ARM AND HAND MOVEMENT: CURRENT KNOWLEDGE AND FUTURE PERSPECTIVE

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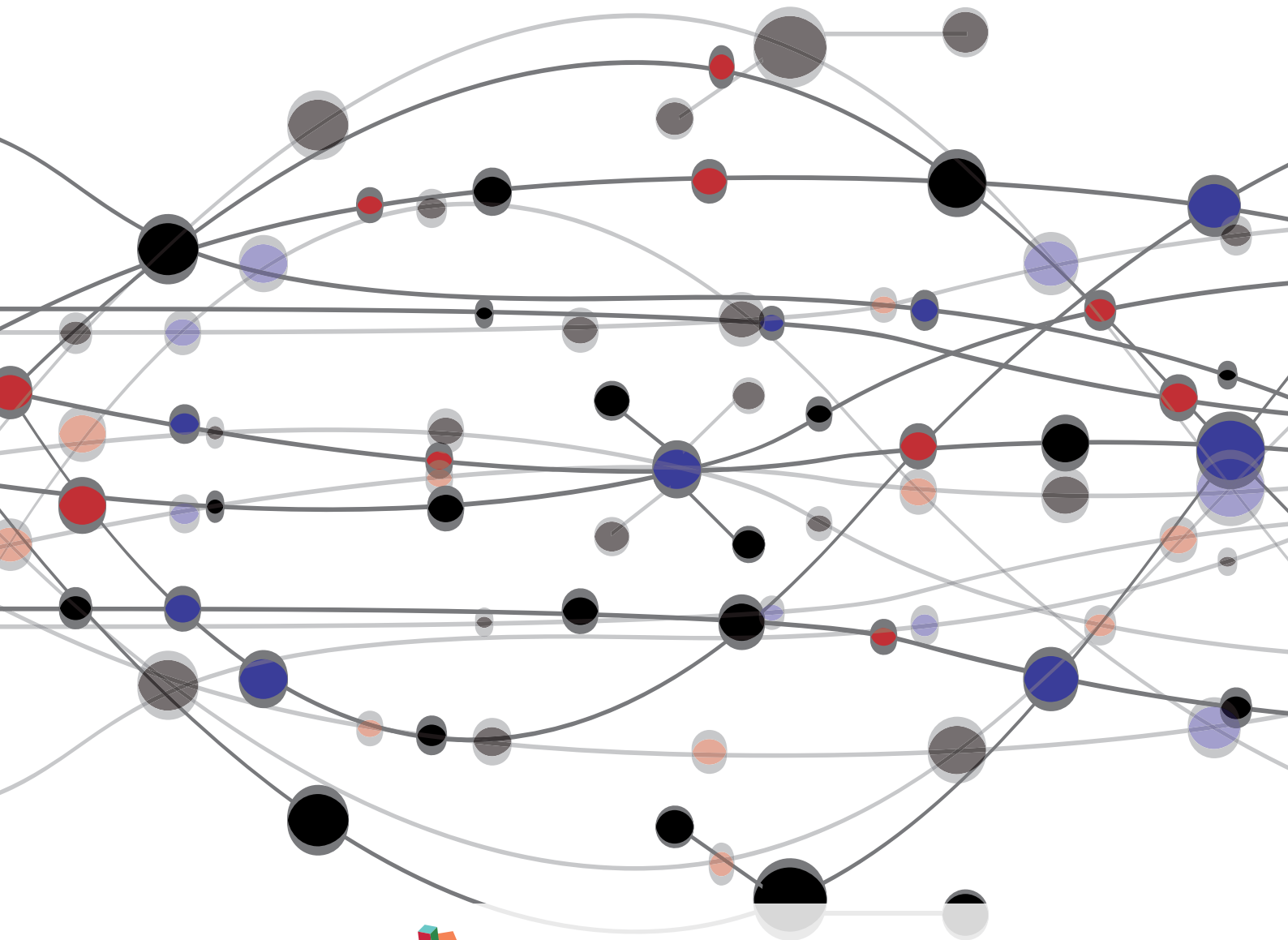

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ARM AND HAND MOVEMENT: CURRENT KNOWLEDGE AND FUTURE PERSPECTIVE

Topic Editors:
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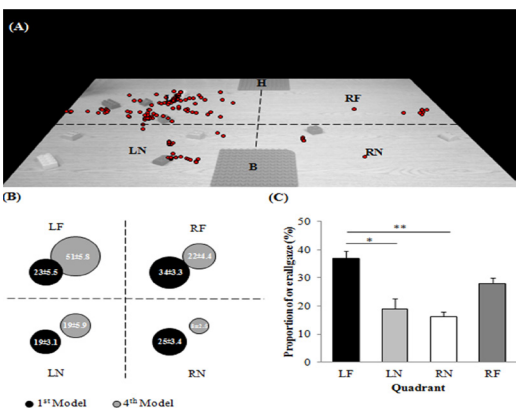

(**A**) Example of gaze position data during LEGO model construction where workspace is notionally divided into quadrants; left near (LN), left far (LF), right near (RN), and right far (RF). Model to be replicated is located at home base plate (H) and model being constructed is at build base plate (B). Proportion of gaze directed toward each quadrant during construction of (**B**) first and last model and (**C**) all four models.

Image taken from: de Bruin N, Bryant DC and Gonzalez CLR (2014) "Left neglected," but only in far space: spatial biases in healthy participants revealed in a visually guided grasping task. Front. Neurol. 5:4. doi: 10.3389/fneur.2014.00004

This Research Topic is devoted to arm and hand movement in health as well as in several disease conditions. It is a collection of several original research papers and reviews, clinical case studies, hypothesis and theory articles, opinions, commentaries, and methods papers that cover some important aspects of the topic from distinct scientific perspectives. We invite the readers to appreciate the range in methodologies and experimental designs that together have led to widen our understanding of this especially broad field of research.

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EDITORIAL
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Arm and hand movement: current knowledge and future perspective

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Keywords: reaching, grasping, arm and hand movement, neuromuscular dysfunction, reach-to-grasp, motor control of movement

Reaching with the arm and grasping with the hand and fingers is a complex behavior that appears *in utero*, is elaborated over the first few years of life, and serves useful everyday functions throughout the course of human life. Several neurological conditions can impair the ability to produce arm and hand movements and so greatly impact on the quality of life and well-being of the affected individuals. Given the fundamental role that arm and hand movements play in everyday life, deficits related to arm and hand function are one of the most debilitating motor conditions. Neurological conditions that can affect arm and hand movements include autism spectrum disorder, Parkinson's and Huntington's diseases, amyotrophic lateral sclerosis, cerebral palsy, and stroke-related motor cortex damage as well as spinal cord injury at cervical levels. While arm and hand movement has received considerable attention from both clinicians and researchers from diverse scientific backgrounds, there are a number of broad research questions that still need to be addressed in this research field. The present Research Topic is entirely devoted to arm and hand movement in health as well as in disease. It is a compilation of original research papers and reviews, clinical case studies, hypothesis and theory articles, opinions, commentaries, and methods articles that cover important aspects of the topic from different perspectives.

In this volume, de Bruin et al. (1) present data that describe how healthy adults use space while performing a visually guided grasping task. A model for understanding hand functioning in children with cerebral palsy is proposed by Arnould et al. (2) while Johansson et al. (3) explore the effect of timing training on upper limb movement in three children with diplegic cerebral palsy. Parma et al. (4) compare the kinematics of the reach-to grasp movement in patients with vascular and idiopathic Parkinson's disease whereas Aluru et al. (5) evaluate the effect of auditory constraints on motor performance at different stages after a stroke. Lawrence et al. (6) measure dexterous manipulation in a cross-sectional study comparing gender, age, and absence and presence of disease. Kirsh et al. (7) provide evidence to support the view that neurons outside the primary motor cortex – such as those populating the pontomedullary reticular formation and the spinal cord-drive movement and muscle synergies that primary motor cortex neurons then break up to create individual wrist and finger movements. Sacrey et al. (8) summarize current knowledge related to reaching and grasping in autism spectrum disorder. On the other hand, Whitwell et al. (9) reinstate patient DF's amazing ability to use information regarding form and orientation of objects to guide skilled reaching actions despite her visual agnosia. In an opinion article, Moore (10) argues that nerve transfer is increasingly popular and is becoming the best treatment strategy for most brachial plexus damage as well as for patients with spinal cord injury at cervical levels. Vicario (11) provides a personal commentary on a paper from Hayashi et al. (12) and, in a review article, Karl and Whishaw (13) summarize the evidence that show that reaching and grasping are from distinct neural and evolutionary origins. Irvine et al. (14) contribute a methods article that assesses the reliability of the Irvine, Beatties, and Bresnahan (IBB) forelimb recovery scale. Fouad et al. (15) demonstrate that continuous viral-mediated brain-derived neurotrophic factor (BDNF) over-expression promotes spasticity in rats with spinal cord hemisections at cervical levels. Alstermark and Pettersson (16) bring evidence to show that lesions to the corticospinal tract that spare the cortico-reticulospinal pathway in the rat have no deleterious effects on skilled reaching and grasping. Finally, Tosolini et al. (17) describe how targeting the full length of the motor endplate region in the mouse forelimb with Fluoro-Gold increases the uptake of this neuroanatomical retrograde tracer in corresponding motor neurons.

We are delighted to present "Arm and Hand Movement: Current Knowledge and Future Perspective" as a Research Topic in Frontiers in Neurology. We feel that this wide-ranging compilation of articles by leading experts in upperlimb/forelimb movement and working either in clinical or basic research settings has offered fresh perspectives on the topic. We are thankful for the support of all the scientists who have contributed to this Research Topic and have shared with us their expertise and point of views. Their contributions have deepened our appreciation of the challenge that restoring arm and hand function in different pathologies represents. We invite the readers to experience the diversity in methodological approaches and experimental designs that together have led to broaden our understanding of this particularly wide field of research.

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ORIGINAL RESEARCH ARTICLE
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Quantification of dexterity as the dynamical regulation of instabilities: comparisons across gender, age, and disease

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Dexterous manipulation depends on using the fingertips to stabilize unstable objects. The Strength–Dexterity paradigm consists of asking subjects to compress a slender and compliant spring prone to buckling. The maximal level of compression [requiring low fingertip forces <300 grams force (gf)] quantifies the neural control capability to dynamically regulate fingertip force vectors and motions for a dynamic manipulation task. We found that finger dexterity is significantly affected by age ($p = 0.017$) and gender ($p = 0.021$) in 147 healthy individuals (66F, 81M, 20–88 years). We then measured finger dexterity in 42 hands of patients following treatment for osteoarthritis of the base of the thumb (CMC OA, 33F, 65.8 ± 9.7 years), and 31 hands from patients being treated for Parkinson's disease (PD, 6F, 10M, 67.68 ± 8.5 years). Importantly, we found no differences in finger compression force among patients or controls. However, we did find stronger age-related declines in performance in the patients with PD (slope −2.7 gf/year, $p = 0.002$) than in those with CMC OA (slope −1.4 gf/year, $p = 0.015$), than in controls (slope −0.86 gf/year). In addition, the temporal variability of forces during spring compression shows clearly different dynamics in the clinical populations compared to the controls ($p < 0.001$). Lastly, we compared dexterity across extremities. We found stronger age ($p = 0.005$) and gender ($p = 0.002$) effects of leg compression force in 188 healthy subjects who compressed a larger spring with the foot of an isolated leg (73F, 115M, 14–92 years). In 81 subjects who performed the tests with all four limbs separately, we found finger and leg compression force to be significantly correlated (females $\rho = 0.529$, $p = 0.004$; males $\rho = 0.403$, $p = 0.003$; 28F, 53M, 20–85 years), but surprisingly found no differences between dominant and non-dominant limbs. These results have important clinical implications, and suggest the existence – and compel the investigation – of systemic versus limb-specific mechanisms for dexterity.

Keywords: sensorimotor function, rehabilitation, dexterity, hand, leg, aging, sex differences, sociobiology

INTRODUCTION

Dynamic upper extremity function in general, and of the fingertips in particular, is essential for activities of daily living (ADLs) and quality of life (1, 2). While there are multiple measures of hand function, we have historically lacked a means to quantify the dynamical interaction of the fingertips with objects without the confounds of strength, functional adaptations, whole-arm coordination, visual acuity, etc. We have proposed the Strength–Dexterity (SD) paradigm as a versatile, repeatable, and informative paradigm to quantify finger dexterity across the lifespan in some clinical populations. We define dexterity as the sensorimotor capability to dynamically regulate fingertip force vectors and motions to stabilize an unstable object (3–13). This paradigm consists of testing the extent to which people can compress a slender spring prone to buckling. The spring naturally becomes unstable as it is compressed; thus the maximal level of compression is indicative of the maximal sensorimotor capability to control the fingertips. The springs are designed to require very low forces to reflect the nature of ADLs. Moreover, functional magnetic resonance imaging (fMRI) studies show the SD paradigm can systematically interrogate brain function for dexterous manipulation, which exhibits differential activity across cortical networks depending on the level of difficulty and behavioral goals of the task (4, 7, 8).

Given that we have previously established the reliability and utility of this approach to dexterity (3–13), the purpose of this work is to understand the effects of gender, age, and disease on this sensorimotor ability to control instabilities. The effect of age on motor function in general, and hand function in particular, is well known (2, 13–15). However, recent studies using the SD paradigm have demonstrated its ability to detect previously unknown changes in dexterity lasting into late adolescence in typical development (6, 9, 10), or starting in middle age in healthy older adults (13). One goal of this work is to expand upon those findings by including larger numbers of participants, and including those individuals diagnosed with clinical conditions. While the effect of gender on muscle strength is well known, its effects on sensorimotor function are less clear. There continues to be keen clinical interest given the greater incidence of some musculoskeletal pathologies

and injuries in women, such as osteoarthritis (16) and non-contact ligament tears (17). The literature contains contradictory reports (15, 18) that feed continued debate on the issue. Our own work using the SD paradigm has hinted at gender differences in dexterity in typical development (6, 10), but these remain to be explored in detail.

Lastly, our more recent work has extended the concept of finger dexterity to limbs in general. By simply scaling up the physical size of our test system, we have introduced the concept of limb dexterity (19). The Lower Extremity Dexterity (LED) test has been shown to be a valid and repeatable metric of dynamic leg function (19). Importantly, our report of strong differences in leg dexterity between men and women has begun to provide a neuromuscular explanation for gender differences in agility, and the much higher incidence of non-contact ligament tears in female athletes (19, 20). We are therefore compelled to explore the nature of systemic versus limb-specific dexterity as it relates to age and gender. This is necessary to further our understanding of the neural mechanisms for dynamical function in health and disease.

MATERIALS AND METHODS

All participants gave their informed consent to the experimental protocol, which was approved by the Health Sciences Campus Institutional Review Board at the University of Southern California in Los Angeles, and/or the relevant ethics committees at the Institut de la Main-Clinique Jouvenet in Paris, and the Institute of Sports Science in Innsbruck.

CONTROL SUBJECTS

We measured finger dexterity in 147 healthy volunteers (66F, 81M, 52.7 ± 21.6 years) between 20 and 88 years of age to use as baseline data for comparison. Similarly, we measured single leg dexterity in 188 healthy volunteers (73F, 115M, 42.7 ± 23.6 years) between the ages of 14 and 92 years. Of these, 81 volunteers from 20 to 85 years of age (28F, 53M, 47 ± 22.8 years) completed both the finger and leg dexterity protocols in order to evaluate dexterity systemically. Participants were excluded if they had pathology of the hand or a history of injury that prevented unrestricted use of their fingers or legs.

CLINICAL POPULATIONS

We used a sample of convenience from two clinical conditions known to affect hand function as a first exploration of the clinical utility of this paradigm. Our goal was not to diagnose or evaluate treatment, but simply collect cross-sectional data from patients suffering from these conditions. For these clinical groups, participants were excluded if they were undergoing treatment for injury or surgery and had not been released by their surgeon or physical/occupational therapist to participate in everyday ADL, had a concurrent injury or pathologic condition that caused pain or discomfort in the tested limb during physical activity and/or at rest, had clinical, surgical, physical, cognitive, or other conditions that may have prevented their ability to perform the tasks proposed in this study, including the clinical restriction decided by the surgeon or therapist, or were unable to complete the protocol.

The first clinical group, defined as patients treated for CMC OA, consisted of 33 female participants (65.81 ± 9.72 years, 42 hands) evaluated at an average of 40 months after treatment at Institut de la Main. The same surgeon (Caroline Leclercq) performed the treatments on all the patients. The CMC OA patients underwent one of four treatment types: ligament reconstruction with tendon interposition (LRTI) arthroplasty (21), trapeziectomy (TS) (22), non-surgical medical treatment (i.e., rehabilitation), and no treatment.

The second clinical group, defined as patients treated for PD, consisted of 16 volunteers (10M, 6F; 67.68 ± 8.5 years, 31 hands). All patients were treated at the USC Keck School of Medicine, Department of Neurology in the Parkinson's Disease and other Movement Disorders Clinic.

STRENGTH–DEXTERITY TEST

The SD test is well described elsewhere (3–12). Briefly, it involves using the fingertips to compress as far as possible a slender spring, prone to buckling. This requires control of fingertip motions and force vectors at very low force levels (**Figure 1A**). It was conducted with a custom spring (Century Springs Corp., Los Angeles, CA, USA) outfitted with two miniature compression load cells (ELB4–10, Measurement Specialties, Hampton, VA, USA). The load cells were connected to a signal-conditioning box and USB-DAQ (National Instruments, Austin, TX, USA), sampled at 2000 Hz using custom Matlab (The Mathworks, Natick, MA, USA) software, and calibrated with a deadweight procedure. Participants were asked to compress the spring in a controlled way at their own pace to the point of maximal instability they can sustain (i.e., beyond which they felt it would slip out of their hand), and maintain that compression at a steady level for at least 5 s (**Figure 1B**) (9, 10). They were then to release in a controlled way at their own pace. After familiarization, at least 10 trials were performed for each test limb and the compression force was defined as the mean of the three maximal trials. Participants were allowed as many practice trials as needed to obtain steady state compression for the minimum required compression time of 5 s.

