Original Article

Dose-Response:
An International Journal
January-March 2021:1-10

DOI: 10.1177/1559325821998136
journals.sagepub.com/home/dos
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Buspirone Dose-Response on Facilitating Forelimb Functional Recovery in Cervical Spinal Cord Injured Rats

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Abstract

Buspirone, widely used as a neuropsychiatric drug, has also shown potentials for motor function recovery of injured spinal cord. However, the optimum dosages of such treatment remain unclear. In this study, we investigated the dose-response of Buspirone treatment on reaching and grasping function in cervical cord injured rats. Seventeen adult Sprague-Dawley rats were trained to reach and grasp sugar pellets before a C4 bilateral dorsal column crush injury. After 1 week post-injury, the rats were divided into 3 groups to receive 1 of 3 different dosages of Buspirone (i.p., 1 dose/day: 1.5, n = 5; 2.5, n = 6 and 3.5 mg/kg b.w., n = 6). Forelimb reaching and grip strength test were recorded once per week, within 1 hour of Buspirone administration for 11 weeks post-injury. Different dose groups began to exhibit differences in reaching scores from 4 weeks post-injury. From 4-11 weeks post-injury, the reaching scores were highest in the lowest-dose group rats compared to the other 2 dose groups rats. Average grip strength was also found higher in the lowest-dose rats. Our results demonstrate a significant dose-dependence of Buspirone on the recovery of forelimb motor functions after cervical cord injury with the best performance occurring at the lowest dose tested.

Keywords

serotonin, cervical cord injury, neuromodulation, functional rehabilitation

Introduction

Serotonin (5-hydroxytryptamine or 5-HT) is a monoamine neurotransmitter. Almost all 5-HT axons found within the mammalian spinal cord supraspinally originate from the brainstem.[1] Serotonin plays a vital role in modulating the activity of the spinal network for the gain control of volitional limb movements[2] and inducing partial recovery of plantar stepping.[3] Disruption of serotonin pathways following spinal cord injury (SCI) results in depletion of 5-HT in the spinal neural network, dysregulation of 5-HT transporters as well as elevated expression and sensitivity of specific 5-HT receptors below the spinal lesion.[4]

These changes in the serotonergic system of the spinal cord can produce varying degrees of functional complications after paralysis. To date, various neuropharmacologies have been tested to restore hindlimb functions after SCI.[5-9] 5-HT receptor agonists, when combined with electrical stimulation restores locomotor function in SCI rats primarily by neuromodulating the physiological states of spinal networks that generate stepping.[10,11] Although serotonin agonists have demonstrated improved functional recovery in standing and stepping in SCI paraplegics, limited progress has been made in improving arm and hand functions using such neurochemical modulation.[12] Moreover, $5HT_2$ receptor agonists alone can facilitate the

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Received 26 January 2021; received revised 26 January 2021; accepted 05 February 2021

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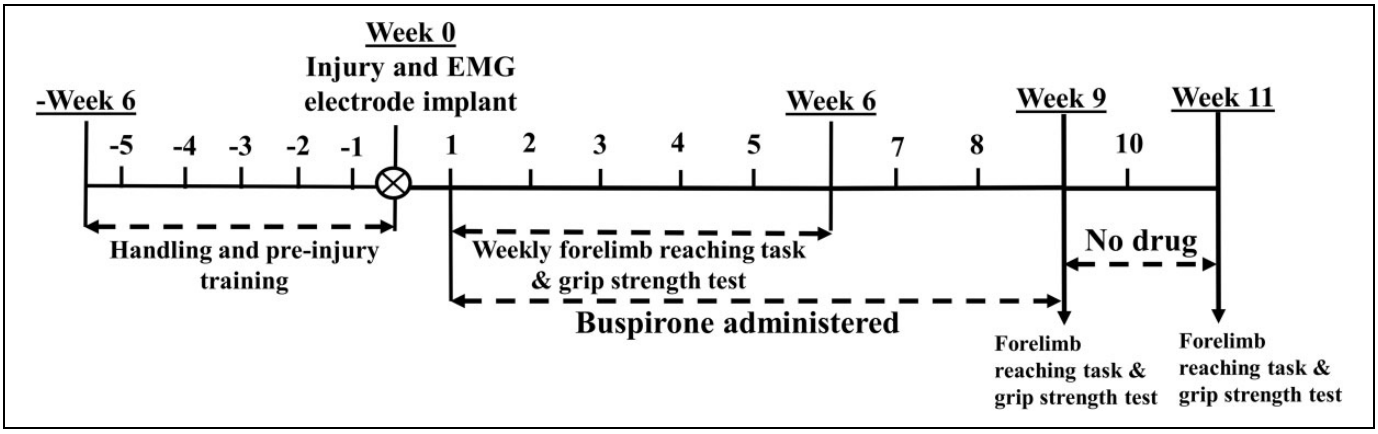

Figure 1. Experimental procedure. Seventeen Sprague-Dawley rats were trained to reach and grasp the pellets for 6 weeks. After mastering the task, all rats received a dorsal funiculus crush injury at the C4 level and subsequent implantation of EMG electrodes in the preferred forearm muscles (extensor digitorum and flexor digitorum). After 1 week of recovering from surgery, the rats were ranked and divided into 3 Buspirone dosages groups: **Group A**, **B** and **C**; low, 1.5, n = 5; medium, 2.5; n = 6 and, high-dose, 3.5 mg/kg b.w., n = 6; respectively. Weekly forelimb reaching task and grip strength tests were conducted for weeks 1-6 continuously and week 9, after which the drug was withdrawn and the rats were tested again in week 11.

persistent sodium inward current which increases the excitation of motorneurons in chronic spinal cord injured rats.[13] The above findings indicate the importance of serotonin receptor agonists in spinal cord injuries.

