37: 165–166, 1996.

53. **Saveika JA**, **Shelton JE**. Cyproheptadine for pediatric intrathecal baclofen withdrawal: A case report. *Am J Phys Med Rehabil* 86: 994–997, 2007.

54. **Sundström E**, **Kölare S**, **Souverbic F**, **Samuelsson EB**, **Pschera H**, **Lunell NO**, **Seiger Å**. Neurochemical differentiation of human bulbospinal monoaminergic neurons during the first trimester. *Dev Brain Res* 75: 1–12, 1993.

55. **Suwa B**, **Bock N**, **Preusse S**, **Rothenberger A**, **Manzke T**. Distribution of Serotonin 4(a) Receptors in the juvenile Rat Brain and Spinal Cord. *J Chem Neuroanat* 55: 67–77, 2014.

56. **Synowiec S**, **Lu J**, **Yu L**, **Goussakov I**, **Lieber R**, **Drobyshevsky A**. Spinal hyper-excitability and altered muscle structure contribute to muscle hypertonia in newborns after antenatal hypoxia-ischemia in a rabbit cerebral palsy model. *Front Neurol* 10: 1–18, 2019.

57. **Tadjalli A**, **Mitchell GS**. Cervical spinal 5-HT 2A and 5-HT 2B receptors are both necessary for moderate acute intermittent hypoxia-induced phrenic long-term facilitation . *J Appl Physiol* 127: 432–443, 2019.

58. **Volgin D V.**, **Fay R**, **Kubin L**. Postnatal development of serotonin 1B, 2A and 2C receptors in brainstem motoneurons. *Eur J Neurosci* 17: 1179–1188, 2003.

59. **Wang M**, **Dun N**. 5-Hyroxytryptamine responses in neonate rat motoneurones in vitro. *J Physiol*

430: 87–103, 1990.

60. **Wei K**, **Glaser JI**, **Deng L**, **Thompson CK**, **Stevenson IH**, **Wang Q**, **Hornby TG**, **Heckman CJ**, **Kording KP**. Serotonin affects movement gain control in the spinal cord. *J Neurosci* 34: 12690–12700, 2014.

61. **Wen M-H**, **Vinit S**, **Lee K-Z**. Modulation of serotonin and adenosine 2A receptors on intermitten hypoxia-induced respiratory recovery following mid-cervical contusion in the rat. .

62. **Whittle N**, **Sartori SB**, **Dierssen M**, **Lubec G**, **Singewald N**. Fetal Down syndrome brains exhibit aberrant levels of neurotransmitters critical for normal brain development. *Pediatrics* 120: e1465–e1471, 2007.

63. **Wienecke J**, **Ren LQ**, **Hultborn H**, **Chen M**, **Møller M**, **Zhang Y**, **Zhang M**. Spinal cord injury enables aromatic L-amino acid decarboxylase cells to synthesize monoamines. *J Neurosci* 34: 11984–12000, 2014.

64. **Wimalasundera N**, **Stevenson VL**. Cerebral palsy. *Pract Neurol* 16: 184–194, 2016.

65. **Wirth A**, **Holst K**, **Ponimaskin E**. How serotonin receptors regulate morphogenic signalling in neurons. *Prog Neurobiol* 151: 35–56, 2017.

66. **Woodrow L**, **Sheppard P**, **Gardiner PF**. Transcriptional changes in rat α-motoneurons resulting from increased physical activity. *Neuroscience* 255: 45–54, 2013.

67. **Xu C**, **Giuliano F**, **Sun X**, **Brisorgueil M-J**, **Leclerc P**, **Verge D**, **Conrath M**. Serotonin 5-HT2A and 5-HT5A Receptors Are Expressed by Different Motoneuron Populations in Rat Onuf's Nucleus. *J Comp Neurol* 502: 620–634, 2007.

68. **Yang CJ**, **Tan HP**, **Du YJ**. The developmental disruptions of serotonin signaling may involved in autism during early brain development. *Neuroscience* 267: 1–10, 2014.

69. **Ziskind-Conhaim L**, **Seebach BS**, **Gao BX**. Changes in serotonin-induced potentials during spinal cord development. *J Neurophysiol* 69: 1338–1349, 1993.