LOWER EXTREMITY DEXTERITY TEST

Similar to the SD test, the LED test is a single leg dynamic contact control task that is based on the ability of participants to compress a slender spring (19, 20, 23). The LED test device consists of a helical compression spring (Century Springs Corp., Los Angeles, CA, USA) mounted on a single-axis force sensor (Transducer Techniques, Temecula, CA, USA) affixed to a stable base with a 15 cm × 30 cm platform affixed to the free end (**Figure 2A**). Participants were positioned in an upright partially seated posture on a bicycle saddle intended to stabilize the body and minimize the extraneous use of the contralateral limb and upper extremities during testing. A computer monitor provided visual force feedback of the vertical force (19, 20, 23). As with the SD test, participants were instructed to slowly compress the spring with their foot with the goal to raise the force feedback line as high as possible and maintain that compression for at least 10 s (**Figure 2B**). After familiarization, between 10 and 20 trials were performed for each test limb (19, 20, 23) and the compression force was defined as the mean of the three maximal trials. Participants were allowed as many practice trials as needed to obtain steady state compression for the minimum required compression time of 10 s.

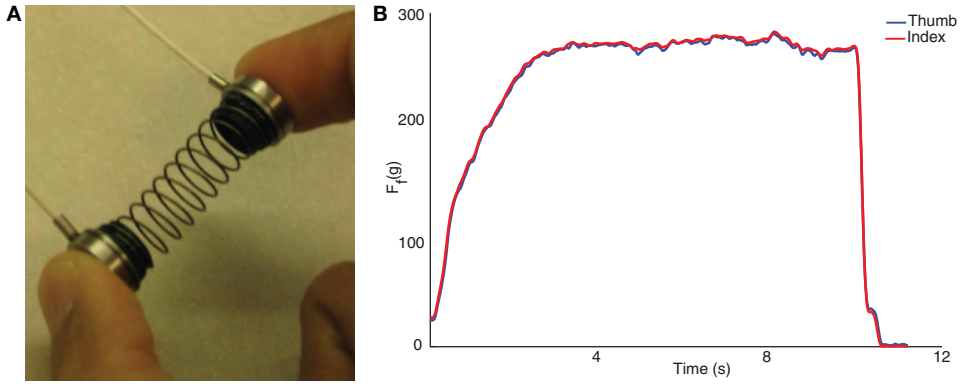

FIGURE 1 | The SD test (A) consists of compressing a compliant, slender spring prone to buckling, and sustaining the maximal level of compression for >5 s. The pulps of the thumb and index finger press against miniature load cells. Sample data from spring compression are shown to the right **(B)**. The forces from the thumb and index finger, in gf, are averaged to calculate the maximal compression force.

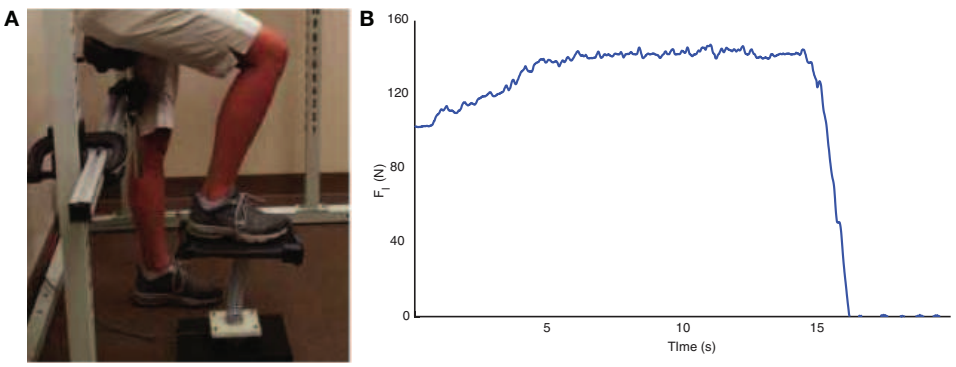

FIGURE 2 | The LED test (A) consists of pressing an appropriately scaled-up spring with the foot against the ground. Compression forces, in N, are quantified with a load cell located under the spring. Sample data from spring compression are shown to the right **(B)**.

DATA ANALYSIS AND VARIABLE DESCRIPTIONS

The dependent variables for the SD and LED tests are defined in **Table 1**. Linear regressions, two-tailed t-tests, and analysis of variance (ANOVA) were applied to the data set, as appropriate, to identify and quantify the relationships between test performance, age, gender, and dominance and to compare performance between clinical and control populations. Significance was set at $p < 0.05$ for all analyses. Matlab R2013a and SPSS version 22 (IBM, Armonk, NY, USA) were used for these analyses.

RESULTS

OVERVIEW

The ANOVA results are summarized in **Table 2** and discussed in detail in this section. We report strong age and gender effects in leg and finger compression force in healthy participants. Furthermore, we report strong effects of clinical condition (both CMC OA and PD) on the force velocity, acceleration, and RMS of the SD test. Interestingly, we report no differences in any variable between the dominant and non-dominant sides of control participants, patients diagnosed with CMC OA, and between self-reported affected and unaffected sides of patients diagnosed with PD.

The results from the linear regression analyses of compression force with respect to age are summarized in **Table 3**. We report significant increases in compression force in both the finger and leg in healthy participants under the age of 40, and vice versa for those over the age of 40 years – but as clarified in the Section "Discussion," this effect is not always seen when separating subjects by gender. Furthermore, there were greater decreases in force with age in the clinical groups compared to unimpaired participants.

FINGER SD TEST WITH CONTROL SUBJECTS IN THE SELF-REPORTED DOMINANT HAND

We tested for the effects of age and gender on finger dexterity in the self-reported dominant hand of 147 healthy individuals between the ages of 20 and 88 years. When needed, some variables (F_f, $\dot{F}_f$, $\ddot{F}_f$, and RMS_f) were transformed using the natural logarithm function to meet the assumptions of normality required for parametric statistics. As shown in **Table 2**, an ANOVA with finger compression force as the dependent variable and age and gender as factors performed on the transformed data revealed a significant effect by both age ($p = 0.017$) and gender ($p = 0.021$). Furthermore, we report no gender effects on the compression dynamics ($\dot{F}_f$, $\ddot{F}_f$,

Table 1 | Definition of variables used in analyses.

Variable	Symbol	Description
Finger compression force	F_f	Mean compression force during the hold phase of the SD test (units: gf)
Finger force velocity	$\dot{F}_f$	Mean of the absolute value of the first time derivate of compression force during the hold phase of the SD test (units: gf/s)
Finger force acceleration	$\ddot{F}_f$	Mean of the absolute value of the second time derivate of compression force during the hold phase of the SD test (units: gf/s^2)
Finger force RMS	RMS_f	Magnitude of the mean of the force dispersions during the hold phase of the SD test (units: gf)
Leg compression force	F_l	Mean compression force during the hold phase of the LED test (units: N)
Leg force velocity	$\dot{F}_l$	Mean of the absolute value of the first time derivate of compression force during the hold phase of the SD test (units: N/s)
Leg force acceleration	$\ddot{F}_l$	Mean of the absolute value of the second time derivate of compression force during the hold phase of the SD test (units: N/s^2)
Leg force root-mean square (RMS)	RMS_f	Magnitude of the mean force dispersions during the hold phase of the SD test (units: N)

Note that force magnitudes for the finger and leg tasks (cf. ***Figures 1*** *and* ***2****) are two orders of magnitude apart. Therefore, we use the SI units of gf and N, respectively, to accommodate those differences.*

Table 2 | Summary of multifactor ANOVA results.

Variable	Age	Gender	Side	Clinical condition
Finger compression force (F_f)	$*p = 0.017$[a]	$*p = 0.021$[a]	Control: $p = 0.461$[a] PD: $p = 0.784$ CMC OA: $p = 0.327$	$p = 0.081$
Finger force velocity ($\dot{F}_f$)	$*p = 0.048$[a]	$p = 0.542$[a]	Control: $p = 0.408$[a] PD: $p = 0.668$ CMC OA: $p = 0.786$	$*p < 0.001$
Finger force acceleration ($\ddot{F}_f$)	$p = 0.061$[a]	$p = 0.158$[a]	Control: $p = 0.672$[a] PD: $p = 0.725$ CMC OA: $p = 0.849$	$*p < 0.001$
Finger force RMS (RMS_f)	$p = 0.880$[a]	$p = 0.989$[a]	Control: $p = 0.183$[a] PD: $p = 0.696$ CMC OA: $p = 0.755$	$*p < 0.001$
Leg compression force (F_l)	$*p = 0.005$	$*p = 0.002$	$p = 0.295$	–
Leg force velocity ($\dot{F}_l$)	$p = 0.595$	$p = 0.536$	$p = 0.945$	–
Leg force acceleration ($\ddot{F}_l$)	$p = 0.519$	$p = 0.441$	$p = 0.872$	–
Leg force RMS (RMS_l)	$p = 0.532$	$p = 0.135$	$p = 0.237$	–

[a] *Indicates transformed data set.*

**indicates significance level of 0.05.*

Table 3 | Summary of linear regressions of compression force with age results.

Variable	Controls <40 years			Controls >40 years			Clinical participants	
	Males	Females	All	Males	Females	All	CMC OA	PD
Finger compression force (F_f)	$p = 0.328$	$p = 0.316$	$*p = 0.019$	$p = 0.09$	$*p = 0.008$	$*p = 0.002$	$*p < 0.001$	$*p < 0.001$
Leg compression force (F_l)	$*p = 0.001$	$p = 0.09$	$*p < 0.001$	$p = 0.055$	$p = 0.076$	$*p = 0.007$	–	–

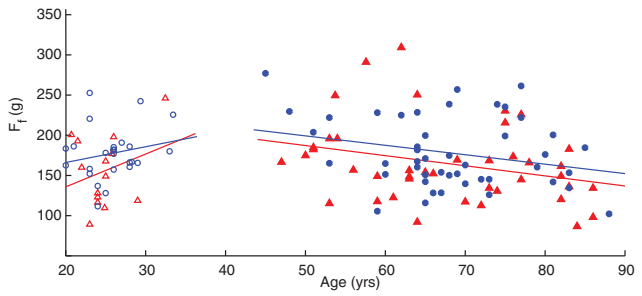

FIGURE 3 | Linear regression of finger compression force with respect to age. Younger adults (empty symbols) tended to show an increase in compression force while older adults (filled symbols) showed a decrease. Male participants (blue circles) tended to have greater values than females (red triangles) as indicated by the position of the fit lines. See **Table 3**.

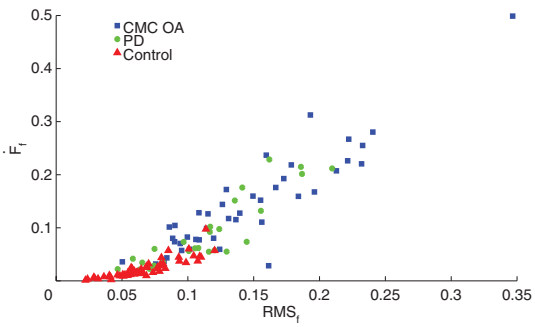

FIGURE 4 | Dynamic characteristics of the SD test. Control participants (red triangles) had significantly greater stability during SD compression compared to patients with CMC OA (blue squares) and PD (green circles).

and RMS_f) and no age effects on force accelerations and RMS, but age does affect the finger force velocity ($p = 0.048$) (**Table 2**).

A linear regression of finger compression force with respect to age, grouped by gender, is shown in **Figure 3**. Without accounting for gender, adults under the age of 40 years have an increase in finger compression force with age ($p = 0.019$) while adults over 40 have a decrease in force with age ($p = 0.002$). When the groups are separated by gender, however, the increases in compression force in younger males and females and decreases in older males are no longer significant (**Table 3**). Note the offset in regression lines, which agrees with the significant on the gender effect on compression force as per the ANOVA.

FINGER SD TEST WITH CLINICAL SUBJECTS

We compared performance on the SD test (F_f, $\dot{F}_f$, $\ddot{F}_f$, and RMS_f) between clinical patients diagnosed with either CMC OA or PD and a subset from our dataset of 29 healthy, age-matched volunteers (10M, 19F; 65.6 ± 9.7 years, 48 hands) with no history of hand injury or disease or neurological disorder. Interestingly, we found no significant differences in finger compression force among groups, however we found differences between the clinical and control groups in compression dynamics ($\dot{F}_f$, $\ddot{F}_f$, and RMS_f) during the sustained compression as illustrated in **Figure 4**. We found no differences in compression dynamics between the PD and CMC OA groups; however, both groups showed significant differences from the control participants ($p < 0.001$), indicating distinctly different dynamical behavior during manipulation in these clinical populations (**Table 2**).

Additionally, as in Ref. (9, 10, 13), we characterized the force dynamics during the sustained compression by plotting the phase portraits of F_f versus $\dot{F}_f$ versus $\ddot{F}_f$ (**Figure 5**). The character of the phase portrait was quantified by the mean Euclidean distance from the origin per unit time (9, 10, 13). A greater Euclidean distance is suggestive of weaker corrective actions by the neuromuscular controller enforcing the sustained compression (9, 10, 13). There are clear differences in the phase portraits of the control and clinical participants, with greater dispersion associated with the clinical groups.

We also performed linear regressions of finger compression force versus age in these three populations, which revealed that individuals with CMC OA and PD showed greater rates of decline compared to control subjects ($p < 0.001$), **Figure 6**. Patients with CMC OA and PD had average rates of decline of -1.4 and -2.7 gf/year, respectively, compared to -0.86 gf/year in control participants (**Table 3**).

To further expand the analysis and investigate the effect of laterality, we compared performance on the self-reported affected hand to the unaffected hand in a subset ($n = 8$) of the PD group. An ANOVA revealed no effect of side in any variables (F_f, $\dot{F}_f$, $\ddot{F}_f$, and RMS_f; **Table 2**). We performed a similar analysis on the self-reported dominant and non-dominant hands of a subset of the CMC OA group ($n = 17$) and report no effect of laterality in any variable (F_f, $\dot{F}_f$, $\ddot{F}_f$, and RMS_f; **Table 2**).

LEG LED TEST WITH CONTROL SUBJECTS IN THE RIGHT LEG

Mirroring the work on finger dexterity, we also tested for effects of age, gender, and dominance on leg dexterity in the right leg of 188 healthy individuals from 14 to 92 years. In order to account for the age and gender effects on body weight, which may influence leg compression force, we included body mass index (BMI) in the analysis. The data were normally distributed, and an ANOVA with leg compression force as dependent variable, age and gender as factors, and BMI as a covariate showed that compression force is strongly affected by both age ($p = 0.005$) and gender ($p = 0.002$; **Table 2**), but not by BMI ($p = 0.198$). Furthermore, ANOVA on the force dynamics ($\dot{F}_l$, $\ddot{F}_l$, and RMS_l) during sustained compression showed no effect of gender, age, or BMI.

Linear regressions of leg compression force versus age revealed significant increases in force in adults under the age of 40 ($p < 0.001$) and decreases in participants over 40 years ($p = 0.007$). However, when separated by gender, increase in compression force in young females and decreases in older males and females are no longer significant (**Table 3**). As with the hand, there are increases in compression force with respect to age in younger adults and decreases in older adults; and the regression lines of male participants are slightly shifted above those of females, corroborating the ANOVA results that compression forces for male participants tended to be greater on average than that of female participants when using age as a factor (**Figure 7**). Note that in these subjects we only tested one leg, the right leg, for expediency because the effect

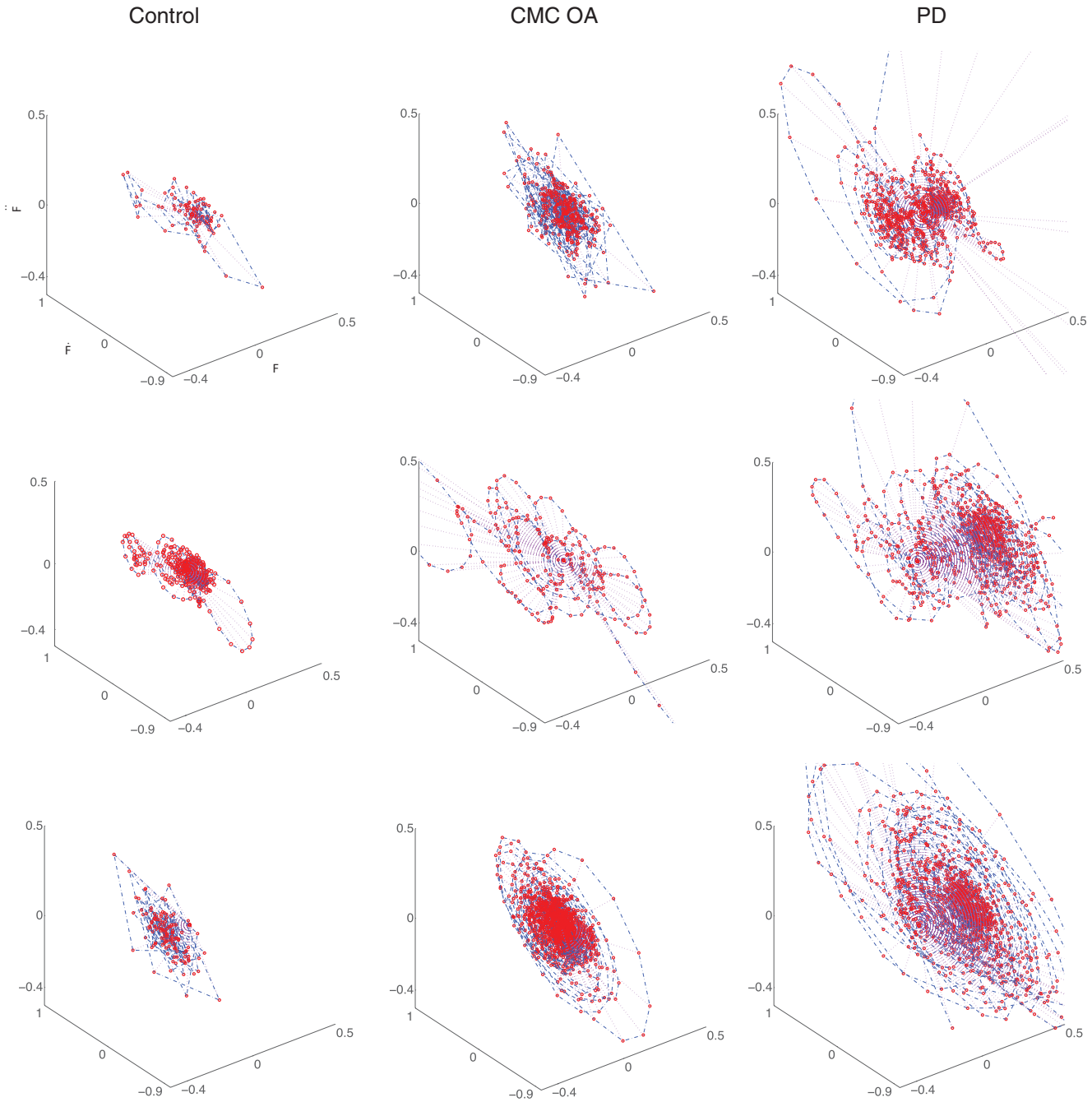

FIGURE 5 | Representative phase portraits of three participants from each group (ages between 70 and 75 years): healthy control subjects (first column), participants diagnosed with CMC OA (second column), and participants diagnosed with PD (third column). The clinical subjects exhibit greater dispersion in the phase portrait than the control subjects.

of leg dominance was explored in a different subset of subjects (see below).