Buspirone, a serotonin 5-HT_{1A} receptor partial agonist acts as an antagonist for dopamine D_2 autoreceptors. There is some evidence of a weak affinity for 5-HT_2 receptors, commonly used as an anxiolytic drug, and has the potential to improve brain functions after injuries or diseases.[14] In recent studies on SCI patients, it has been suggested that electrical stimulation of the spinal cord, when combined with regular Buspirone administration can restore voluntary control of the hand[12] and locomotor function.[15,16] Previous reports have shown that low doses of Buspirone can stimulate the somatodendritic 5-HT_{1A} receptors in an *ex-vivo* bovine brain[17] and can trigger motor activity in rats.[18] However, high doses of Buspirone can block the postsynaptic terminals on dopaminergic neurons and induce behavioral changes.[19] In Parkinson's diseases, a dose of 2 mg/kg b.w. of Buspirone has also been reported to reduce locomotor activity. Similar effects have also been reported in other neurological disorders.[20] Likewise, high doses of a $5HT_2$ receptor agonist may activate $5HT_{1A}$ receptor agonists and modulate the interneuronal activity to fire repetitively in chronic conditions.[13] The optimal dose response of Buspirone for improving arm and hand functions in cervical SCI is unclear. The lack of clarity in the dose-response relationships among the experiments noted above demonstrate that to understand the efficacy of a given dose of Buspirone, in spite of its long history of clinical use, care must be taken to control the dosage, physiological system, the timing of the response and the species of interest. In the current study, we investigated the dose-responses of Buspirone treatment on reaching and grasping function after a cervical spinal cord injury in rats.

Methods

All experimental procedures were carried out according to the guidelines and approval of the Animal Subjects Ethics Subcommittee of The Hong Kong Polytechnic University.

Animal Subjects

Seventeen adult female Sprague-Dawley rats (230 $\pm$ 20 grams b.w.) were used in this study. Acclimation of the animals was done for 1 week prior to the behavioral training. Adlibitum food and water were provided before starting the forelimb training. Body weights were monitored after the surgery weekly. The room temperature (24°C) and humidity (40%) were carefully maintained.

Reaching and Grasping Task

All the rats were trained (thrice/week) to reach, grasp and eat the 45 mg dustless sugar pellets (Bio-serv®, Flemington, USA) from a pit flatform (3 cm $\times$ 2 cm) of a specialized box (18 cm $\times$ 15 cm $\times$ 31 cm) as described before.[21,22] Food restriction was provided before each task to master the technique. During each test, 30 pellets were provided to grasp and eat. After 6 weeks of training, the rats demonstrated at least a 60% success rate in their reaching and grasping task. Beginning at 1-week post-injury, weekly reaching and grasping scores were measured for 11 weeks (Figure 1).

Grip Strength Test

Prior to the surgery, forelimb grip strength tests were conducted to acclimatize the animal to the test apparatus using a custom-made grip strength meter as described previously.[23] By holding the base of the tail, the rat was gently pulled away from

the grid which was connected to a force sensor. From 1-week post-injury, weekly maximum grip strength was measured for each rat (Figure 1).

Cervical Cord Injury

All surgical procedures were carried out under aseptic conditions. To induce anesthesia, at first the rats were anesthetized with 5% isoflurane gas which was maintained at 1.5-2% throughout the surgery via a face mask. To prevent hypothermia, the body temperature was maintained at 37°C by placing the rat on an automated heating pad (ThermoStar Homeothermic Monitoring System, RWD Life Science Co. Ltd., China). To minimize the pain, an analgesic, Buprenorphine HCl (Buprenex®, 0.5mg/kg, s.c.) was administered before the surgery. In deep anesthetic condition, an incomplete spinal cord injury at the C4 level was carried out as described previously.[24] In brief, after a 3-4 cm skin incision was made using a sharp scalpel blade, the skin and fascia were retracted by a forceps to reveal the muscles beneath. After retracting the paravertebral muscles a laminectomy was performed at the C3-C4 vertebrae to reveal the spinal cord. The tips of fine forceps were used to crush the dorsal funiculi of the spinal cord (2 mm apart, 2 mm depth). A thin 2-mm length of a stainless steel sterile rod was used to ensure the consistency of the lesion size during each surgery at the C4 level. The SCI resulted in significant loss of supraspinal control with moderate motor and sensory impairments.[25,26] Saline water was used to moisturize the skin area and the muscles were sutured by using 4.0 Vicryl sutures.

Electrode Implantation

To record the EMG signals from the preferred paw, intramuscular EMG electrodes were implanted into the forelimb flexor and extensor digitorum muscles. A skin incision was made on the skull to place a head-plug. The skin and connective tissues were retracted from the skull. Cotton gauze was soaked and placed on the skull to prevent dryness. Two longitudinal skin incisions around 2 cm were carried out to place the Teflon-coated stainless steel wires (AS631, Cooner Wire, USA) in the flexor and extensor digitorum muscles. The fascia of the muscles was retracted to reveal the belly of the desired muscle. By using forceps the wires were then passed to the muscles subcutaneously. A 27-gauge needle was then inserted in the muscle belly to implant the electrode wires. After insertion, a part of the Teflon ($\sim$0.5 mm) from the wire was removed to make a recording electrode. The wire electrodes were then anchored by using 4.0 Ethilon sutures and stimulation was given through the connector to verify the position of the electrode. The EMG wires were coiled to relieve the stress and the skin incisions were closed by using 4.0 Nylon sutures. Finally, to anchor the head-plug, 4 screws were firmly placed into the skull after drilling and thoroughly dried. Dental cement was then applied to immerge the screws to support the head plug after drying.

The analgesic, Buprenorphine HCL, was administered for 3 days post-surgery. At the same time an antibiotic, Enrofloxacin (Baytril®, 0.5mg/kg, s.c) was administered and continued twice daily for 3 days to prevent any infection. The rats were then moved to a temperature and humidity-controlled incubator (AEOLUS Incubator, ICU-1801, USA) to recover from the anesthesia. After recovering, the animals were transferred to their individual home cages. Fresh fruit and juice were provided for a quick recovery. The rat's condition was monitored continuously for at least 1 week.

Drug Treatment

After recovering from the surgery at day 7, the animals' ability to reach and grasp the sugar pellets was tested. One of the rats died during the recovery period from the surgery and hence one group was slightly imbalanced. Based on the scores, the animals were than ranked and divided into 3 balanced groups to be administered with different dosages of Buspirone (i.p., 1 dose/day): **Group A** with a low dose (1.5 mg/kg b.w.; $n = 5$), **Group B** with a medium dose (2.5 mg/kg b.w.; $n = 6$) and **Group C** with a high dose (3.5 mg/kg b.w.; $n = 6$). The drug, Buspirone (Tocris®, UK), was previously prepared by dissolving 1 mg/1 ml of ultrapure distilled water (Invitrogen®, USA). The drug was administered intraperitoneally into different dose groups of SCI rats up to 9 weeks post-injury. Behavior tests and electrophysiological recordings were conducted from 0.5 to 1 hour of Buspirone administration. Each animal was videotaped with a camera[27] while retrieving food pellets. After each forelimb reaching task and electrophysiological recording, a grip strength test for each rat was conducted once per week, for 9 weeks. To see the effects of the drug, after 9 weeks, the drug administration was ceased, and the behavior tests and electrophysiological recording were done only at week-11 post-injury. At the end of all the experiments, the rats were euthanized with a high dose of Ketamine and Xylazine mixture and confirmed by a surgeon according to the guideline of the Animal Subjects Ethics Sub-Committee of The Hong Kong Polytechnic University.

Data Acquisition and Statistical Analysis

Videotape footage of the forelimb reaching task of each rat was examined frame by frame in windows media player to identify the components of the grasping task. The EMG signals were bandpass filtered at 10-1000 Hz and amplified (1,000 times) by an analog amplifier (Model 1700 Differential AC Amplifier, AM Systems, USA). The signals were then digitized by a data acquisition system (Power1401-3A, Cambridge Electronics Design Ltd., UK). The data were then visualized and recorded on a computer for further analysis via a software interface (Signal, Cambridge Electronics Design Ltd., UK). Based on the synchronization of the video, extensor and flexor muscle EMGs for every trial were plotted and calculated by a custom script written in MATLAB (MathWorks Inc., USA). From the EMG signals of extensor digitorum and flexor digitorum

muscles, area-under-the curve (AUC) values were calculated as described previously.[24] The normalized AUC values for different dose groups were then tested for statistical significance using 2-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test using 2-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test. Pre- and post-injury reaching scores were compared for significance of difference by using a paired t-test. The success rates of forelimb reaching of the 3 dose groups were normalized to their pre-injury success rate using the following equation:

$$\text{Normalized reaching score} = \left(\frac{\text{Original success rate}}{\text{Preinjury success rate}}\right) \times 100.$$

The scores were then evaluated for significant differences by 2-way ANOVA. One-way ANOVA was used to analyze the dose responses in each group. All the statistical measurements were carried out using software (GraphPad Software Inc., USA) with significance levels set at $p < 0.05$ for all the comparisons.

Results

A Low Dosage of Buspirone Facilitates Forelimb Reaching and Grasping Function

Following cervical cord injury, after a few attempts the animals could place their forelimbs on the food platform; however, mostly failed to grasp the pellets. The post-injury forelimb grasping success rates were compared with the pre-injury scores. Forelimb reaching scores dropped significantly 1 week after the cervical cord injury (68.82 ± 2.33 vs. 2.05 ± 1.25; $^{***}p < 0.001$, paired t-test).

All the rats were administered Buspirone, according to their dose group, till 9 weeks post-injury and the grasping success rate was measured. The animals started to increase their grasping function noticeably after 3 weeks post-injury. The success rates of each dose group were compared with 1-week post-injury scores. In **Group A**, significant improvements of reaching function were found at weeks 4 and 5, respectively (40.33 ± 6.33 and 41.99 ± 4.89, $^{**}p < 0.01$, 1-way ANOVA). The reaching score remained significantly high till week 9, and even at week 11 after cessation of the drug treatment (respectively 46.33 ± 5.22 and 45.33 ± 4.54;$^{**}p < 0.01$, ANOVA) (Figure 2a).

The medium-dose rats, **Group B**, significantly improved their forelimb grasping function at and after 4 weeks post-injury compared to week 1 (Figure 2b). At week 11 after the cessation of Buspirone administration, the reaching scores dropped but still remained significantly high compared to the week 1 post-injury score. **Group C** did not exhibit any significant improvement of reaching function (Figure 2c). The average normalized reaching scores of all groups were compared with each other (Figure 2d), showing that **Group B** and **C** scores increased up to 3 weeks post-injury, while **Group A** continued to improve up to 6 weeks. The reaching scores of **Group B** rats started to decay from 5 weeks post-injury and never recovered. At the end of the study, **Group A** low-dose rats exhibited an average of 69% improvement of reaching and grasping function over **Group B** and **C** medium- and high-dose rats. Although the low-dose rats displayed noticeable improvements in later weeks after treatment compared to the higher-dose rats, no statistical significance was found between their normalized reaching scores (at 11 weeks post-injury: **Group A** vs. **Group B**, $p = 0.8786$; **Group A** vs. **Group C**, $p = 0.6715$).