DEXTERITY ACROSS BOTH FINGERS AND LEGS

Finally, we explored dexterity across the upper and lower extremities by comparing SD and LED performance in both fingers and legs of 81 healthy volunteers between the ages of 20 and 85, each labeled as self-reported dominant or non-dominant (**Figure 8**). Surprisingly, ANOVA (in this case a repeated measures ANOVA given that we collected finger and leg data in the same subjects) revealed no effects of laterality (i.e., dominant versus non-dominant) for any variable, when controlling for gender and age in these participants (**Table 2**). However, we found statistically significant ($p < 0.001$) Pearson's product–moment correlation of

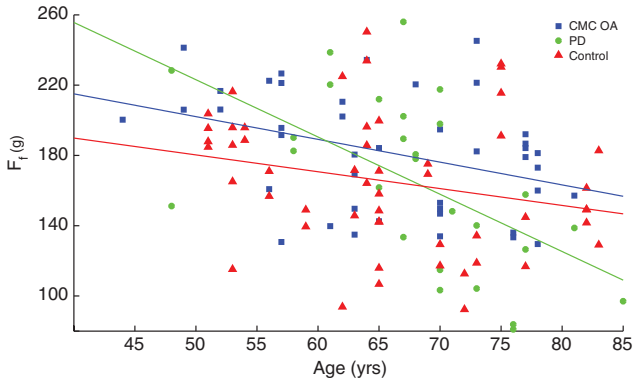

FIGURE 6 | Comparison of rate of decline between clinical and control populations. Finger compression force was plotted against age and revealed that the clinical groups (PD and CMC OA, green circles and blue squares, respectively) had a greater rate of decline with age than control participants (red triangles).

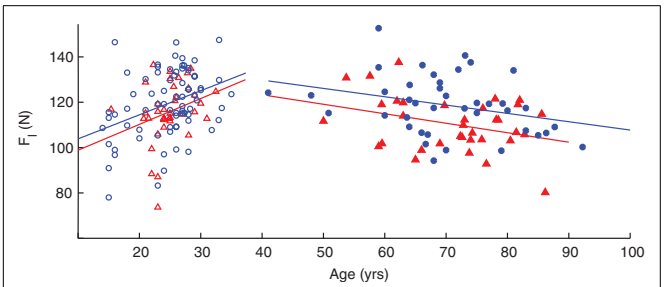

FIGURE 7 | Age- and gender-related changes in leg compression force. Regressions against age indicated an increase in younger adults (empty symbols) and a decrease in older adults (filled symbols). Male participants (blue circles) tended to have greater values than females (red triangles) as indicated by the position of the fit lines.

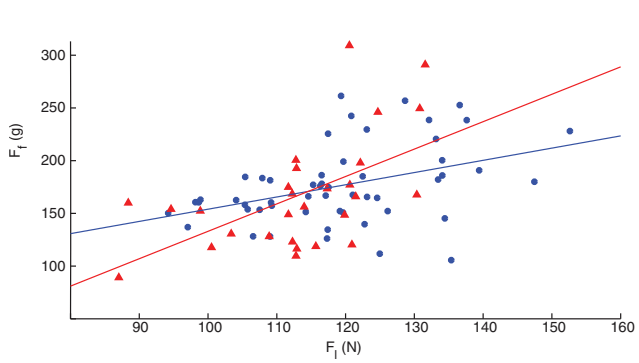

FIGURE 8 | Correlation of finger and leg dexterity. Both male (blue circles) and female (red triangles) participants showed significant association between finger and leg compression force in the self-reported dominant limb, with females exhibiting higher correlation than males, $\rho = 0.529$ and 0.403, respectively.

$\rho = 0.458$ between finger and leg compression forces in all subjects. When separating them by gender, the Pearson's product–moment correlation was higher in females ($\rho = 0.529$, $p = 0.004$, $n = 28$) than in males ($\rho = 0.403$, $p = 0.003$, $n = 53$).

DISCUSSION

There are multiple definitions for, and connotations of, the concept of dexterity. In a series of recent publications using the SD paradigm, we have argued that quantifying the sensorimotor ability to stabilize objects with the fingertips is a valid definition of one aspect of finger dexterity (3–10). By focusing on how the fingertips act on an object by dynamically regulating the magnitude and direction of fingertip forces, we can quantify important features of using precision pinch (or tip-to-tip, or pincer grasp) to manipulate objects. Therefore, the purpose of this comparative cross-sectional study was to quantify how these features of dexterous manipulation are affected by age, gender, and disease. We have previously attributed the sensitivity of the SD test to detect functional changes among both healthy and clinical populations across the life span to its ability to focus on the sensorimotor function of the isolated CNS-limb system without the confounds of visual acuity, whole-arm function, or finger strength (3, 5, 6, 9–12). Furthermore, it has allowed the detection and identification of specific and context-sensitive brain circuits for dynamic control of the fingers (4, 7, 8). Those prior findings inform our interpretation of our important results now quantifying the effects of gender, age, and disease.

EFFECT OF AGE

Our results corroborate the effect of age we have reported for finger dexterity in young children and adolescents (10), and older adults (13). However, we extend those results in crucial ways. It is important to note that our prior work (9) revealed no significant changes in dexterous manipulation in middle age and therefore, we used samples of convenience (college-aged students and older control subjects for comparison to clinical populations of interest), which resulted in an under sampling of subjects between 35 and 50 years of age, but does not affect the results we report. First, we emphasize our study of adults starting at 20 years of age, where we continue to see an improvement in young adulthood. In an earlier study, we report the strong association between improvements in finger compression force and compression dynamics with maturation of the brain in children and adolescents (10). To our knowledge, this is the first report of continual improvement of dexterity into young adulthood after the age of 20. The continual behavioral improvements we see here are, therefore, credibly associated – at least in part – with such neural maturation and have important clinical implications for rehabilitation. For example, traumatic injuries [such as spinal cord injury in males (24) and anterior cruciate ligament (ACL) tears in females (17)] are most prevalent in young adults. Our results indicating the presence of motor learning and neural plasticity in early adulthood suggest that these individuals would naturally have a propensity to respond to therapy better than older adults. Similarly, our results now come from 147 adults from 20 to 88 years of age. These include 108 subjects not previously analyzed and 39 from our previous reported pool of 98 subjects (13). This was critical to reveal the gender effect in finger compression not previously significant (see below and **Table 2**),

and now confirm what was a near significant effect of age on finger force dynamics hinted at in our previous work (6, 9, 10, 13), **Table 2**.

While we also corroborate the finding that finger dexterity begins to decline in middle age (13), this study goes on to reveal differences in that decline in individuals aging with a disability. We find that one condition (PD) exhibited a rate of decline two times greater than another (CMA OA), and three times greater than non-symptomatic control subjects (**Figure 7**). This has important implications to the differential role in which different disease mechanism produce disability (see below). Aside from the clinical details we discuss below, the idea that finger dexterity is an indicator of the integrity of the sensorimotor system (3), together with the idea that loss of dexterity in older adults is not linked to muscular weakness (13) or BMI, leads to the implication that in older adults the ability of the nervous system to respond to therapy is increasingly muted.

In our prior work (10) we have noted that, in parallel with the development of the ascending and descending pathways between brain and hand, there are striking developmental processes taking place in the brain gray and white matter during childhood up to adolescence, e.g., expansion of the white matter and pruning of the cortical gray matter (25–30). Ehrsson et al. (31) demonstrated that there is greater activity in the fronto-parietal sensorimotor areas during the control of smaller forces than larger forces, with control of larger forces associated with increased activity in the M1 region. Fronto-parietal regions demonstrate significant developmental changes in the adolescent years (28, 29, 32), and the pruning of the gray matter occurs later in the frontal and parietal areas (33) than in M1. These associations between the development of cortical neural networks, including ascending and descending pathways on one hand, and the dexterity measured by our method are, of course, mostly empirical and speculative. Our results now raise the possibility that these processes continue into young adulthood. Moreover, they also seem to be reversed (or counteracted) by the mechanisms of aging in a way that is behaviorally measurable, in a way that has important clinical and therapeutic implications.

EFFECT OF GENDER

The effect of gender on motor skill is not well documented, necessarily predictable, or expected in dynamic finger function – contrary to the well known effect of gender on muscle strength or BMI. Given those differences in strength across genders, we designed our test of dynamic sensorimotor function to require only very low levels of force (<300 gf). We have reported hints of a gender effect on dexterity in typically developing children (6) – which may have been colored by a test protocol that tended to require large forces. However, these new results now establish without a doubt that females exhibit lower ability to control instabilities with the fingertips than males at any age. The literature does not report consistent gender effects, and the issue remains very much debatable (6, 15, 18, 34). Our results add to this literature by providing a new example of performance differences between women and men.

Given that we have found the SD paradigm to be informative of local and systemic neuromuscular mechanisms [e.g., brain maturation, muscle contractile speeds, functional brain connectivity and networks, etc. (3–10)], this clear gender effect is remarkable as it strongly suggests those sensorimotor differences in women are a function of specific mechanisms at the level of the muscles, spinal cord, and/or brain. This leads directly to testable hypotheses at each of these hierarchical levels. For example, does the excitability of motoneuron pools during the control of unstable forces change differently in men versus women? What are the roles of hormonal cycles in the general excitability and controllability of the sensorimotor system? Are there differences in brain connectivity in sensorimotor areas across genders as is now reported for cognitive areas? There is a growing consensus that male brains are structured to facilitate connectivity between perception and coordinated action, whereas female brains are designed to facilitate communication between analytical and intuitive processing modes (35). Our methodology now allows us to systematically interrogate those differences in the context of the functionally critical areas of dexterity.

EFFECT OF CLINICAL CONDITION

Our study also raises the similarly noteworthy question of why a condition that is presumably purely orthopedic (i.e., CMC OA) produces deficits in dynamic manipulation – and accelerated losses with age – comparable to those in a purely neurological condition (i.e., PD). Both the CMC OA and PD groups displayed significant differences ($p < 0.001$) in the compression dynamics ($\dot{F}_f$, $\ddot{F}_f$, and RMS_f) compared to the control participants (**Figure 4**), although no differences in compression force. That is, all three populations were able to compress to the same amount, but not in the same way. Similarly, detailed visualization of the finger force dynamics during compression via phase portraits (**Figure 5**) shows subjects with CMC OA and PD tend to demonstrate weaker correction strategies. The greater amount of dispersion in the phase portraits of clinical patients suggests a compromised ability to execute corrections, or a different neural control strategy toward instability, not seen in control subjects (10, 13). Whether these differences in neural control, or the mechanisms of executing neural control, are similar or different in CMC OA and PD remains an open question.

These results also challenge the notion that CMC OA is a strictly orthopedic condition given that we now see it produces sensorimotor deficits. The link between a disease of articular cartilage and deficits in sensorimotor integration capabilities is underappreciated and understudied in the literature. To elaborate, **Figure 4** illustrates that the CMC OA and PD populations are essentially indistinguishable when plotting finger force velocity versus finger force RMS. These results raise the question, what is it about chronic pain and damage to the joint that leads to changes in sensorimotor capabilities? Others have begun to speak about this and a picture is now emerging showing that chronic pain leads to reorganization of brain circuits. For example, subacute low back pain induces changes in connectivity and functional reorganization of the insula and sensorimotor cortex, even after only 1 year with moderate pain (36). Also, spontaneous pain due to knee OA is known to engage brain regions distinct from those activated by pressure-evoked pain, specifically prefrontal-limbic structures (37). The presence of acute pain will naturally compromise function – but we now

see that chronic pain also affects the performance of a dexterous task even if it requires very low forces and does not elicit pain. Our prior work suggests these deficits are credibly attributable to structural or functional changes in portions of the nervous system responsible for the neural control of dexterity.

At the other end of the clinical spectrum, PD starts out as a purely neurological degenerative disease characterized by upper and lower extremity rigidity, tremor, bradykinesia, and/or postural instabilities (38, 39). Our prior work has shown that the cortical networks associated with controlling instabilities in dexterity can involve the basal ganglia (8), where degeneration of dopamine-producing cells plays a central role in PD (39). Thus it is expected that we would detect deficits in sensorimotor function and, in turn, dexterous manipulation in this population. However, our results allow us to go deeper than this. They allow us to, for the first time, (i) systematically quantify behavioral deficits in PD and other neurological conditions, (ii) disambiguate the contributions of different elements of the neuromuscular system to these deficits, and (iii) easily and objectively quantify the effectiveness of different treatment regimens (e.g., absorption of medication or titration of deep brain stimulation level) during the daily – and even hourly – fluctuations in motor deficits in PD that traditional measures cannot. However, it is also critical to note that PD leads to significantly greater rates of decline of dexterity with age when compared to healthy aging or with patients diagnosed with CMC OA. This highlights the neurodegenerative nature of the disease, and underscores the need to quantify the effects of PD on sensorimotor processing and dexterous manipulation to better understand its neurodegeneration and treatment.

How do our results speak to ADLs? The SD paradigm falls clearly within the Body Functions and Structure Components of the International Classification of Function [ICF (40)]. Understanding the link between SD performance and the Activity Limitations and Participation Restriction Components of the ICF requires further research. But as of now, we can say that the SD paradigm is likely very informative of systemic mechanisms that make dexterous function possible – as argued throughout the Section "Discussion." That is, the SD paradigm reflects the potential to execute ADLs without the confounds of functional adaptations that mask the detrimental effects of disease. A clear example for the upper extremity is that of manipulating small and/or deformable objects such as beads or squeezing lemons, respectively. In both these cases, the manipulation task is unstable in the same sense that the SD paradigm specifies: they require accurate dynamical regulation of the magnitude and direction of fingertip forces and motions (9, 10, 13). For the lower extremity, we have proposed that the SD paradigm may explain the risk of injury or falls (19, 20, 23) because the regulation of dynamical interactions with the ground is critical to locomotion and many sports activities, as mentioned above.

SYSTEMIC VERSUS LIMB-SPECIFIC DEXTERITY

Another fundamental aspect of this work is that we extended the concept of finger dexterity to limbs in general. We use the same definition of dexterity to quantify the sensorimotor ability of the leg to regulate dynamical interactions with the ground in a subset of our participants. In the context of lower extremity function, the LED test evaluates the ability of the sensorimotor system to control an unstable ground contact with the isolated leg; and avoids potential confounds often found in gait, posture, and balance studies such as vestibular function, visuo-spatial perception, strength, whole-body balance, locomotor confidence, and inter-limb coordination. Clearly, our aim is not to study locomotion, but to focus on the fundamental sensorimotor capabilities of the leg. Further work is needed to establish its relationship to whole-body gait, posture, and balance capabilities. Nevertheless, our recent work on the lower extremity has demonstrated the validity and reproducibility of the LED test as a metric of dynamic leg function, and its correlation to whole-body agility. It has also clearly detected differences between young men and women (19, 20, 23). As in the case of the fingers (6), we have shown that the LED test quantifies a previously unrecognized functional domain related to dexterity of the isolated leg that cannot be seen as simply a covariate of available functional tests of strength, gait, or balance (41). Here we extend that prior work on leg dexterity by measuring the same set of variables as for the finger in 188 healthy volunteer participants (**Tables 1–3**). To our knowledge, this is the first comparison of finger versus leg dexterity that allows us to distinguish between systemic and limb-specific sensorimotor capabilities. Interestingly, we find similar effects of age and gender in both finger and leg dexterity.

The age and gender effects on leg compression force (**Figure 7**; **Table 3**) naturally suggest that the same neural mechanisms and networks for the fingers (discussed above) are at work in the leg to some extent. Traditionally we have come to think of "dexterity" as specific to fingers [e.g., Ref. (42–45)], and surely some features are. Phylogenetically speaking, however, legs evolved earlier and for the same purpose: to produce dynamical interactions with the ground. Thus, the prior existence of neural circuits to regulate instabilities in ground contact during quadruped gait and brachiation likely served as the foundation from which specializations evolved for manipulation in the human hand. Therefore, our discussions above about the neurophysiological bases of age and gender effects apply here as well. However, there are also important differences. We found no age and gender effects on compression dynamics ($\dot{F}_l$, $\ddot{F}_l$, and RMS_l), and most of these effects are far from significant even in this relatively large sample size (**Table 2**).

These similarities and differences between finger and leg dexterity, as quantified by the SD and LED tests, suggest the existence of specialized mechanisms for systemic versus limb-specific dexterity. First, it is clear that these results compel us to study in detail the neurophysiological bases of leg dexterity in health and disease, to at least to the level we have for the fingers. Moreover, the multiple time scales and latencies with which these dynamical tasks need to be controlled suggest a hierarchical organization of neural control, in agreement with current thinking (46–48). However, we must not be content with this generalization. Future work must leverage available techniques [e.g., electromyography (EMG), fMRI (7, 8), Hoffmann-reflex, transcranial magnetic stimulation (TMS), coherence analysis (49), EMG-weighted averaging (50)] in specific and well-directed studies to disambiguate among peripheral, spinal, and cortical contributions and mechanisms of dexterity. The SD paradigm allows such studies for the legs as it has for the fingers.