Low- to Medium-Dose BUSPIRONE Treatment Improved Distal Muscle Co-Ordination

Examples of raw EMG signals from the extensor digitorum and flexor digitorum muscles during reaching and grasping for different dose groups are presented in Figure 3. At 1 week post-injury, the amplitude and bursting properties during the sugar pellet reaching task were relatively low compared to 9 weeks post-injury.

The EMG signals of the extensor digitorum and flexor digitorum muscles were normalized for each rat to calculate the area-under-the-curve (AUC). The normalized AUC values of different dose groups were analyzed for statistical significance. In each group of rats the normalized AUC of the extensor muscle tended to increase gradually after week 6 and even after withdrawal of the drug **(**Figure 4a**)**. However, the EMG from the flexor digitorum muscle did not show any consistent trend over the 11 weeks of post-injury measurements, presumably in large part due to the low number of rats, particularly for **Group C**. In **Group C**, only 2 rats were found to have good EMG signals and were included in Figure 4 for analysis. In the other rats, the EMG signals were noisy and were not reliable for analysis. In **Group A**, the flexor muscle activity dropped at week 9 and dramatically increased at week 11 after withdrawal of the drug, while in **Group C** the opposite phenomenon happened **(**Figure 4b**)**. The medium-dose group, **Group B**, however, showed a similar increasing trend like the extensor digitorum muscle. This may imply that a low- to medium-dose of Buspirone may have more consistent effects on the forelimb muscles on reaching and grasping function. Although no statistically significant differences between the dose groups were found, the similarity of the relative patterns of differences across time in the extensor muscle and a consistently different pattern observed in the flexor, regardless of group identity, suggest that the dosage-time effect was different between the extensor and flexor muscles.

Low-Dosage of Buspirone Treatment Improved Forelimb Grip Strength

Following the forelimb reaching task, the maximum value of the forelimb grip strength test was carried out weekly for 6 weeks and finally at week 9. After the withdrawal of Buspirone, the tests were carried out at week 11. The forelimb grip strength of all 3 groups exhibited continuous improvement up to week 4 (Figure 5).

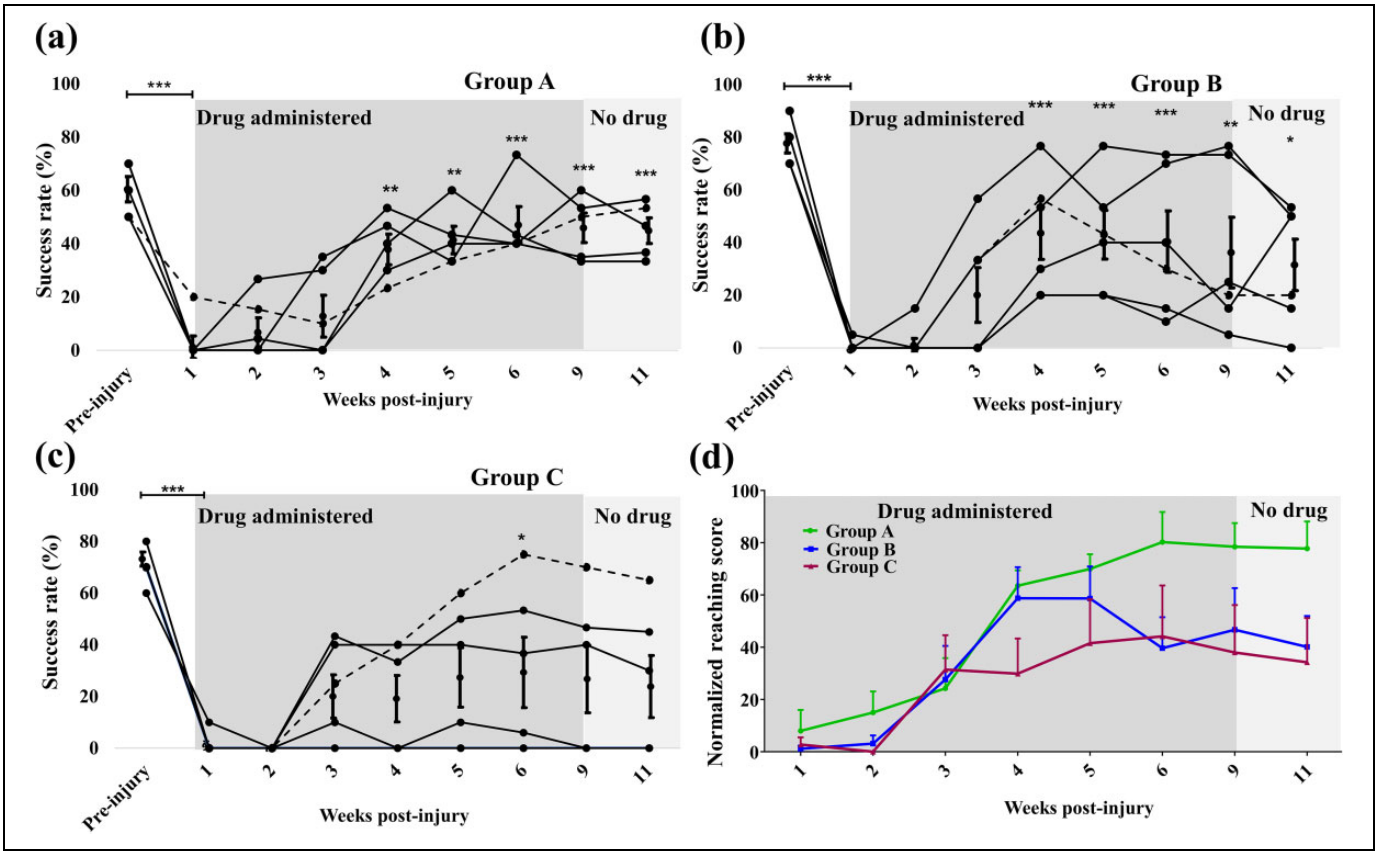

Figure 2. Reaching and grasping scores of 3 different dose groups after Buspirone administration. **a)** Mean (±SEM) success rates of **Group A** rats (n = 30 trials/rat/test session, 5 rats). 1-week vs. 4 and 5-week post-injury scores (**$p < 0.01$). 1-week vs. 6, 9 and 11-week post-injury scores (***$p < 0.001$). **b)** Mean (±SEM) success rates of **Group B** rats (n = 30 trials/rat/test session, 6 rats). 1-week vs. 4 and 5-week post-injury scores (***$p < 0.001$). 1-week vs. 6 and 9-week post-injury score (**$p < 0.01$). 1-week vs. 11-week post-injury score (*$p < 0.05$). **c)** No significant difference was found in the mean (±SEM) success rates of **Group C** (n = 30 trials/rat/test session, 6 rats). **d)** The progression of the mean (±SEM) normalized reaching scores of all 3 groups. The dotted line is the score of a representative rat for each group.

A similar effect of Buspirone was also found in **Group B** and **C**. For **Group B**, the force was boosted from 6.67 N (weeks 1&2 post-injury) to 13.42 N (weeks 6&9 post-injury). For **Group C**, the maximum grip force increased from 5.60 N (weeks 1&2 post-injury) to 12.16 N (week 4 post-injury) and dropped to 11.09 N at weeks 6&9 post-injury. After withdrawal of the drug, at 11 weeks post-injury, the average grip force increased slightly to about 11.24 N for **Groups A** and **B**. Significantly higher grip strength (**$p < 0.01$ and *$p < 0.05$ ANOVA) in **Group A**, compared to **Group C** was found at 5 and 11 weeks post-injury (Figure 5d).

No significant relation was found between the forelimb reaching score and the forelimb grip force in the early post-injury weeks (data not shown). From 6 weeks of drug treatment, both reaching success and grip strength values increased in almost all the dose groups (Figure 6). **Group A** exhibited the most consistent functional recovery as assessed for both fine motor control of reaching and for gross forelimb grip strengths. These improvements were also sustained after withdrawal of the low dosage of the drug. The success rate and grip strength of **Group B** rats increased at 6 weeks post-injury to an optimum level, but dropped afterward. The results indicate that each week the motor task had an influence on the functional recovery. Unlike **Group A** and **B rats**, high-dose **Group C** rats did not undergo any improvement of fine motor control. Overall, a higher success rate and grip strength were found for the low-dose rats (Figure 6). The results indicate that the lowest dose of buspirone tested provided the most consistent pattern of functional improvements compared to the medium and high dosages, but also suggest that maximum grip force was entirely unrelated to performance success. It also suggests that regardless of group identity, the mean highest grip force occurred at the 11th week, i.e. grip strength increased with time, but without Buspirone.

Discussion

As a serotonin agonist, Buspirone, primarily known as an anxiolytic drug, has high affinity for $5HT_{1A}$ receptors and weak affinity for $5HT_{2A}$ receptors. In contrast, the drug has antagonistic properties against dopamine D2 receptors.[14] A recent study has shown the potential of the drug to improve forearm grip strength