Second, our findings about leg dexterity nevertheless have immediate utility, both scientifically and clinically. Understanding the orthopedic and neurological effects of aging with a disability on quality of life is now emerging as an important public health issue (51–55) of immediate interest is the study of leg dexterity in patients with PD, where shuffle gait, ataxia, and bradykinesia are common – and the SD paradigm combined with clinical outcome measures and the techniques mentioned above will serve to clarify the mechanisms enabling leg dexterity and their neuroanatomical and functional hierarchy. Similarly, it is important to follow up with studies in patients with hip or knee OA, where we can begin to understand the effects of chronic pain on locomotor abilities both because OA is so prevalent, and because gait deficits that lead to falls in the elderly are a pressing public health problem (56).

In addition to providing insight into the nature of sensorimotor dysfunction in clinical populations, the fact that the LED test is able to discern gender differences (**Figure 7**; **Table 2**) may provide insight into why young women have a much greater likelihood of non-contact ACL tears than men (57). Though the reasons are not clear, some theories include differences in knee alignments, ligament laxity, hormone levels, muscle strength and conditioning, and neuromuscular control (17, 20). The clearly reduced dexterity we report in young women (both in fingers and legs) expands on previous results (20) with a smaller sample size where gender differences in dexterity were used to provide a neuromuscular explanation for the higher incidence of ACL tears and reduced agility in young female athletes. Moreover, given that we now show that these gender differences in leg dexterity are present throughout the lifespan also speaks to the fact that women over the age of 65 have a disproportionately greater occurrence of unintentional falls than men (16, 58). Future work will include identifying those with reduced leg dexterity who may have a greater risk for ACL tears or falls and would benefit from preventative neuromuscular training programs.

Interestingly, we saw no clear effect of limb dominance on finger and leg dexterity in the subset of 81 participants who completed the SD paradigm with all four limbs. After all, voluntary fine-motor tasks such as writing, cutting, catching, and kicking exhibit strong effects of laterality. In fact, there is a multitude of evidence supporting both functional (e.g., strength and motor control) and anatomical differences at the cortical level between dominant and non-dominant limbs (15, 59–64). It is reported that long-term preferential use of muscles results in a higher percentage of type 1 muscle fibers in the dominant hand and, in turn, changes in motor unit firing behavior (61). Furthermore, imaging studies have shown that the hemisphere contralateral to the dominant hand demonstrates more efficient motor control at lower activation levels and less crosstalk than the non-dominant hemisphere (62, 63). One potential explanation is that we simply did not have enough subjects to demonstrate that latent effect, much as we did not find an age or gender effect in this same group of 81 subjects spanning multiple ages. This mirrors our prior work where we were not able to detect gender effects for the upper extremity in studies with smaller sample sizes (9). What is more striking, however, is that larger numbers may be needed to detect an effect of limb dominance, if it is even present.

Our lack of detection of limb dominance nevertheless raises important questions. As mentioned recently, it is likely that hemispheric specialization emerged to accommodate increasing motor complexity of tasks during primate evolution. That is, instead of the non-dominant limb being a lesser analog of the dominant limb, Sainburg and colleagues (65) have proposed an alternative view that motor lateralization reflects proficiency of each arm for complementary functions in response to distinct movement control mechanisms associated with specific unimanual tasks. We speculate that the lack of effect of dominance suggests that the SD and LED tests reveal and quantify subcortical mechanisms for dynamical function that are not influenced by hemispheric differences – in accordance with theories of hierarchical neural control and phylogenetic development of the nervous system. There is evidence of subcortical contributions to motor control (i.e., dexterity) independent of limb dominance. In this hierarchical view of motor control, the cerebellum, basal ganglia, spinal cord, etc. are essential to executing and regulating motor function. In agreement with Sainburg and colleagues (65), we speculate that hand (or leg) dominance is therefore likely a late arrival to the motor repertoire in humans that affects fine-motor tasks but not "low-level" stabilization mechanisms tested by the SD paradigm. This is supported by recent studies using Blood Oxygenation Level-Dependent (BOLD fMRI) to evaluate how hand dominance and task difficulty affect activation levels at the spinal cord (66) level. They found significant differences in spinal cord activation levels when performing simple unilateral tapping tasks with the dominant and non-dominant hands – but they found no effect of hand dominance during a more complex unilateral tapping task. The SD paradigm may be engaging these systemic hierarchically common circuits to all limbs independently of cerebral lateralization. A clinical consequence of this may be the fact that we did not see differences across the self-reported affected versus unaffected hand in patients with PD – although this requires further clinical investigations with greater numbers of individuals.

How does this concept that dexterity requires both subcortical and cortical mechanisms agree with or revise current thinking? Very briefly, the literature on cortical involvement in dexterous manipulation is large [e.g., the reviews in Ref. (45, 67, 68)]. Our own fMRI studies agree with many others suggesting direct cortical involvement by showing the SD paradigm can systematically interrogate brain function for dexterous manipulation, which exhibits differential activity across cortical networks depending on the level of difficulty and behavioral goals of the task (4, 7, 8). We have also proposed the likely evolutionary advantage of the monosynaptic corticospinal tract to manipulation by enabling the time-sensitive transitions from the control of motion to the control of static force (5); and that the competition between descending commands for manipulation likely involves the phylogenetically older reticulospinal and the newer corticospinal tracts (69). However, our results here compel us to confront several inconvenient facts to the cortico-centric view of the neural control of the hand including time delays, our evolutionary history, and clinical symptomatology. These issues can be resolved by paying more

attention – and due credit – to subcortical mechanisms. For example, many dynamic manipulation tasks (such as stabilization in the SD paradigm) occur at time scales for which spinal–cortical–spinal delays would compromise closed-loop control. Neural control must, therefore, involve motoneuronal modulation by the spine in human and non-human primates to some extent (70, 71). In fact, neuroanatomists and electrophysiologists since the time of Sherrington have sought to map the circuitry in the spinal cord (72) to understand the spinally mediated excitation–inhibition mechanisms that enable voluntary function [e.g., Ref. (73, 74)] – and produce the clinical symptomatology of, for example, spastic hypertonia present in many neurological disorders including stroke, traumatic brain injury, cerebral palsy, multiple sclerosis, and spinal cord injury [e.g., Ref. (75) and references therein]. Therefore, much as Lemon has written "it may be too sweeping a generalization to suggest that cortico-motoneuronal connections are the *sine qua non* of independent digit movements" (70), our results indicate that it may be too sweeping a generalization to suggest that cortical mechanisms are the *sine qua non* of dexterity. Once again, this compels future work to disambiguate among peripheral, spinal, and cortical contributions and mechanisms of finger and leg dexterity.

Finally, this is the first time that to our knowledge a same paradigm is used to quantify *both* finger and leg dexterity. We report their correlation in **Figure 8**, indicating that the sensorimotor system may have a combination of systemic versus limb-specific mechanisms, although the contribution of each remains unclear. The fact that this correlation is greater in female than in male participants ($\rho = 0.529$ versus $\rho = 0.403$, respectively) suggests a much greater systemic component in women. We speculate that dexterity is actually the sum of two components: the basic systemic, plus the limb-specific. The stronger systemic component in women may then suggest that men are able to add more of the limb-specific component and thus show less correlation overall. What could be the causes of this added plasticity for limb-specific dexterity in men? In addition to genetically imposed dimorphism (e.g., nature), sociobiological elements (e.g., nurture) such as differential exposure to physical activity, cultural biases, social expectations, etc., may play a role in the development and learning of motor function (76). Thus, the differences in dexterity across genders that we report, and in brain connectivity that others report, may be – at least in part – its phenotypical neurobiological consequence.

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Primary motor cortex neurons during individuated finger and wrist movements: correlation of spike firing rates with the motion of individual digits versus their principal components

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The joints of the hand provide 24 mechanical degrees of freedom. Yet 2–7 principal components (PCs) account for 80–95% of the variance in hand joint motion during tasks that vary from grasping to finger spelling. Such findings have led to the hypothesis that the brain may simplify operation of the hand by preferentially controlling PCs. We tested this hypothesis using data recorded from the primary motor cortex (M1) during individuated finger and wrist movements. Principal component analysis (PCA) of the simultaneous position of the five digits and the wrist showed relatively consistent kinematic synergies across recording sessions in two monkeys. The first three PCs typically accounted for 85% of the variance. Cross-correlations then were calculated between the firing rate of single neurons and the simultaneous flexion/extension motion of each of the five digits and the wrist, as well as with each of their six PCs. For each neuron, we then compared the maximal absolute value of the cross-correlations (MAXC) achieved with the motion of any digit or the wrist to the MAXC achieved with motion along any PC axis. The MAXC with a digit and the MAXC with a PC were themselves highly correlated across neurons. A minority of neurons correlated more strongly with a PC than with any digit. But for the populations of neurons sampled from each of two subjects, MAXCs with digits were slightly but significantly higher than those with PCs. We therefore reject the hypothesis that M1 neurons preferentially control PCs of hand motion. We cannot exclude the possibility that M1 neurons might control kinematic synergies identified using linear or non-linear methods other than PCA. We consider it more likely, however, that neurons in other centers of the motor system – such as the pontomedullary reticular formation and the spinal gray matter – drive synergies of movement and/or muscles, which M1 neurons act to fractionate in producing individuated finger and wrist movements.

Keywords: cortico-motoneuronal, electromyography, hand, joint angle, kinematic synergy, principal component, spike-triggered average

INTRODUCTION

The digits of the hand commonly have been thought to move independently of one another. But kinematic analysis has shown that simultaneous motion of multiple fingers occurs in virtually all human hand and finger movements. These include not only activities of daily living such as grasping and haptic exploration (1–4), but also sophisticated performances including finger spelling, typing, or piano playing (5–7), and even the individuated movements made when normal human subjects are asked to move only one finger (8, 9).

When simultaneous variation occurs in many independent elements – whether joint angles, muscles, or neurons – a limited variety of fixed patterns, or synergies, potentially can account for much of the simultaneous variation. The concept of synergies is useful, simplifying the problem of controlling all the original elements, primarily if the number of synergies needed to account for most of the variation in the data is substantially less than the number of original elements. Several different mathematical approaches, both linear and non-linear, might be used to identify such synergies, and which approach is most likely to capture synergies potentially used by the nervous system cannot be predicted.

Almost all prior studies of the kinematic synergies involved in hand movements have used a comparatively straightforward, linear approach – principal component analysis (PCA) (10). In the human studies cited above, application of PCA has identified patterns of correlated motion among multiple joints of the fingers and wrist. In general, a small number of such patterns, captured as principal components (PCs), accounts for the vast majority of the variance in the larger number of original elements, here

mechanical degrees of freedom (DoFs), typically the rotation of individual joints. Similarly in the grasping movements of non-human primates, the simultaneous correlated motion of multiple DoFs in the thumb, fingers, and wrist can be attributed largely to a small number of PCs (11–15).

These observations have led to the hypothesis that, at some level, the central nervous system (CNS) may simplify the computational burden of controlling the hand by driving PCs of hand kinematics. Patterns of simultaneous correlated movement kinematics, isometric forces, or muscle activity have been attributed variously to the spinal gray matter (16), the pontomedullary reticular formation (PMRF) (17–19), and the motor cortex (20, 21). If the PCs of hand and finger movements are controlled at some level of the CNS, then downstream neural, muscular, or mechanical elements would be responsible for distributing motion to multiple mechanical DoFs simultaneously. Upstream levels of the CNS then also might work in terms of PCs. Alternatively, some upstream centers might bypass the levels driving PCs and superimpose additional control on hand kinematics. Here we examined the PCs of individuated finger and wrist movements in non-human primates, as well as the extent to which neurons in the primary motor cortex (M1) are correlated with these PCs as compared to the original kinematics.

MATERIALS AND METHODS

Many of the methods used in the present study for behavioral training, data collection, and initial analyses have been described in previous reports, and are summarized here as needed.

ANIMALS AND BEHAVIORAL PROCEDURES

All care and use of these purpose-bred monkeys complied with the U.S.P.H.S. Policy on Humane Care and Use of Laboratory Animals, and was approved by the University Committee on Animal Resources at the University of Rochester. Each monkey was trained to perform visually cued individuated flexion and extension movements of the right hand fingers and/or wrist (22). As the monkey sat in a primate chair, the right elbow was held in a molded cast, and the right hand was placed in a pistol-grip manipulandum, which separated each finger into a different slot (**Figure 1A**). At the end of each slot, the fingertip lay between two microswitches (**Figure 1B**). By flexing or extending the digit a few millimeters, the monkey closed the ventral or dorsal switch, respectively. The manipulandum, in turn, was mounted on an axis that permitted flexion and extension wrist movements, transduced with a co-axial precision potentiometer. Each monkey viewed a display (**Figure 1C**) on which each digit (and the wrist) was represented by a row of five light-emitting diodes (LEDs). When the monkey flexed or extended a digit, closing a microswitch, the central yellow LED went out and a green LED to the left or right, respectively, came on, cueing the monkey as to which switch(es) had been closed. For the wrist, the voltage read from the potentiometer crossed fixed levels that substituted for flexion and extension microswitches. Red LEDs to the far left or right were illuminated one at a time, instructing the monkey to close that one switch (or move the wrist). If the monkey closed the instructed switch within the 700 ms response time allowed after illumination of the red instruction LED, and held it closed for a 500 ms final hold period without closing any other switches, the monkey received a water reward. After each rewarded trial, the movement to be instructed for the next trial was rotated in a pseudorandom order. We abbreviate each instructed movement with the number of the instructed digit (1 = thumb through 5 = little finger, 6 or w = wrist), and the first letter of the instructed direction (f – flexion; e – extension), for example, "4f" indicates instructed flexion of the ring finger. The behavioral task was controlled by custom software written in TEMPO (Reflective Computing, Olympia, WA, USA), which also generated 8-bit behavioral event marker codes.

While behavioral performance depended only on the closing of the microswitches for the fingers and the level crossings for the wrist, a continuous analog signal representing the flexion/extension position of each digit was generated using a semiconductor strain gage (BLH SPB3-20-35) mounted on the lever-arm of each microswitch (22). The gages mounted on the flexion and extension switches for each digit were configured as two legs of a Wheatstone bridge, the output of which was amplified, low-pass filtered (5 kHz cutoff), and biased with a commercial circuit (Analog Devices 2B31J). Although the spring qualities of the microswitches and their lever-arms produced a linear relationship between fingertip position and force, here we will consider these signals to represent fingertip position. A separate analog signal representing the flexion/extension position of the wrist was provided by the potentiometer coupled to the wrist axis.

DATA COLLECTION

After training, aseptic surgery under isoflurane anesthesia was used to open a craniotomy over the left central sulcus at the level of the hand representation, and to implant both a rectangular Lucite recording chamber over the craniotomy and two head-holding posts. Once the monkey had recovered from this procedure and had become accustomed to performing the finger movement task with its head held stationary, EMG electrodes made of 32 gage, Teflon-insulated, multi-stranded stainless steel wire (Cooner AS632, Chatsworth, CA, USA) were implanted percutaneously using aseptic technique in 8–16 forearm and hand muscles under Ketamine anesthesia, using techniques adapted from those of Cheney and colleagues (23–25). Muscles implanted typically included 8–16 of the following: thenar eminence (Thenar); first dorsal interosseus (FDI); hypothenar eminence (Hypoth); flexor digitorum profundus, radial region (FDPr); flexor digitorum profundus, ulnar region (FDPu); flexor digitorum profundus, proximal ulnar region (FDPpu); flexor digitorum superficialis (FDS); flexor carpi radialis (FCR); palmaris longus (PL); flexor carpi ulnaris (FCU); abductor pollicis longus (APL); extensor pollicis longus (EPL); extensor digiti secundi et tertii (ED23); extensor digitorum communis (EDC); extensor digiti quarti et quinti (ED45); extensor carpi radialis (ECR); extensor carpi ulnaris (ECU), and supinator (Sup).

Thereafter in daily recording sessions, conventional techniques were used to record a single M1 neurons simultaneously with the analog signals representing the flexion/extension position of each digit and the wrist (sampled at 1 kHz) and with EMG activity from the implanted forearm and hand muscles (EMG amplification 2,000–100,000×, bandpass 0.3–3 kHz, sampling frequency ~4 kHz per channel) as the monkey performed individuated finger

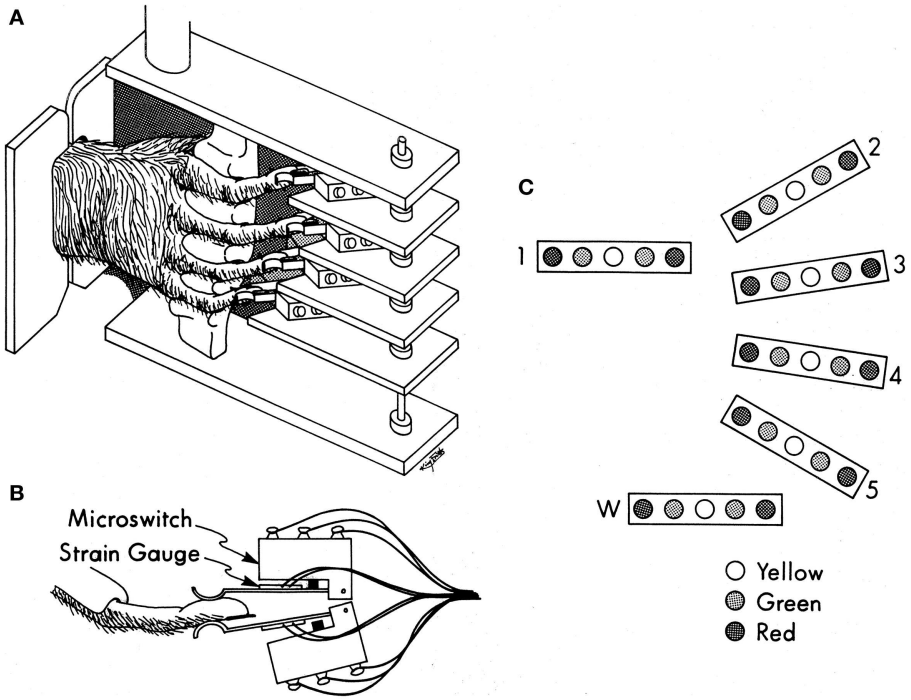

FIGURE 1 | Manipulandum and display for the individuated finger and wrist movement task. **(A)** Pistol-grip manipulandum. **(B)** Overhead view of a monkey's finger between two microswitches with semiconductor strain gages mounted on the lever-arm of each microswitch. **(C)** Display of LEDs used to instruct movements (red) and to inform the monkey for which fingers both switches were open (yellow), or if not, then which one was closed (green). Reproduced with permission from Ref. (22).

and wrist movements. During each recording session, two data acquisition interfaces were used to store data to disk on two host PCs, which also provided scrolling displays of all neuron, kinematic and EMG recordings (Power1401 interface, Spike2 software, Cambridge Electronic Design, UK). The same neuron data and behavioral event marker codes were stored in parallel in these two data streams, while the six kinematic signals were stored together on one system along with four EMG channels, and the remaining EMGs were stored on the other system. A third data acquisition interface and host PC running AVE software (courtesy Shupe, Fetz, and Cheney) were used concurrently to form initial on-line averages of rectified EMG for each channel using data segments extending ± 50 ms from the time of all neuron spikes.