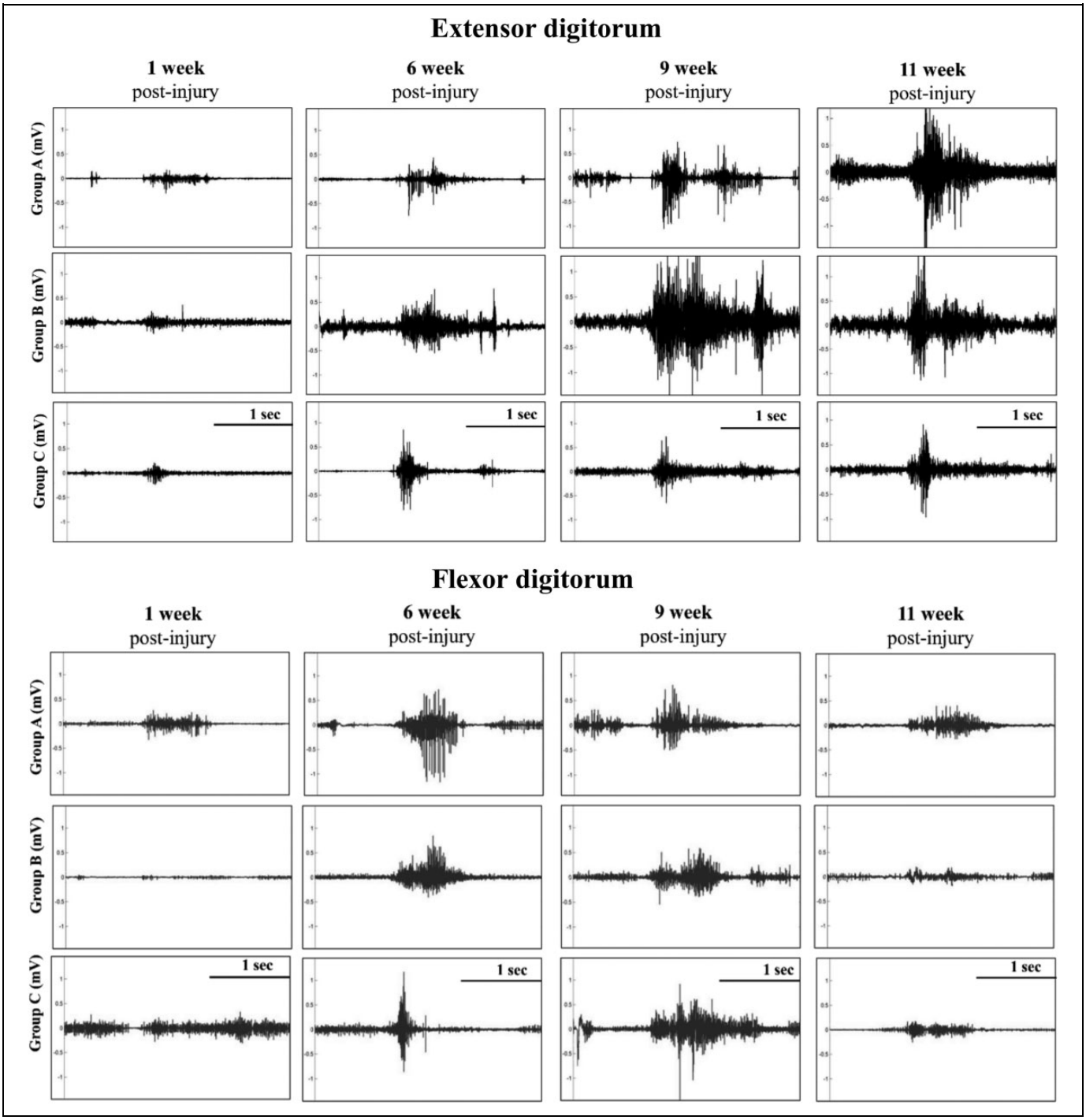

Figure 3. Raw EMG signals (n = 30 trials) during forelimb reaching and grasping task at 1, 6, 9 and 11 weeks post-injury from one representative rat (marked as a dotted line in Figure 2) from each treatment group. Only at 6 and 9 weeks post-injury was the EMG recorded during the Buspirone administration period, while week 1 was before, and week 11 was 2 weeks after the drug treatment.

function along with electric stimulation in tetraplegic patients, but these effects do not provide strong evidence for significant functional effects in performing daily motor tasks.[12]

Skilled forelimb function such as forelimb reaching and grasping task is commonly used to examine the functional recovery after cervical cord injury in rats.[28] In a previous study, we showed that an incremental dose of Buspirone (1 to 2 mg/kg) can improve forelimb reaching function in rats with cervical cord injury.[29] The spontaneous recovery of forelimb function of rats without any drug intervention was found to be less than 20% while the rats which were administered Buspirone reached a success rate of over 60% by 6 weeks post-injury. Thus, serotonergic modulation enabled over 3-fold improvements in forelimb reaching and grasping after post-injury Buspirone

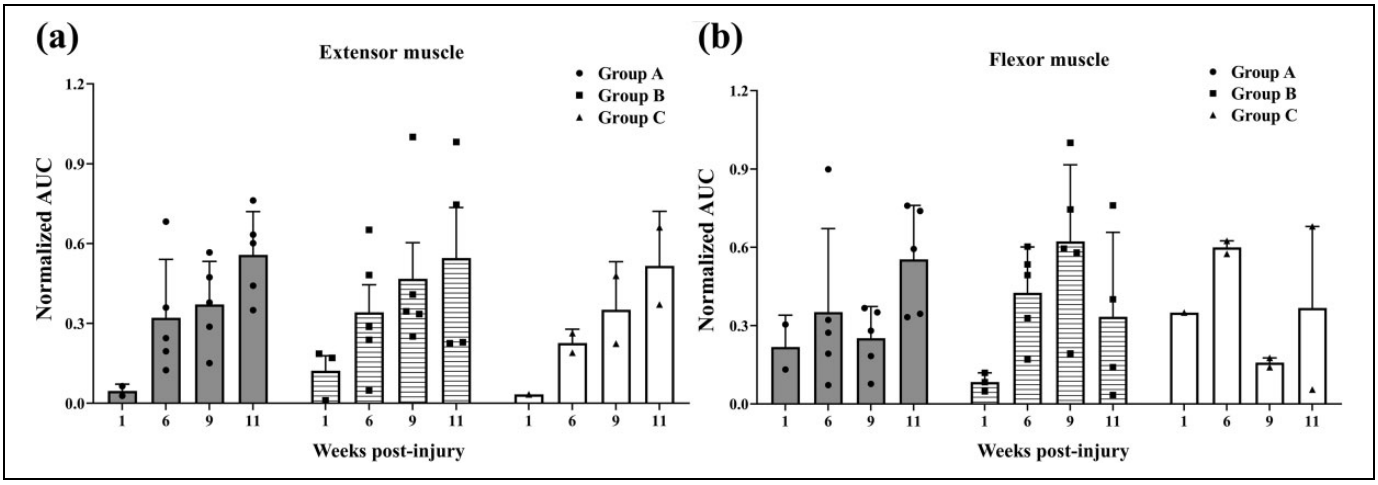

Figure 4. Average area-under-the-curve (AUC) of normalized EMG signals for **a)** extensor digitorum and **b)** flexor digitorum muscles during the single pellet reaching task in 3 different Buspirone dose groups (**Group A**, n = 5; **Group B**, n = 5; and **Group C**, n = 2).

administration. In the present study, we demonstrated that different dosages of Buspirone have different effects on forelimb functional recovery after a dorsal funiculus injury in rats. Our main finding is that the low dose (1.5 mg/kg b.w.) of Buspirone markedly improved forelimb fine motor control and grip strength compared to a higher dose. Furthermore, a low to medium dose (1.5-2.5 mg/kg b.w.) of Buspirone improved the forelimb muscle synergies of the distal muscles.

When considering the possibility of using Buspirone as a pharmacological way to enhance the recovery of motor functions, it is important to consider the dose-response characteristics of other serotonergic systemic responses that have been observed that may be detrimental or beneficial either acutely or chronically. Based on the history of its development the dose regimen for the treatment of behavioral effects will be of obvious importance. The effects on cerebral glucose metabolism in response to doses ranging from 0.4 mg/kg to 40 mg/kg have shown reduced cerebral metabolic rates for glucose in rats. A dose of 0.4 mg/kg Buspirone can preferentially activate the 5-HT_{1A} autoreceptors.[30] Pain sensation has been studied in rats using dosages ranging from 0.1-2.0 mg/kg.[31] Such dose can also improve the acquisition and retention of memory in a water maze.[31] Given the distinctly dose-dependent differences observed in the present study, each of the very few cases noted here emphasizes the criticalness of dosage in defining its effectiveness for a specific function.

From neurochemical evidence, it was found that a low (1 mg/kg) but not high dose (5 mg/kg) of Buspirone stimulates 5-HT_{1A} receptors and decreases striatal metabolism. The finding also suggests that a low dose could help to release dopaminergic neurons that could be useful in reducing Parkinsonian-like effects.[18] Furthermore, a model of L-DOPA-induced dyskinesia in Parkinson's disease 1 mg/kg b.w. of Buspirone did not impair the rotarod performances in rats, while higher doses (2 and 4 mg/kg b.w.) significantly reduced the rotarod performance by 25% and 20%, respectively.[32] A low dose of Buspirone can significantly increase the synthesis and availability of dopamine in the pre-synaptic terminal, whereas a higher dose blocks postsynaptic dopaminergic receptors.[19]

Generally, these findings demonstrate some of the complexities of Buspirone given the multiple autoreceptors in the nervous system, which could contribute to the dose dependencies of its physiological responses. The present findings suggest that the functional window of efficacy for Buspirone, and probably other potential pharmacological candidates with limited specificity for spinal neuromodulation, can be narrow. These data, for example, show that the efficacy of Buspirone not only is a function of relatively narrow ranges of dose; the timeframe over which functional changes are likely to occur gives a clue as to the persistence of the responses.

Other variables that need to be examined are the potential interactive effects associated with activity-dependent mechanisms that might be in play. Regarding this specific interaction of a pharmacological and activity-dependent interaction, there have been clear examples of these 2 interventional modes having antagonist or synergistic effects depending on whether they were presented sequentially or simultaneously. Finally, it is logical to assume that the highly complex serotonergic receptor types within the spinal cord and the fact that almost all of the serotonin is derived from supraspinal nuclei, and their axonal projections rapidly degenerate post injury. Thus, it seems likely that the serotonergic spinal network response to a serotonergic acting drug will be significantly different when tested in acute phases, as in this study, compared to the more frequently available chronic state in humans with a spinal injury. In the present study, it was expected that the higher dose could activate the cervical spinal network as occurs among the lumbosacral networks.[33] The present results showed a robust effect of a low to medium dosage effect on forelimb grasping function in the acute phase of adaptation to the injury imposed. It appears that