DATA ANALYSIS

Principal component analysis

If we consider each original element (here the motion of each of the five digits and of the wrist) as a dimension in an abstract Euclidean space with orthogonal axes, we can consider our data (here the simultaneous positions of the five digits and the wrist at each time step) as a cloud of points in the six-dimensional space. If some of the original elements are correlated, then there will be a direction in this space that accounts for their simultaneous, correlated variation. PCA can be thought of as a translation of the origin and a rotation of the orthogonal axes such that as much of variance in the data points as possible lies along a single axis, which then is defined as that of the first PC (PC1) (10). A unit vector that points in the direction of this new axis is termed the eigenvector of PC1. A second orthogonal axis (PC2) will be found that accounts for as much of the remaining variance as possible, and so forth for as many PCs as there are original dimensions. The orthogonal PC axes thus are another orthogonal coordinate system (a basis) for viewing the same data. Just as a single data point can be considered to have a projection on each of the original axes, so the same data point can be considered to have a projection on each of the PC axes (in the direction of each of the eigenvectors). And as successive points progress in a time series, their projections on both the original axes and on the PC axes progress as time series.

Two important differences exist, however, between the original axes and the PC axes: first, whereas projections of the data along the original dimensions may be correlated, projections of the data in the directions of the PC eigenvectors are uncorrelated. And second, whereas the original elements may each have any amount of variance, the PCs are rank-ordered according to the fraction of the total variance accounted for by each, with PC1 accounting for the most variance and progressively higher-order PCs accounting for progressively less variance. For purposes of identifying synergies and thereby reducing dimensions, low-order PCs are most likely to represent meaningful synergies while high-order PCs that account for little variance can be considered to be "noise" and disregarded.

For the present study, the kinematic data representing the flexion/extension position of each digit and of the wrist was normalized from -1 (greatest extension achieved by that digit) to $+1$ (greatest flexion achieved by that digit) across each recording

session, and downsampled to 200 Hz. PCA performed on these normalized, six-dimensional kinematic data from each recording session then resulted in six PC eigenvectors (the translated and rotated basis of orthonormal unit vectors) rank-ordered according to the variance accounted for by each, and the temporal weighting of each eigenvector as a function of time throughout the recording session.

Cross-correlation of neuron firing rate with kinematic variables

To enable cross-correlation of neuron firing rate with kinematic variables, each neuron's spike train was converted to an analog representation of firing rate as a function of time as:

$$y(t) = \begin{cases} (t_{n-1} - t_{n-2})^{-1}, & t - t_{n-1} < t_{n-1} - t_{n-2} \\ (t - t_{n-1})^{-1}, & t - t_{n-1} \geq t_{n-1} - t_{n-2} \end{cases}$$

where $y(t)$ is the estimate of the instantaneous firing rate at time t, t_{n-1} is the time of the most recent spike preceding time t, and t_{n-2} is the time of the spike preceding t_{n-1}. Hence at each 5 ms time step, t, the time elapsed since the most recent spike, $t - t_{n-1}$, was compared to the interval between the two most recent spikes, $t_{n-2} - t_{n-1}$. If the time elapsed was less than the most recent inter-spike interval, then the instantaneous frequency was set to the inverse of this interval. If the time elapsed was greater than or equal to the most recent inter-spike interval, then the instantaneous frequency was set to the inverse of the interval between the most recent spike and the current time, providing a gradual decay of instantaneous frequency until the occurrence of the next spike.

We then performed cross-correlation of each neuron's instantaneous firing rate against each of the kinematic variables – both the six original digit and wrist positions and their six PCs – for leads and lags up to ±500 ms. Prior to cross-correlation, each signal was mean-zeroed and normalized such that the auto-covariance at zero lag was 1. Each cross-correlation was performed using data over the entire duration of the recording, which in monkey C averaged 777 ± 228 s (mean ± SD; range: 370–1550 s) and in monkey G averaged 690 ± 273 s (range: 178–1515 s).

RESULTS

The present data include 49 single-neuron recording sessions made during 38 daily microelectrode penetrations in monkey C, and 155 single-neuron recording sessions made during 83 microelectrode penetrations in monkey G (24, 25).

PRINCIPAL COMPONENTS OF INDIVIDUATED FINGER AND WRIST MOVEMENTS

Principal component analysis was performed on the kinematic data from each recording separately. **Figure 2** shows the cumulative variance accounted for as the number of PCs included in rank order increased from 1 to 6. Each point here represents the mean across all sessions from a given monkey. In both monkeys, PC1 accounted for approximately 50% of the variance, and the first three PCs together accounted for approximately 85% of the variance. Consistent with other studies that have applied PCA to the hand movements of both humans and non-human primates, a few low-order PCs thus accounted for the large majority of the variance in the present individuated finger and wrist movements.

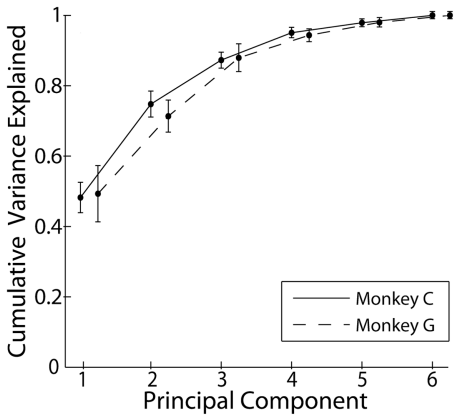

FIGURE 2 | Cumulative variance explained by the rank-ordered principal components. Each point represents the mean across all recordings each monkey. Error bars indicate 1 SD.

The six eigenvectors derived by PCA are illustrated for four selected sessions from each monkey in **Figure 3**. Within each frame, the eigenvector for a given PC (row) in a given session (column) is shown as a bar graph of its components along the original digit and wrist dimensions. In some cases, the patterns of correlated motion represented by a given PC changed rank order, indicating session to session differences in the relative amount of variance explained by the different patterns (black arrows in **Figure 3**). But on the whole, inspection of these data suggested considerable consistency from session to session and from monkey to monkey.

To examine the consistency of the patterns identified by PCA across all recording sessions more objectively, we performed average-linkage cluster analysis on all six eigenvectors from all sessions, using 1 minus the absolute value of the dot product between eigenvectors as a distance measure. Because the dot product between two unit vectors will be 1 if they point in the same direction and −1 if they point in exactly opposite directions, two eigenvectors that point along the same line in the six-dimensional space will have a distance measure of 0, and two eigenvectors that are orthogonal to one another (dot product of 0) will have a distance measure of 1.

Initially, this cluster analysis was performed on all the sessions from each monkey separately. **Figure 4** illustrates the results, with a dendrogram above, a distance matrix below, and color bands along the margins of the distance matrix that show which rank-ordered PCs from different sessions were grouped together by the clustering process. Although our clustering method did not specify the number of groups expected, in each monkey six major groups of similar eigenvectors resulted, evident in the distance matrix as six dark regions of similar size along the main diagonal.

We therefore defined six kinematic synergies in each monkey by dividing the clustered eigenvectors into six groups of equal size, as illustrated by lines drawn on each distance matrix to create an evenly spaced, 6 × 6 square grid. If the eigenvectors had clustered into six perfectly distinct groups, with one eigenvector from each session in each group, then the six large dark regions along the

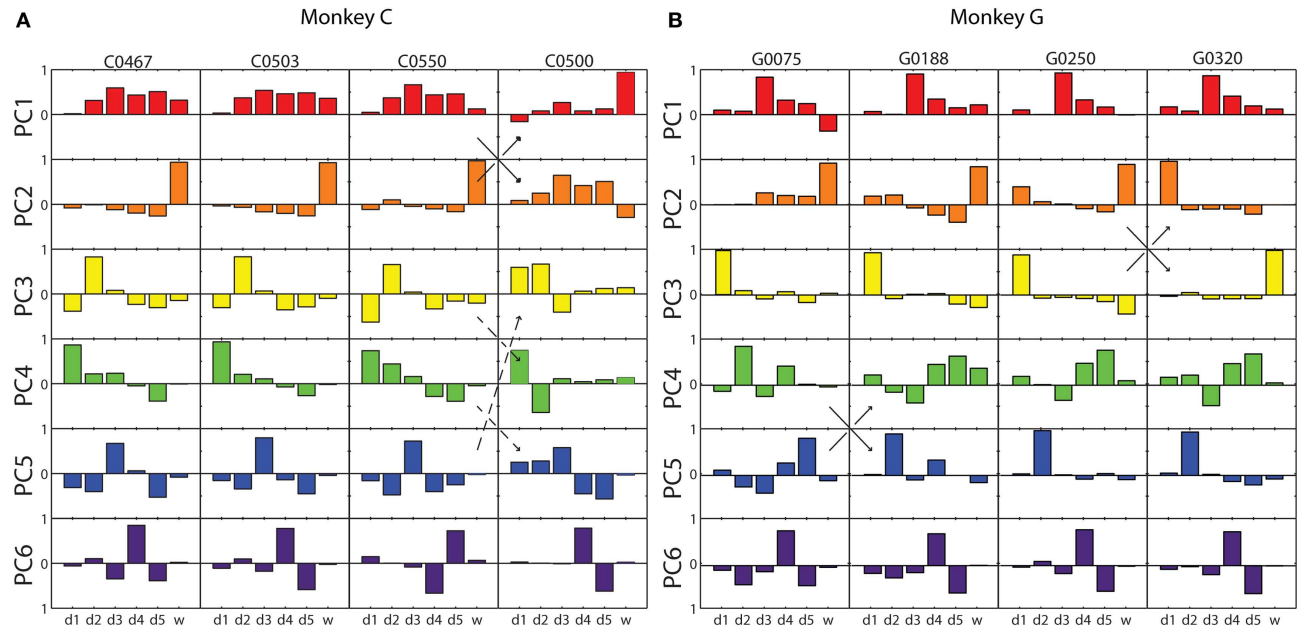

FIGURE 3 | Eigenvector components for each principal component in four illustrative sessions from each monkey. **(A)** Monkey C; **(B)** Monkey G. Each of the eight columns displays the components of the six PC eigenvectors as a separate bar graph in each row, from PC1 (red) at the top to PC6 (purple) at the bottom, from a single session. Within each bar graph, the six bars represent the six components of the eigenvector projected onto each of the original six DoF axes, from d1 through w. Solid arrows indicate instances in which two similar eigenvectors swapped rank order reflecting that one accounted for somewhat more variance in one session, whereas the other accounted for more variance in the other session. Dashed arrows indicate an instance in which the composition of three eigenvectors changed between two sessions.

main diagonal would have been perfectly delimited by these lines. While less than perfect, we felt that the borders of the dark regions were close enough to the squares delimited along the main diagonal for us to consider that the lines delimited six different kinematic synergies that were relatively consistent in each monkey. We refer to these six kinematic synergies as S1–S6.

To visualize each kinematic synergy, we vector-averaged all the eigenvectors assigned to a given synergy. **Figures 5A,B** show these averaged eigenvectors for each of the six kinematic synergies derived from the cluster analysis of the data from each monkey, C and G, respectively. In addition, we pooled the eigenvectors from the cluster analysis of both monkeys' sessions and repeated the cluster analysis. Here, we again divided the distance matrix into an evenly spaced, 6×6 square grid (not illustrated), and vector-averaged the eigenvectors in each square along the main diagonal to define synergies for all sessions from both monkeys considered together. These average synergies across both monkeys are shown in **Figure 5C**.

The first synergy, S1, was characterized by motion of digits 3, 4, and 5 in the same direction, with d3 moving the most. In monkey C, S1 also included some motion of d2 and d6 in the same direction. S2 was dominated by movement of the wrist, d6. S3 was dominated by movement of the thumb, d1, with slight movement of d5 in the opposite direction. In monkey C, S3 also included some motion of d2 and d3 in the same direction as d1. S4 consisted primarily of motion of d2, in monkey C also including lesser motion of d4 and d5 in the opposite direction. S5 can be characterized as motion of d3 and d5 in opposite directions, in monkey G including motion of d4 in the same direction as d5. S6 comprises motion of d4 in one direction with motion of d3 and d5 in the opposite direction. The six average kinematic synergies found in the two monkeys thus were similar.

CROSS-CORRELATION OF M1 NEURON FIRING RATE AND MOVEMENT KINEMATICS

For each M1 neuron, we preformed cross-correlation of its firing rate separately against the simultaneously recorded position of each digit and of the wrist, as well as against the temporal weighting of each of the six PCs derived from that simultaneous position data. **Figure 6** shows the 12 resulting cross-correlation functions for neuron C0485, selected because it had relatively strong cross-correlations with finger kinematics. The cross-correlation functions with digits 1–6 in the left column show that this neuron correlated inversely with motion of digits 2, 3, 4, and 5, indicating that firing rate increased with extension of the digits. Because negative correlations here are just as meaningful as positive correlations, we focused on absolute values. The largest absolute value of any of these six cross-correlations ($\rho = -0.34$) occurred with d3 at a lead of -76 ms (indicated by the circle). The cross-correlations with the six PCs are shown in the right column. Here, the largest absolute value of any of the six cross-correlations ($\rho = -0.32$) occurred with PC1 at a lead of -84 ms (circle). The maximal absolute cross-correlation (MAXC) between the firing rate of this neuron and any of the digits thus was similar in both magnitude and timing to the MAXC obtained with any of the PCs.

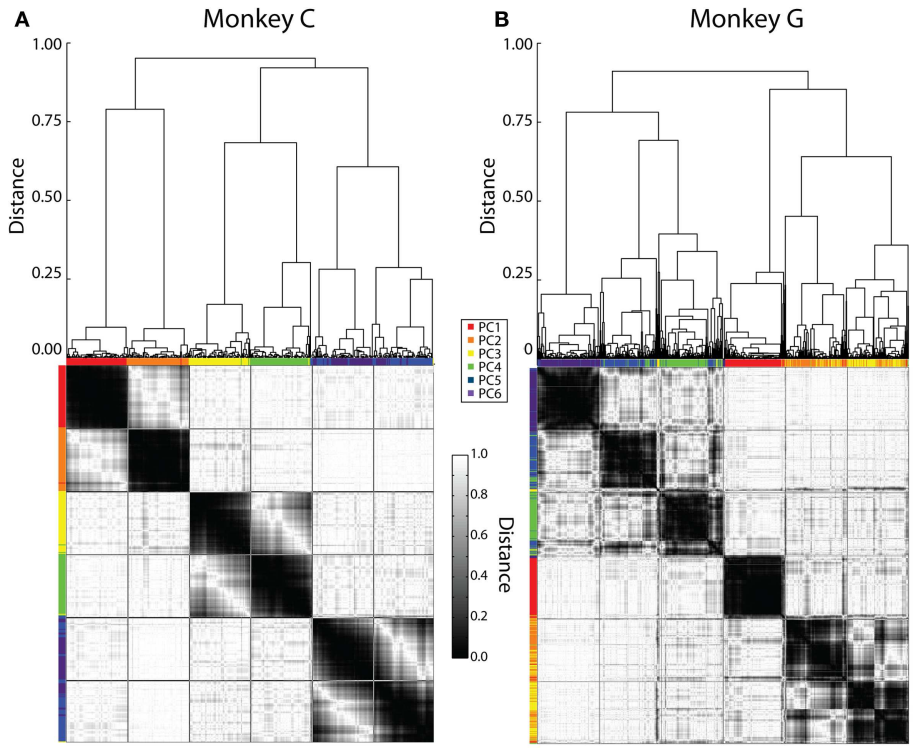

FIGURE 4 | Clustering of PC eigenvectors. Shown here are the results of separate clustering for monkey C **(A)** and monkey G **(B)**. The resulting dendrogram is shown above and the distance matrix below. Colored ticks along the top and left sides of the distance matrix indicate the original PC rank-order from PC1 (red) to PC6 (purple) of each eigenvector represented by each column or row of the distance matrix. The distance matrix is symmetric about its main diagonal. The six major dark squares along this diagonal indicate that six relatively consistent kinematic synergies were present across sessions from both monkeys. Lines have been drawn on the distance matrix dividing both the rows and the columns into six groups of equal number.