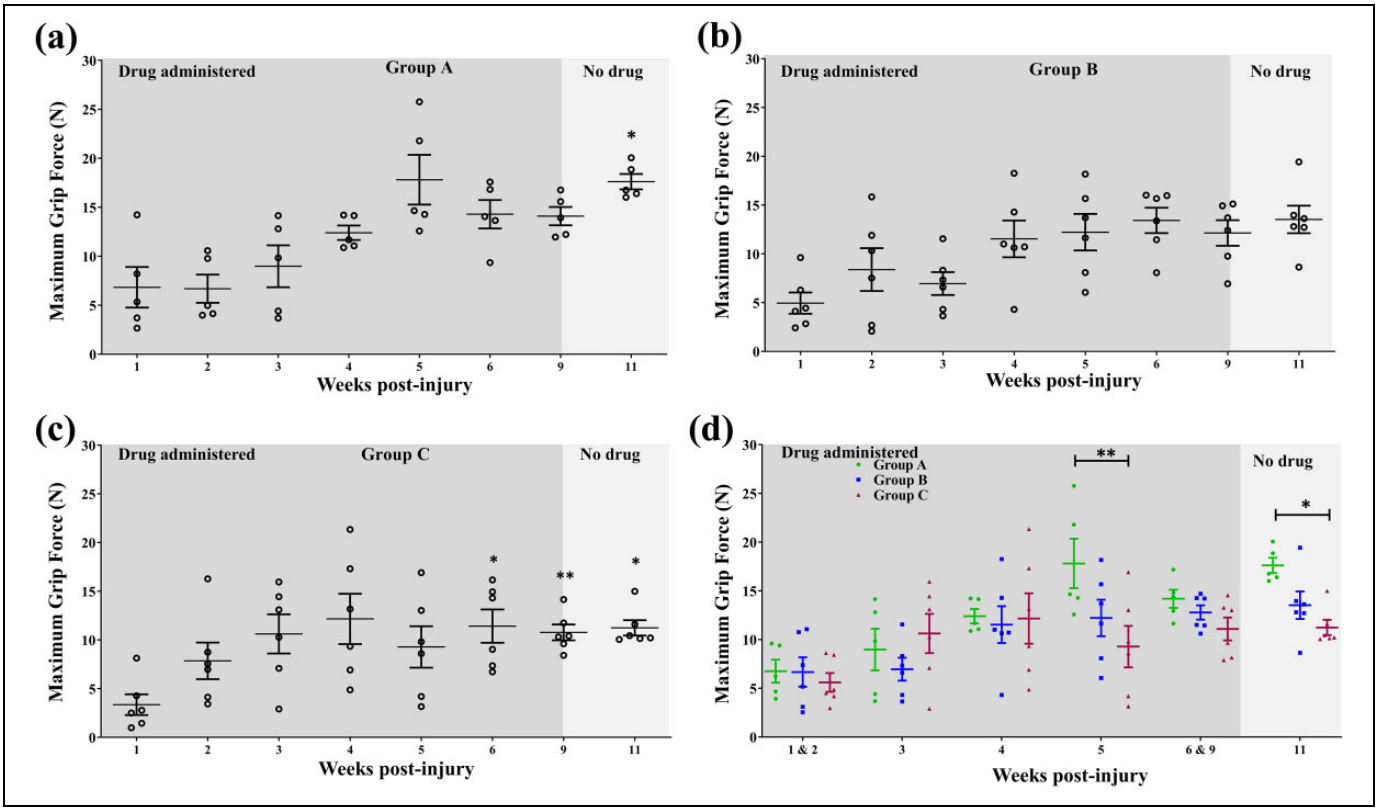

Figure 5. Maximum grip force of the 3 different dose groups after Buspirone administration. a) Mean (±SEM) maximum grip force of **Group A** (n = 3 trials/rat/test session, 5 rats). 2 weeks vs. 11 weeks and 9 weeks vs. 11 weeks post-injury (*$p < 0.05$) **b)** Mean (±SEM) maximum grip force of **Group B** (n = 3 trials/rat/test session, 6 rats). No significant difference was found. **c)** Mean (±SEM) maximum grip force of **Group C** (n = 3 trials/rat/test session, 6 rats). 1 week vs. 6 weeks and 11 weeks post-injury (*$p < 0.05$); and 1 week vs. 9 weeks post-injury (**$p < 0.01$). **d)** Post-injury mean (±SEM) maximum grip force of 3 dose groups. **Group A** showed significantly higher grip strength at week 5 (**$p < 0.01$) and at week 11 post-injury (*$p < 0.05$) compared to **Group C**.

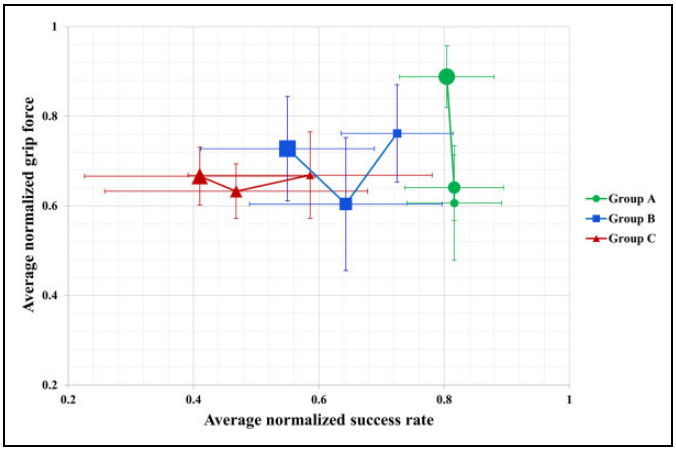

Figure 6. Overall forelimb functions of each drug dose group. Normalized success rate vs. normalized grip force at 6, 9, and 11 weeks post-injury. Data are presented as the mean (±SEM). The marker sizes (smaller to larger) represents 6, 9 and 11 weeks post-injury, respectively.

a critical question now is, what would the dose responses be for motor tasks in the acute vs. chronic stage of a spinal injury. We have demonstrated previously, for example, that the efficacy of an intervention after a spinal injury is highly dependent on the timing at which a biochemical and an activity-dependent intervention is presented.[34,35]

Conclusions

Buspirone had clear effects on the recovery of forelimb grasping and grip strength following administration at a dose of 1.5 mg/kg b.w. after an incomplete cervical cord injury. The magnitude of these effects were highly dose- and post treatment time-dependent. At the longest post-treatment time studied among all 3 dosage groups there was a direct relationship between grip strength and reach and grasp success rate. However, a larger sample size and more functional tests may confirm the significance and synergy of the drug dosage. Based on the EMG data represented as the total EMG activity of a primary extensor and flexor muscle per reach and grasp effort, no clear pattern of changes was found to be closely linked to successful performances, an issue that needs comprehensive assessment to understand the subject-specific functional neural reorganization adopted strategies that shaped the patterns of recovery. From the EMG responses, it can be observed that the low-dose rats had most consistent patterns of forelimb function

compared to the medium- and high-dose rats. The two behavioral motor tests and forelimb electrophysiology combined may suggest that a low dose of Buspirone has better influence on forelimb functional recovery than a higher dose.

Declaration of Conflicting Interests

The author declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. V.R.E. holds shareholder interests in NeuroRecovery Technologies and holds certain inventorship rights over intellectual property licensed by the Regents of the University of California to NeuroRecovery Technologies. V.R.E holds shareholder interests in spineX Inc. and holds certain inventorship rights over intellectual property licensed by the Regents of the University of California to spineX Inc. V.R.E. serves on the scientific advisory board of in vivo Therapeutics and ArianRF and serves as the Chair of the Scientific Advisory board at spineX.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research study was partially supported by the Hong Kong Polytechnic University (H-ZG4 W), Hong Kong Innovation and Technology Fund (ITS/276/17), and Telefield Charitable Fund (ZH3 V).

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