COMPARING M1 NEURON CROSS-CORRELATIONS WITH ORIGINAL KINEMATICS VERSUS KINEMATIC SYNERGIES

We reasoned that if an M1 neuron represented one of the kinematic synergies identified by PCA, then the cross-correlation of its firing rate with that synergy should be stronger than its cross-correlation with any of the individual digits or the wrist. For each monkey, we therefore plotted each M1 neuron's MAXC with any of the digits against its MAXC with any of the average synergies. The resulting scatterplots are shown separately for the two monkeys in **Figure 7**. Here, values along the ordinate represent MAXC values obtained with the kinematic data projected along the six averaged eigenvectors shown in **Figures 5A,B**. Similar results were obtained, however, using the projection along the PC eigenvectors from each neuron's individual recording session (as illustrated in **Figure 3**). Across the population of neurons from each monkey, MAXC values with the digits and with the synergies were correlated strongly with one another (using averaged synergies: monkey C, $\rho = 0.94$, $p < 10^{-22}$; monkey G, $\rho = 0.93$, $p < 10^{-69}$; using individual session PCs: monkey C, $\rho = 0.94$, $p < 10^{-22}$; monkey G, $\rho = 0.87$, $p < 10^{-48}$). Some points fell above the line of unity slope (solid line), indicating that for these neurons the MAXC with one of the synergies was greater than the MAXC with any of the digits. But paired testing showed that most points fell below the line of unity slope, indicating that for most M1 neurons the MAXC with one of the digits was greater than the MAXC with any of the synergies (synergies: monkey C, $z = 3.01$, $p < 10^{-2}$; monkey G, $z = 4.02$, $p < 10^{-4}$; PCs: monkey C, $z = 1.99$, $p < 0.05$; monkey G, $z = 2.17$, $p < 0.05$, Wilcoxon signed rank tests). Furthermore, in each monkey, points representing neurons with higher MAXC values tended to fall farther below the line of unity slope. The line best-fitting the data in each monkey (dashed line) had a slope significantly less than 1 ($p < 0.05$; synergies: monkey C, $m = 0.85$; monkey G, $m = 0.80$; PCs: monkey C, $m = 0.84$; monkey G, $m = 0.74$). Overall, rather than correlating more strongly with the kinematic synergies identified by PCA, the firing rates of M1 neurons, particularly those more strongly cross-correlated with finger and wrist kinematics, thus had somewhat stronger cross-correlations with the position of one of the digits or the wrist than with any of the average synergies or individual PCs.

Because some M1 neurons correlated most strongly with a kinematic synergy (points above the line of unity slope) whereas others correlated most strongly with an original DoF (points below the line), we also considered the possibility that the transformation from synergies to the muscle activation needed to drive them might occur at least in part within M1. More specifically, M1 neurons with relatively direct output to spinal motoneuron pools, particularly groups of cortico-motoneuronal (CM) cells with output to a similar subset of muscles, might produce patterns of

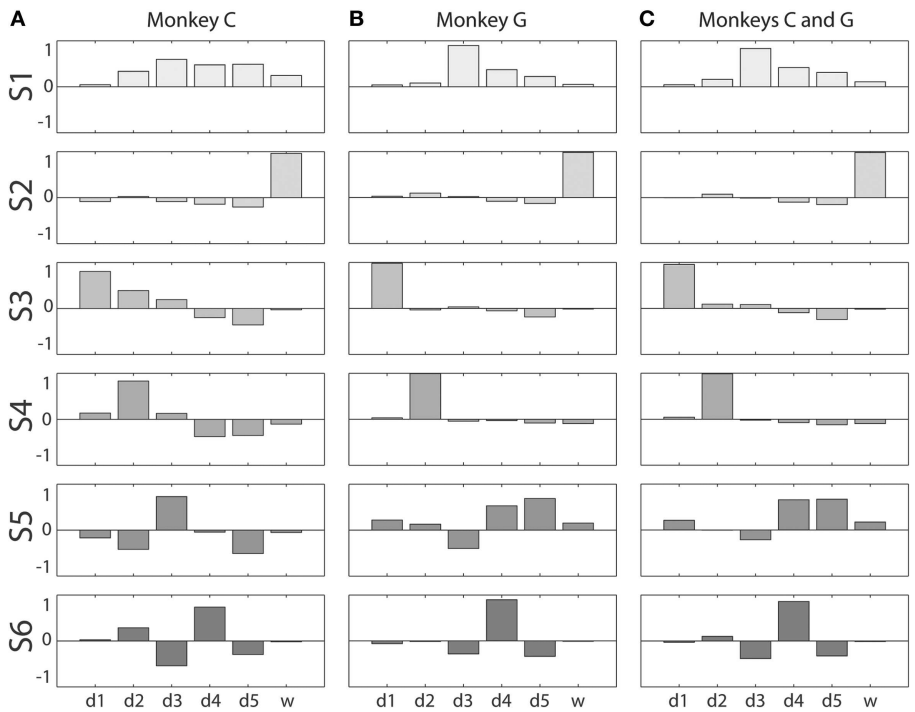

FIGURE 5 | Kinematic synergies. Vector averaging of the eigenvectors clustered into each of the six squares along the main diagonal of the distance matrices of **Figure 4** resulted in the six synergies – S1 through S6 – shown for monkeys C and G in **(A,B)**, respectively. **(C)** shows the six synergies resulting from the same process applied to the eigenvectors from all sessions from both monkeys together. Within each bargraph, the six bars represent the six components of that synergy's eigenvector projected onto each of the six original DoF axes, from d1 through w.

activation in multiple muscles that would facilitate a given synergy (26, 27). If so, then those neurons that had stronger correlations with synergies might be those with relatively direct output to spinal motoneuron pools, whereas those neurons that had stronger correlations with an individual digit or the wrist might be less likely to have relatively direct outputs to muscles.

Each of the present neurons had been tested for such outputs with spike-triggered averaging of rectified EMG activity (24, 25). We classified the spike-triggered average (SpikeTA) effects of each neuron as being pure (consistent with direct, monosynaptic connections to motoneurons), synchrony (including synchronization with other neurons that had connections to the motoneuron pools), mixed (pure and synchrony effects in different muscles), or none. Open shapes in **Figure 7** indicate which neurons had which type of SpikeTA effect. We observed no relationship between the presence or absence of any type of SpikeTA effect in M1 neurons and their correlations with synergies versus original DoFs.

We also examined the distribution of MAXCs over the digits and kinematic synergies. The upper marginal histograms of **Figure 8** show that in each monkey, the largest number of M1 neurons had their MAXC with d1, the thumb, and the next largest number with d6, the wrist. This is notable because in previous work the thumb and wrist have been found to exhibit higher degrees of independence than the other digits (22). The two monkeys did not show similar distributions of MAXCs across the synergies, however, as shown by the rightward marginal histograms in **Figure 8**. In monkey C, the largest number of neurons was best correlated with S2, which was dominated by d6, whereas in monkey G, the largest number of neurons was best correlated with S6, consisting primarily of motion in d4 with oppositely directed motion in d3 and d5 (Using individual session PCs, neurons in monkey C also were most often best correlated with PC2, and in monkey G with PC6.). Considering each neuron's MAXC with a digit and its MAXC with a PC simultaneously, the two-dimensional histograms of **Figure 8** show that in monkey C, the largest number of neurons was best correlated with d6 and S2 (dominated by d6 motion), and in monkey G the largest number was best correlated with d1 and S3 (dominated by d1 motion).

DISCUSSION

KINEMATIC SYNERGIES OF THE HAND IN HUMANS AND MONKEYS

Previous studies have identified kinematic synergies of human hand motion by applying PCA to joint angles monitored during various activities, including grasping (1, 2, 11, 28), haptic exploration (4), activities of daily living (3), and finger spelling (29). PCA also has been applied to the kinematics of grasping in non-human primates (12, 14, 15). To our knowledge, none of the many other possible linear and non-linear mathematical methods for dimensionality reduction have been applied to hand kinematics. In preliminary studies, we examined the synergies identified in the present individuated finger and wrist movement task by independent component analysis (a linear method of dimensionality reduction that does not require the new basis to be orthogonal), but we found that for the present data the resulting independent

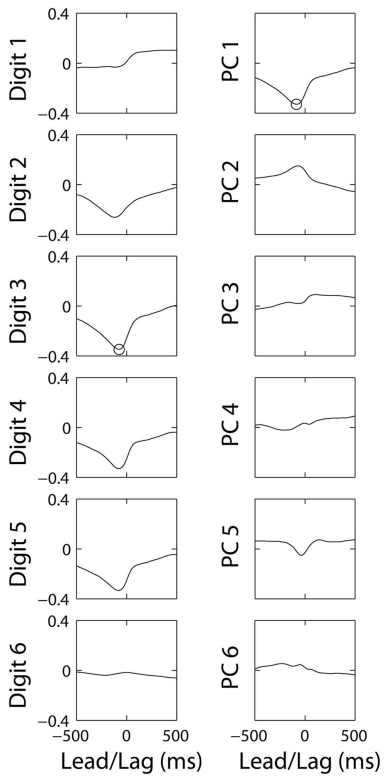

FIGURE 6 | Cross-correlations of the same M1 neuron's firing rate with each original DoF and with each of the PCs from the same session. The correlation coefficient (ordinate) is plotted as a function of the lead or lag (abscissa). Negative times represent those at which discharge of the neuron led the kinematic variable. Circles indicate the maximal absolute cross correlation for this neuron with any of the digits (Digit 3) and with any of the PCs (PC1).

components were not substantially different from the six original DoFs, i.e., the five individual digits and the wrist. For these reasons, the present study focused on the kinematic synergies identified with PCA. We found that these kinematic synergies were remarkably consistent across sessions and between monkeys. Nevertheless, we recognize that future studies using other approaches might better identify kinematic synergies used by the nervous system.

The studies cited above generally have found that: (i) a small number of the lowest order PCs account for a substantial majority of the variance in the motion of multiple joints; (ii) the synergies identified by PCA generally were similar from one subject to another; and (iii) the lowest order PCs represent a fundamental opening and closing of the hand involving similar motion in the thumb and all four fingers. In the present study, we likewise found that (i) the first PC accounted for ~50% of the variance, and the first three PCs for ~85%; (ii) the synergies identified by PCA were relatively consistent across sessions and between monkeys, and (iii) the first synergy (typically PC1) represented motion of the fingers in the same direction, albeit to different degrees in the two monkeys. In these three respects, the synergies identified here with PCA are similar to those identified in previous studies, although the present monkeys were instructed to move only one finger at a time insofar as possible.

We examined the structure of the kinematic synergies identified by PCA (**Figure 5**). Whereas S1 comprised simultaneous motion of the fingers all in the same direction, S2 in both monkeys consisted almost entirely of motion at the wrist, indicating that the wrist often moved relatively independently of the digits. S3 and S4, particularly in monkey G, likewise consisted almost entirely of motion of the thumb or of the index finger, respectively, indicating that each of these two digits also moved relatively independently. Compared to S3 and S4 in monkey G, S3 and S4 in monkey C included some motion of other radial digits (d1, d2, and/or d3) in the same direction, with motion of the ulnar digits (d4 and d5) in the opposite direction. In both monkeys, S5 and S6 represented simultaneous, oppositely directed motion in even closer subsets of the fingers. S5 comprised motion of the middle finger in one direction, with motion of other digits, most consistently the little finger, in the opposite direction. S6 comprised motion of the ring finger in one direction, with motion of the middle and little fingers in the opposite direction. In sum, whereas S1 comprised motion of multiple digits in the same direction, S2, S3, and S4 consisted of relatively independent motion of the wrist, thumb, and index finger, respectively, particularly in monkey G with some degree of radio-ulnar "contrast" (i.e., oppositely directed motion) in monkey C, and S5 and S6 consisted of increasingly close contrast among the more ulnar digits.

These features of the synergies identified with PCA may be related to findings on the relative independence of the digits and the structure of muscles in the macaque hand. Our previous studies of the individuated finger and wrist movement task performed by different monkeys demonstrated that the thumb, index finger, and wrist moved with more independence than the more ulnar digits – the middle, ring, and little fingers (22). Instructed movements of these ulnar digits generally involved motion of them all in the same direction (e.g., S1), with slightly more motion of the instructed digit, as might be created by the combination of S1 with one or more of the higher-order, "contrast" synergies. The combination of S1 and S6, for example, could produce more motion of the ring finger (d4) than other digits.

None of the kinematic synergies identified with PCA appeared to correspond to the activation of a particular muscle, however. Although S1 might be thought to reflect the action of the extrinsic multitendoned finger muscles – FDP, FDS, and EDC – in macaques FDP consists of two major compartments: FDPr, which exerts the most tension on d2, less on d3, and still less on d4; and FDPu, which exerts the most tension on d5 and d4 and less on d3 (30, 31). And the largest part of FDS acts on d3 and d4. Indeed, prior studies have indicated that flexion of each finger is produced by a different combination of activity in FDPr, FDPu, and FDS (32). S2 might be thought to reflect the action of muscles that act only across the wrist – FCR, FCU, ECR, and ECU – but ED23, EDC, and ED45, all are activated during wrist extension along with ECR and ECU. And higher-order synergies that include motion of some digits in one direction with motion of other digits in the opposite direction would have to be produced by coordination of forces acting on

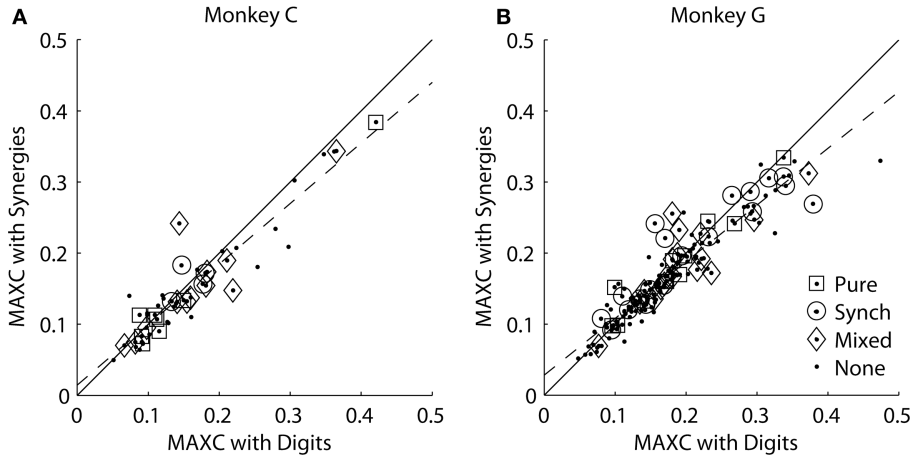

FIGURE 7 | Maximal absolute cross-correlations with original digit DoFs versus kinematic synergies in each monkey. **(A)** Monkey C. **(B)** Monkey G. In each scatterplot, each point represents an M1 neuron plotted at the coordinates of its MAXC with any digit (abscissa) versus its MAXC with any of the average kinematic synergies from that monkey (ordinate). The solid line has a slope of 1.0, and the dashed line is the linear regression best fit to the data. Open symbols indicate the points representing neurons that had different types of effects in spike-triggered averages of EMG as indicated by the legend.

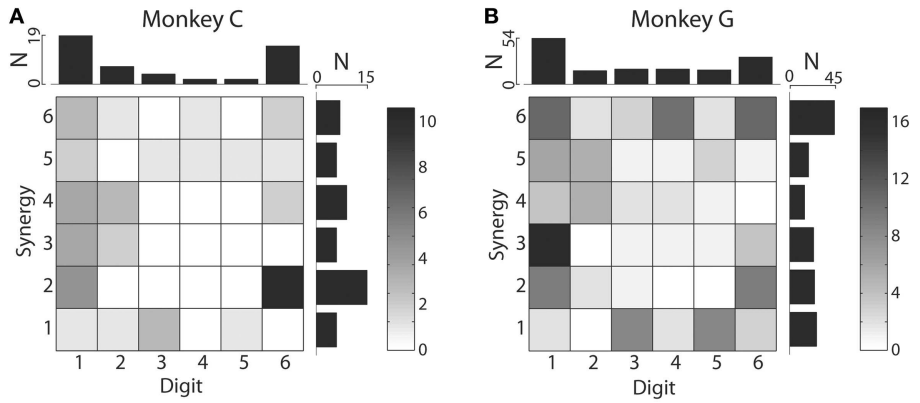

FIGURE 8 | Distributions of digits that cross-correlated most strongly with each original digit DoF and with each PC. Two-dimensional histograms shown as grayscale matrices indicate the number of M1 neurons that had their MAXC with each joint DoF and with each PC in monkey C **(A)** and monkey G **(B)**. Note that the marginal histograms above shown that in both monkeys, the largest number of M1 neurons were best correlated with the thumb and then with the wrist, whereas the synergy with which the largest number of M1 neurons were best correlated differed between monkeys: S2 for monkey C, S6 for monkey G.

different digits in different directions. Few if any of the kinematic synergies identified by PCA, thus appear to represent the action of single muscles. Rather, each kinematic synergy is likely to involve coordinated activation in multiple muscles.

To some extent, the kinematic synergies identified here may reflect the particular mechanical constraints of the present individuated finger and wrist movement task (**Figure 1**) in which the instructed digit was required to move more than others, not only in flexion but also in extension. More ethologically natural human hand movements monitored during haptic exploration also showed synergies dominated by the motion of the thumb or the index finger, not unlike the present S3 and S4 (4) (S2 and S3 in their **Figure 3**). Although the same study showed that individuated movements of each digit could be reconstructed from the synergies identified, neither this nor other previous studies of kinematic synergies have elicited individuated movements of the middle, ring, and little fingers. Hence the "contrasts" between these digits represented by the present synergies S5 and S6, may not have appeared in more ethologically natural hand movements.

REPRESENTATION OF KINEMATIC SYNERGIES IN THE PRIMARY MOTOR CORTEX

Overall, M1 neurons that had progressively stronger correlations with finger and wrist kinematics had stronger MAXCs with both synergies (or PCs) and original DoFs. If an M1 neuron specifically represented one of the kinematic synergies identified by

PCA, then its firing rate would be expected to correlate more strongly with some synergy than with the motion of any of the individual digits or wrist. A minority of M1 neurons in each monkey – those represented by points lying above the line of unity slope in **Figure 7** – in fact did show MAXCs with one of the synergies larger than with any of the individual digits or wrist. The majority of M1 neurons, however, showed a stronger correlation with the motion of an individual digit or the wrist than with any PC or kinematic synergy. Furthermore, in each monkey the M1 neurons that had progressively stronger correlations with kinematics showed particularly strong correlations with an original DoF rather than with a synergy. So although some M1 neurons might represent kinematic synergies in the present task, most M1 neurons represent these kinematic synergies no better than the original DoFs.

SYNERGIES AND NEURAL CONTROL OF MOVEMENT

Although we found little evidence that kinematic synergies are represented by M1 neurons more strongly than the original digit and wrist DoFs, our findings do not exclude a number of other possible ways in which synergies might be used by the CNS in controlling movement of the wrist, hand and fingers. First, methods other than PCA, either linear (e.g., independent component analysis), or non-linear (e.g., Isomap), may be necessary to identify kinematic synergies used by the nervous system. Second, although here we used digit and wrist positions as the original DoFs, synergies of other kinematic, and/or dynamic DoFs – such as velocity (15), acceleration, or force – might be represented more strongly in M1 neuron firing. Alternatively, rather than working in the domain of kinematic and/or dynamic synergies, the nervous system instead may control muscle synergies.

Much of the basic generation of such muscle synergies might occur at subcortical levels, including the PMRF and the spinal gray matter. Neurons in the intermediate zone of the lumbar spinal gray of the spinalized frog provide premotor drive for a limited number of muscular synergies (16), and rostral midbrain transection in the frog leaves most natural muscular synergies intact (19). These observations indicate that certain muscular synergies are mediated in the spinal cord. In monkeys, outputs from the PMRF produce relatively stereotyped facilitation of ipsilateral flexors and suppression of ipsilateral extensors (17, 33, 34), including hand muscles (35, 36), and PMRF neurons participate in visually targeted reaching movements (37, 38). Muscle synergies also have been identified during reach-to-grasp movements in both monkeys (20, 39) and humans (40–42), and remain largely unchanged after stroke damages the frontal cortex (18). Together, these studies suggest that in primates, the PMRF may generate important muscular synergies. M1 neurons, acting on subcortical centers, on spinal interneurons, and on the motoneuron pools themselves, then might sculpt the output to muscles so as to produce a wide variety of individuated movements (43).

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Long-term viral brain-derived neurotrophic factor delivery promotes spasticity in rats with a cervical spinal cord hemisection

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We have recently reported that rats with complete thoracic spinal cord injury (SCI) that received a combinatorial treatment, including viral brain-derived neurotrophic factor (BDNF) delivery in the spinal cord, not only showed enhanced axonal regeneration, but also deterioration of hind-limb motor function. By demonstrating that BDNF over-expression can trigger spasticity-like symptoms in a rat model of sacral SCI, we proposed a causal relationship between the observed spasticity-like symptoms (i.e., resistance to passive range of motion) and the over-expression of BDNF. The current study was originally designed to evaluate a comparable combined treatment for cervical SCI in the rat to improve motor recovery. Once again we found similar signs of spasticity involving clenching of the paws and wrist flexion. This finding changed the focus of the study and, we then explored whether this spasticity-like symptom is directly related to the over-expression of BDNF by administering a BDNF antagonist. Using electromyographic measurements we showed that this treatment gradually diminished the resistance to overcome forelimb flexion in an acute experiment. Thus, we conclude that neuro-excitatory effects of chronic BDNF delivery together with diminished descending control after SCI can result in adverse effects.

Keywords: spinal cord injury, BDNF, combined treatment, TrkB-Fc, EMG

INTRODUCTION

Axonal regeneration in the adult mammalian central nervous system is inhibited by numerous factors (1) impeding the development of effective treatments for brain or spinal cord injuries (SCI). Consequently, it is likely that repair of the injured spinal cord by axonal regeneration and plasticity will require a combined treatment approach. Several combinatorial strategies have been pursued with a wide range of outcomes [e.g., Ref. (2–5)]. These experiments are not only technically demanding, but also challenging in the interpretation of the results, considering the unpredictable effects of promoting neurite outgrowth and possible interactions between treatment components (6, 7).

One prominent molecule that has been the subject of many studies in spinal cord repair is brain-derived neurotrophic factor (BDNF). BDNF not only has neuro-protective, regeneration, and plasticity promoting effects but also neuro-excitatory properties and binds with high affinity to the TrkB receptor and with lower affinity to p75 [reviewed in Ref. (8)]. As such, BDNF is a prominent candidate for combined treatment approaches [e.g., Ref. (6, 9, 10)]. However, due to the broad range of its effects, the consequences of BDNF delivery can be complex. For example, we observed that a combinatorial treatment involving BDNF (achieved by direct viral vector injection and bone marrow stromal cell grafts genetically modified to express BDNF) promoted spasticity in rats with a cervical hemisection. Because the combined treatment also promoted axonal regeneration and plasticity, the origin of the observed spasticity remains unclear (11). We could, however, provide an indirect explanation by demonstrating that the over-expression of BDNF could promote the development of hyperreflexia in tail muscles in a sacral model of SCI (11). Yet, it remains unclear whether this effect of BDNF was caused by its neuro-excitatory properties or through an augmentation of neuroplasticity and/or regenerative growth. However, this was not the original question of the current study. We initially set out to test a combined treatment that addressed multiple limitations for neurite growth following cervical SCI. The focus of the analysis shifted when severe spasticity-like symptoms in the injured forelimb became evident, which led us to focus on determining the cause of spasticity. To address this question we administered a BDNF antagonist into the spinal cord of rats that showed spasticity-like symptoms, and these were gradually reduced by this treatment.

MATERIALS AND METHODS

ANIMALS

Adult female Fischer 344 rats (Charles River, 180–220 g) were group housed and kept at 12/12 h light/dark cycle with *ad libitum* water. The study was approved by the Animal Care and Use Committee for Health Sciences of the University of Alberta, and complies with the guidelines of the Canadian Council for Animal Care.

LESION SURGERY AND TREATMENT

Rats were anesthetized (using Hypnorm 0.16 mg/kg; Vetapharma, Leeds, UK; and Midazolam 2.5 mg/kg; Sandoz, Canada; diluted in sterile water) and mounted into a stereotaxic frame (Kopf Instruments), and the spinal cord between C5 and C6 was exposed by performing a laminectomy of half of the C5 segment. The spinal cord was lesioned using a microsuction pipette and a spring scissor. A lateral hemisection lesion was performed ipsilateral to the preferred paw as determined by a forelimb reaching task. A small gap was created to ensure lesion completeness and provide space for the cell graft. The dura was sealed with a thin agarose film (Sigma) and fibrin glue (Baxter, USA), overlying muscles were sutured and the skin was stapled. Rats were placed on a heating pad until they were awake and received buprenorphine (0.03 mg/kg) as analgesic and saline over the next 2 days.

Preparation of cell grafts to provide a tissue bridge for regenerating axons

Fibroblasts were isolated from skin biopsies of Fischer 344 rats and cultivated in D'MEM/10% FBS with antibiotics. Cells were transduced with retroviral vectors to express BDNF as described by Lu et al. (11, 12) or NT-3 (13) and selected for G418 resistance. BDNF and NT-3 expression was measured *in vitro*, in 24 h cell culture supernatants by ELISA as described (14, 15). BDNF-transduced fibroblasts expressed 27 ng/10^6 cells/24 h, NT-3 transduced cells expressed 134 ng/10^6 cells/24 h. For grafting, BDNF and NT-3 expressing cells were mixed 1:1 and 2–3 μl were injected into the lesion site using a 5-μl Hamilton syringe at a concentration of 2.5×10^4 cells/μl.

scAAV vector preparation and injections

Self-complementary adeno-associated viral vectors (scAAV) were generated as described by Lu et al. (11). In order to attract regenerative growth out of the graft into the caudal spinal cord, BDNF, and NT-3 (and GFP as control) transfected scAAV vectors (1.3 μl/site) were injected 1.25, 2.5, and 4 mm caudal to the lesion on the side ipsilateral to the lesion through pulled glass capillaries using a Picospritzer II similar to Lu et al. (11). The injection volume was divided over a depth of 1 and 1.5 mm.

One week post-lesion surgery, cell grafts and viral vector injection surgeries were performed in all lesioned animals in the following groups:

Group 1 is a CONTROL group used to demonstrate the ability of self-complementary adeno-associated virus (scAAV) vectors to successfully infect spinal neurons. This group did not receive a cell graft, only PBS, followed by injections of 4.3×10^{11} vg/ml scAAV vectors expressing Green Fluorescent Protein (scAAV2-GFP) at 1.25, 2.5, and 4 mm caudal to the lesion; $n = 16$.

Group 2, scAAV-BDNF group received an injection of PBS in the lesion site, followed by 4.3×10^{11} vg/ml scAAV-BDNF injections at 1.25, 2.5, and 4 mm caudal to the lesion to serve as trophic support for regenerating fibers; $n = 5$.

Group 3, the GRAFT/BDNF/NT-3 or FULL treatment group, received a fibroblast cell graft (expressing BDNF and NT-3) into the lesion site to provide a permissive cellular matrix that allows axonal growth, and a trophic stimulus to stimulate axonal growth. This group also received injections of a mixture of scAAV2 BDNF (f.c. 4.3×10^{11} vg/ml), scAAV2-NT-3 (f.c. 4.3×10^{11} vg/ml), and scAAV2-GFP (f.c. 4×10^{10} vg/ml) mixed with 20 U/ml ChABC (Seigaku, USA) caudal to the lesion (1.25, 2.5, and 4 mm). The neurotrophic mixture is intended to provide trophic stimulus beyond the lesion and ChABC degrades growth inhibitory proteoglycans in the scar tissue (3). In addition, this group also received a subcutaneous injection of the phosphodiesterase (PDE) inhibitor, Rolipram, (AG Scientific, San Diego, CA, USA; 3 ml of 3 mg/kg in 2% DMSO and saline) once per day for 14 days following the lesion. This drug elevates cAMP levels in the CNS and augments regeneration-related gene expression in injured neurons (16); $n = 18$.

Group 4, the GRAFT/scAAV-NT-3 group received a fibroblast cell graft expressing BDNF and NT-3 into the lesion site, injections of scAAV expressing NT-3 and GFP only, mixed with ChABC caudal to the lesion and subcutaneous injections of rolipram; $n = 7$.

Group 5, the GRAFT/ChABC group received a fibroblast cell graft expressing BDNF and NT-3 into the lesion site and ChABC injections 1.25, 2.5, and 4 mm caudal to the lesion, followed by daily rolipram administration; $n = 5$.

BEHAVIORAL TESTING

Single-pellet grasping task

This test was performed as described previously (17, 18). In order to motivate rats to grasp for sugar pellets (45 mg, banana flavor; TestDiet, Richmond, CA, USA) in the task, restricted amounts of food were allowed directly after the daily training session (10 g/rat/day). A transparent Plexiglas chamber (30 cm × 36 cm × 30 cm) with a narrow opening in the front wall and an attached shelf outside (2 cm distance from the inside of the front wall, 3 cm above the floor) was used. Pellets were placed at a distance such that the rats had to grasp them with their preferred forelimb and could not reach them with their tongue. The preferred paw was determined for each rat during the first few training sessions. Successful grasps were scored over a 10-min period only when a rat was able to grasp the presented pellet, bring it to its mouth and eat it. Pre-operative baseline values were obtained after a 4-week training period (five times per week, 10 min per rat). Post-operative rehabilitative training of the injured forelimb started 1 week after graft implantation surgery (2 weeks post-lesion).

Forelimb placement

To further assess forelimb function, all rats were scored over a 4-min period in a Plexiglas open field according to Martinez et al. (19), with scores ranging from 0 (no function) to 20 (no deficit). Behavioral components were categorized from global fore- and hind-limb movements to antero-posterior coordination, which included weight support, fine distal positioning, and stepping abilities. Nine weeks post-lesion, asymmetries in spontaneous forelimb use were evaluated by placing individual rats into a clear Plexiglas cylinder (24 cm high/19 cm inner diameter). A mirror was placed underneath the cylinder at an angle to facilitate the videotaping of the rat's vertical exploratory activity in the cylinder (**Figure 1**). Ten rearing movements were recorded in each test session. Each forepaw placement on the wall of the cylinder was scored during a rearing movement. If the affected forepaw was

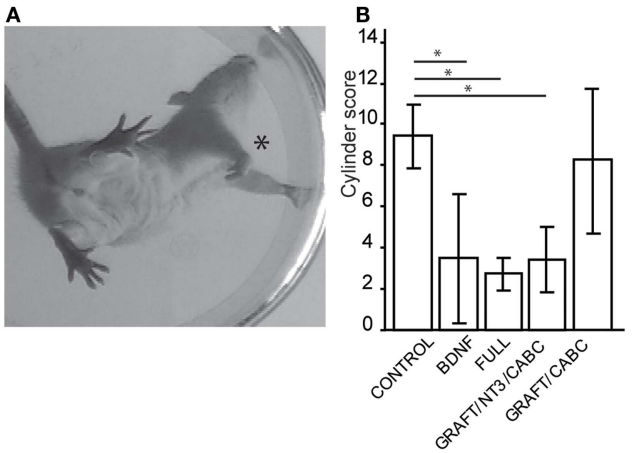

FIGURE 1 | Treatments involving BDNF promoted exaggerated wrist flexion and affected forelimb use. Signs of wrist flexion became apparent as early as 2 weeks after treatment **(A)**. By 9 weeks post-injury the cylinder exploration task indicated that animals in the FULL treatment group and the scAAV-BDNF only group had significantly reduced use of the injured forelimb (*) when compared to control animals **(B)**. Error bars show average ± SE; $*p < 0.05$, $**p < 0.005$, $***p < 0.0005$.

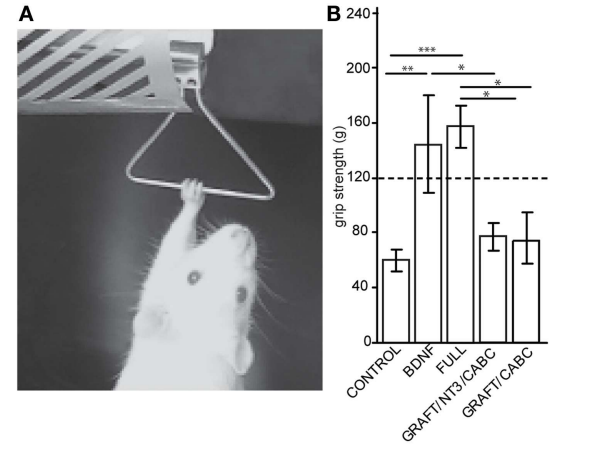

FIGURE 2 | Brain-derived neurotrophic factor expression increases wrist flexion: using an apparatus designed to measure grip strength (A) we compared measurements between control animals and the rats receiving scAAV-BDNF only or the FULL treatment (B). When compared with the control group, a significant increase in "grip" strength was observed in both the scAAV-BDNF and the FULL treatment groups at 9 weeks post treatment. The dashed line indicates a normal grip strength value. Error bars show average ± SE; $**p < 0.01$, $***p < 0.001$.

plantar on contact with the wall of the cylinder, the number of contacts was multiplied by two, if only the dorsal surface of the paw was placed on the wall, the number of contacts was multiplied by one and if the paw did not contact the wall a score of 0 was assigned, thus allowing for a maximum score of 20.

Grip strength

The grip strength of the forelimbs (or in other words, the force needed to overcome wrist and digit flexion when actively or passively holding on to a bar) was assessed 9 weeks post-lesion by measuring the maximum force (in gram) exerted by each forelimb, with a Grip Strength Meter apparatus (Columbus Instruments, OH, USA, **Figure 2**). For the final values, four readings for both paws were obtained over three different trials and averaged for post-operative analysis.

Sensory test

Forelimb withdrawal latency to a heat stimulus was measured prior to injury (baseline) and 9 weeks post-lesion using methods previously described (20). Briefly, the rats were placed on a glass plate over a light box, and a radiant heat stimulus (Ugo Basile) was applied by aiming a beam of light onto the plantar surface of the paw of each forelimb through the glass plate. The light beam was turned off automatically when the rat lifted the limb, allowing the measurement of time between the start of the light beam and the paw withdrawal. Five minutes were allowed between three trials, baseline values were averaged for each limb and compared to post-injury values.

ELECTROPHYSIOLOGY

Electromyographic (EMG) recordings of flexor muscles of the injured paw were carried out prior to perfusion. Animals were anesthetized with 5% isoflurane gas anesthesia for induction and 1.5% for maintenance of anesthesia and placed into a stereotaxic frame. The spinal cord surrounding the hemisection lesion was exposed. To record EMG responses during manual wrist extension, two electrodes with exposed tips (Teflon coated wire; A-M Systems, Carlsborg, WA, USA) were inserted into wrist flexors of the forelimb ipsilateral to the lesion. The EMG signal was amplified (Grass, Astro-Med Inc., West Warwick, RI, USA), digitized (5 kHz, Digidata 1322A; Axon instruments, Foster City, CA, USA), and filtered (30–300 Hz) as described earlier (17). The muscle responses were recorded under anesthesia before and 10, 20, and 30 min following injection of the TrkB antagonist, TrkB-Fc (1 μl of 0.2 mg/ml; Sigma) or saline into the spinal cord lesion site.

The following animals were selected for EMG analysis: (i) three rats without signs of spasticity (one from the CONTROL group and two from the FULL treatment group) received a TrkB-Fc injection. (ii) Four rats with spasticity (one from the scAAV-BDNF, two from the FULL treatment group and one from GRAFT/NT-3) received a saline injection only. (iii) Ten rats with spasticity (two from scAAV-BDNF and eight from FULL treatment group) received TrkB-Fc injection.

Electromyographic responses were recorded, and the burst duration was measured using Axoscope software (Axoscope 9.0.1.16; Molecular Devices, Sunnyvale, CA, USA). The traces were rectified and the average burst amplitude was quantified and then multiplied by the burst duration using Microsoft Excel.

The animals were euthanized immediately following the EMG recordings with pentobarbital (Euthanyl, Bimeda-MTC; 70/100 mg body weight) and transcardially perfused with phosphate-buffered saline, followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4. The spinal cord was removed, post-fixed in 4% PFA overnight at 4°C and

cryo-protected in 30% sucrose over 2 days. The cervical enlargement encompassing the lesion site (C2–6) was embedded in Tissue Tek (Sakura Finetek USA Inc., Torrance, CA, USA) and frozen in 2-methyl-butane at −60°C.

HISTOLOGY

Spinal cords were cryosectioned in the horizontal plane at 25 μm and mounted in eight series onto Fisherbrand slides (Fisher Scientific, Ottawa, ON, Canada). To evaluate the lesion site, sections were counterstained with 0.1% Cresyl Violet and dehydrated in increasing alcohol concentrations, cleared with xylene, and coverslipped with Permount (Fisher Scientific). Lesion sizes were reconstructed from C2 to C6 spinal cord tissue by analyzing every fourth section through the dorso-ventral plane of the spinal cord by using central canal and gray/white matter interphases as landmarks. From these reconstructions, the percent cross-sectional surface area of the damaged tissue was measured using Image J (NIH) software. Animals with lesions deviating from a complete hemisection by >10% were removed from this study.

Immuno-histochemistry of serotonergic (5-HT) fibers was carried out on every second horizontal section encompassing the lesion site using rabbit anti 5-HT antibody (S5545, Sigma-Aldrich, Oakville, ON, Canada). In brief, following a 10% normal goat serum (NGS, Vector Labs, Burlingame, CA, USA) blocking step, the slides were incubated in 1:1000 dilution of primary antibody containing 1% NGS overnight at 4°C. The slides were then washed in TBS before an overnight incubation at 4°C in the secondary antibody (Vector Labs, Burlingame, CA, USA). Application of ABC and DAB solutions according to manufacturer's recommendations followed (Vector Laboratories, Burlingame, CA, USA). After three washing steps of 10 min in TBS, the slides were serially dehydrated with alcohol, cleared with xylene, and coverslipped in Permount (Sakura Finetek USA, Torrance, CA, USA).

The density of 5-HT fibers rostral and caudal of the lesion was quantified from images taken at 400× using a Leica light microscope. Images were saved as 8-bit gray-scale TIFF files and imported into Image J software version 1.43q (NIH), where threshold values were adjusted. Selected threshold values were kept constant for all images to standardize the amount of background included in quantification. Integrated density measurement, representing the sum of the values of the pixels in an image, was averaged from five different images.

STATISTICS

To determine statistical differences between treated and untreated animals, Student's *t*-test and a one-way ANOVA with Tukey's honestly significant difference *post hoc* test (Prism, V 4.0; GraphPad, San Diego, CA, USA) were used. All results and figures are presented as means ± SE of the mean. Statistical significance is stated for p values <0.05.

RESULTS

FUNCTIONAL RECOVERY

The post-lesion recovery process was overshadowed by the appearance of signs of spasticity in the forelimb ipsilateral to the lesion. The first signs involved clenching of the paw and especially pronounced wrist flexion that appeared 2 weeks post-injury. *Post hoc* analysis indicated that these signs of spasticity occurred in animals in all treatment groups. However, by 6 weeks, only 12% of rats (2 out of 16) in the control treatment group showed abnormal wrist flexion and clenching of the paw. This stood in contrast to 60% (3 out of 5) of rats that were injected with scAAV-BDNF and 72% (13 out of 18) of rats belonging to the FULL treatment group (involving cell grafts expressing BDNF and NT-3 in the lesion site and scAAV-BDNF expression caudal to lesion) and three out of seven rats (43%) in the GRAFT/NT-3 group which received the cell grafts expressing BDNF and NT-3 in the lesion site and scAAV-NT-3 only expression caudal to lesion. In the, GRAFT/ChABC group, consisting of cell grafts expressing BDNF and NT-3 and ChABC injection, only one out of five rats (20%) displayed signs of spasticity. These effects made further functional testing difficult. Rats with these signs of spasticity were unable to plantar step or to grasp for food pellets. Consequently, because most animals were unable to retrieve pellets, the single-pellet task had to be abandoned entirely. Although rats in all groups attempted to reach for pellets, a significant decline in the attempt rate was apparent in the treated groups when compared to their baseline attempt rate measurements (FULL treatment group: 15.4%, scAAV-BDNF group: 35%, GRAFT/NT-3 group: 56.7%; GRAFT/ChABC group: 25.7%, and control group: 86.7%).

A test capable of quantitatively assessing the deficits in hand/wrist function was the cylinder test (**Figure 1A**). When exploring the wall with their paws, rats showing signs of spasticity did not use the affected limb to explore, significant deficits were observed in the group receiving scAAV-BDNF only (3.5 ± 1.3), in the group receiving graft expressing BDNF/NT-3 and scAAV-NT-3 expressing vectors (3.4 ± 1.6), as well as in the FULL treatment group (2.7 ± 0.9) when compared to control group (9.4 ± 1.5) or the graft expressing BDNF/NT-3 and ChABC treated group (8.25 ± 3.4; **Figure 1B**).

When quantifying the forelimb deficits using a forelimb score [Ref. (19); a score of 0 indicates no forelimb function and 20 indicates no deficit] rats in the group receiving scAAV-BDNF only performed somewhat worse than control treated rats (9.2 ± 0.6 vs. 11.2 ± 0.8); however this difference was not found to be statistically significant. When controls were compared to the rats receiving the FULL treatment with markedly more cells expressing BDNF (due to the graft) a significant decrease in the performance was found (7.3 ± 0.5; $p = 0.0008$; data not shown).

A test that dramatically illustrates the inability to open the paw and the rigidity of the wrist flexors in these rats is the grip strength test (**Figure 2A**). By sliding the paw over the bar of the force sensor, the paw basically "hooked" onto the bar and the animal could be pulled away from the sensor in order to measure the force needed to overcome flexion and to release the bar. When comparing these forces between the groups for the unaffected arm (contralateral to the lesion), no differences were found. This is of interest considering that a potential neuro-excitatory effect of BDNF could spread over the entire spinal cord. Because the values were comparable between all groups, an average value from all animals (118.2 ± 4 g) is indicated in **Figure 2B** as "normal" grip strength.

When testing the affected limb we found that control animals with hemisection lesion (and no treatment) usually exert a moderate to weak resistance when being pulled away from the

bar. The force necessary before releasing/sliding the affected paw over the bar was significantly lower than that for the unaffected paw(s) (60.1 ± 31.5 vs. 118.2 ± 4 g; $p = 0.022$). In contrast, rats with signs of spasticity were hardly able to release the bar resulting in higher values than those in the unaffected paw and significantly higher than the control treated rats on the affected side (scAAV-BDNF vs. Control $p = 0.0057$; FULL vs. Control $p = 0.0001$; scAAV-BDNF vs. GRAFT/NT-3 $p = 0.048$; FULL vs. GRAFT/NT-3 $p = 0.001$; FULL vs. GRAFT/ChABC $p = 0.012$). Rats receiving only scAAV-BDNF were statistically indiscernible from those receiving the full treatment (144.2 ± 35.6 vs. 157.2 ± 15.6) as both had abnormally high "grip" strength. Whereas the GRAFT/NT-3 treated group (76.7 ± 10.1 g) was not different from the CONTROL (60.1 ± 31.5 g), or the GRAFT/ChABC treated group (73.5 ± 10.2 g).

In summary, during normal activities, the majority of rats receiving scAAV-BDNF only or in combination with other treatments were unable to extend their wrist or open the digits of their paw. Further, the average force needed to overcome the wrist flexion was significantly increased.

This unexpected treatment-induced motor deficit, likely related to BDNF over-expression by cell grafts and spinal scAAV injection, did not allow the continuation of the originally planned experiment, and consequently, the focus shifted to the possible role of BDNF in the development of spasticity. Therefore, histological results are only reported for serotonergic fibers because of serotonin's neuro-excitatory properties and potential role in the development of spasticity.

Because of the involvement of BDNF in nociception (21), the Hargreaves apparatus was used to analyze the possible treatment effect on the latency of injured forelimb withdrawal in response to a painful stimulus pre and post treatment. Only two groups, FULL and the GRAFT/NT-3, showed a significant reduction in latency of withdrawal of their preferred forelimb (p value: 0.016 and 0.038, respectively), indicating thermal hypersensitivity, in response to thermal stimulation when compared to baseline latency values (FULL: 9.0 vs. 11.4 s, GRAFT/NT-3 9.5 vs. 11.4 s). Other treatment groups did not show any significant change in withdrawal latency following lesion (data not shown). These results do indicate that within our experiment there was no obvious link between changes in nociception and the occurrence of spasticity-like symptoms. The lack of effects on thermo-sensitivity in some of the groups where BDNF was over-expressed could be based on various factors including effects of other treatment components or insufficient BDNF levels.

ANIMAL WEIGHTS

Because earlier findings indicate that BDNF reduces weight gain in rats (22, 23), decreased weight in the groups of animals receiving scAAVs expressing BDNF or a combination of grafts expressing BDNF and scAAVs expressing BDNF, might be one potential indicator for adverse effects of increased BDNF levels (**Figure 3A**).

When we compared the animal weights at the end of the experiment (i.e., 9 weeks post-injury) between the control rats that received a lesion and GFP expressing scAAV vectors (207 ± 1.7 g) and those that received only scAAV-BDNF (201 ± 1.1 g), we found

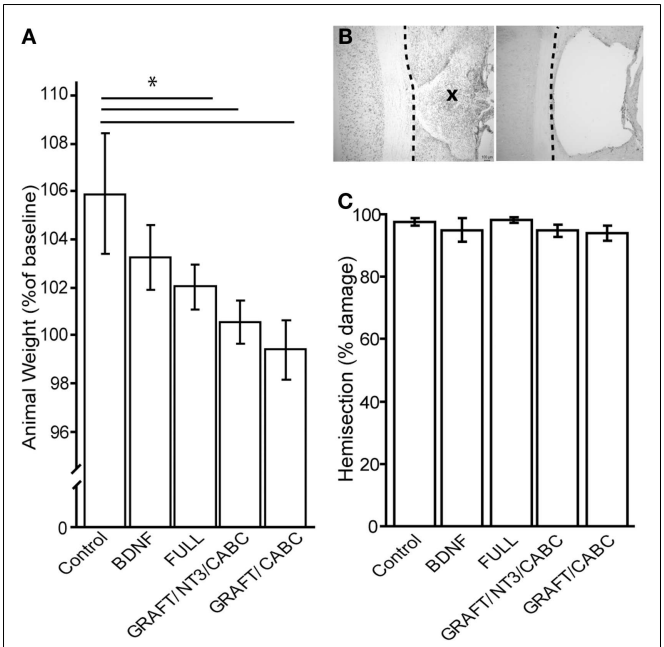

FIGURE 3 | Brain-derived neurotrophic factor application influences weight gain (A). Although baseline weight measurements were not statistically different between the treatment groups (not shown), after 9 weeks of recovery, the control group had gained significantly more weight than the FULL treatment group. Animals that received only scAAV-BDNF but no cell graft BDNF had moderate weight gain and were not significantly different from either group **(A)**. **(B)** Shows a representative example of a horizontal section through a lesion site with cell graft (left; indicated by an X) and without (right). Micrographs were taken at 100× magnification. There was no difference in lesion size among the groups **(C)**. *$p < 0.05$.

a small, non-statistically significant decrease in weight gain. Rats that received the FULL treatment (which entails, among others, BDNF and NT-3 expressing cell grafts in addition to the injection of scAAV-BDNF) showed a significant difference (192 ± 2.3 g). GRAFT/NT-3 and GRAFT/ChABC treated group was not statistically different from any other treatment groups. However, it has to be kept in mind that this weight loss might have also been influenced by other treatment components such as rolipram injections in addition to the chronic BDNF over-expression by either the fibroblast cell graft or scAAV vectors.

LESION SIZES

Lesion sizes were analyzed from reconstruction of serial sections (**Figures 3B,C**). Following our exclusion criteria, animals with lesions deviating from a complete hemisection by >10% were removed from this study ($n = 4$); a comparison of the lesion sizes between the groups showed no statistical difference between either group. The control group had only 2.2% (±1.1) tissue spared in comparison to 4.1% (±2.8) in the scAAV-BDNF only group, 1.1% (±1.4) in the FULL treatment group, 3.7 (±0.01) in the GRAFT/NT-3 group, or 4.12 (±0.01) in the GRAFT/ChABC group. Thus, variations in lesion size were only minor, and differences in functional outcome can therefore confidently be attributed to the different treatments.

SPROUTING OF SEROTONERGIC FIBERS

Nine weeks after SCI, the density of 5-HT positive fibers both immediately rostral and caudal to the lesion site was quantified in order to visualize and compare the possible role of 5-HT fiber sprouting in the observed spasticity following SCI (**Figure 4A**).

The integrated density of 5-HT fibers was compared among all groups (**Figure 4B**). The highest values for 5-HT were observed in the GRAFT/NT-3 group (2923 ± 307) which is statistically significant when compared to the control group (2148 ± 117) and the scAAV-BDNF only group 1621 ± 418 ($p = 0.0330$, 0.0424, respectively). The average density value in the FULL treatment group was 2354 ± 212 and 2423 ± 337 in GRAFT/ChABC (not significant to controls).

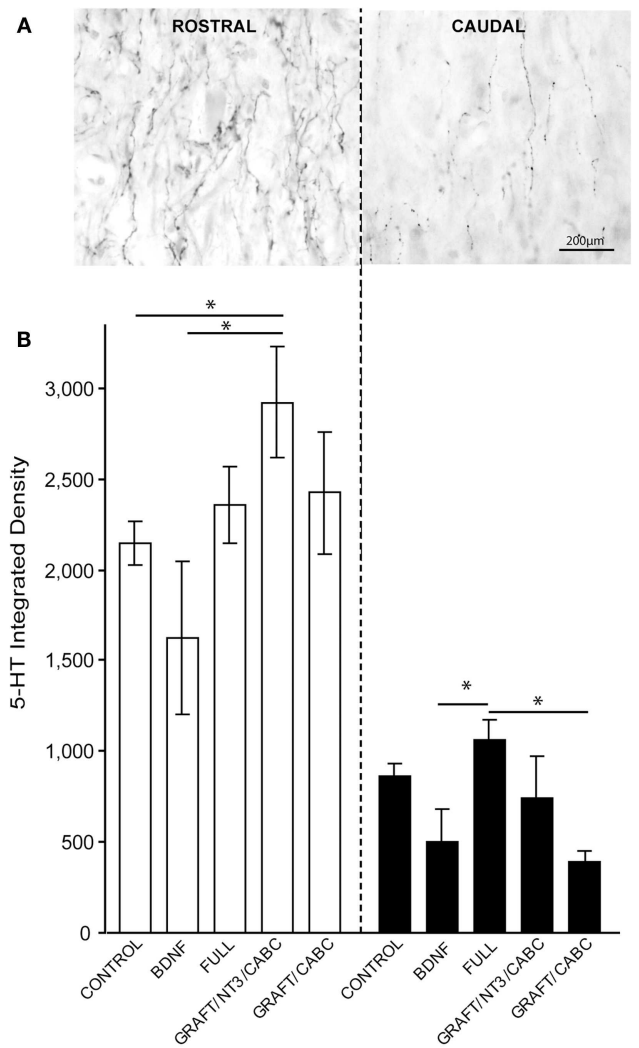

FIGURE 4 | 5-HT fibers do not facilitate treatment-induced spasticity following SCI. **(A)** Gray-scale images following 5-HT immunohistology show a high density of 5-HT fibers immediately rostral to lesion and their subsequent reduction following SCI caudal to the lesion. **(B)** Quantification of integrated 5-HT fiber density rostral and caudal to the lesion does not indicate significant influence of the BDNF treatment. Data presented as mean ± SEM, $*p < 0.05$.

Caudal to the lesion, 5-HT density was reduced and even though the FULL treatment group contained significantly more fibers than the scAAV-BDNF only group ($p = 0.034$; 1059 ± 116 vs. 503 ± 178; respectively), neither group was statistically different from the control group (863 ± 68). The FULL treatment group was however, significantly different from the GRAFT/ChABC group (1059 ± 116 vs. 395 ± 52; $p = 0.0339$), but not different from GRAFT/NT-3 group (1059 ± 116 vs. 739 ± 229). Thus, it appears unlikely that differences in the availability of serotonin contributed to the increase in spasticity of the treated groups.

ANTAGONIZING BDNF

In order to investigate the underlying mechanisms of the observed increase in spasticity-like symptoms of animals that received BDNF treatment either by scAAV vector expression or in combination with grafts expressing BDNF, we quantified the muscle activity elicited by overcoming the wrist flexion before and after the spinal injection of the BDNF antagonist TrkB-Fc.

Before injection of TrkB-Fc into the lesion site, wrist flexion was manually overcome three times in a row with an interval of about 3 s, which was repeated every 10 min following the TrkB-Fc injection for a total of 30 min. When this experiment was performed in rats without signs of spasticity ($n = 3$), there was no resistance to the stretch and no EMG activity was detected before or during the extension of the wrist into a horizontal position (i.e., 180°) even before TrkB-Fc was injected. Therefore, for this experiment only rats that demonstrated resistance and an EMG response in flexor muscles to stretching of the wrist (i.e., displayed spasticity-like symptoms) were included.

When averaging the rectified EMG from all three stretches and comparing this value to values measured at 10 and 20 min following a spinal saline injection, we found in four animals with spasticity-like symptoms (one from the scAAV-BDNF, two from the FULL treatment group, and one from GRAFT/NT-3 group) only an insignificant decrease in EMG response over time (**Figure 5**). Only after 30 min, a significant decline of 11% was observed.

In contrast, in 10 animals with spasticity-like symptoms (two scAAV-BDNF and eight of the FULL treatment group) that received the BDNF antagonist injection, the EMG response dropped by $51 \pm 9.8\%$ starting as early as 10 min after TrkB-Fc injection and continued to decline to $64 \pm 8.4\%$ at 20 min and $80 \pm 6.6\%$ at 30 min. The difference in the stretch induced average and rectified EMG amplitude at each time point was statistically significant between saline and TrkB-Fc injected rats (**Figure 5**).

Interestingly, a significant effect of TrkB-Fc was also found in one out of two control treated rats that showed severe wrist flexion/spasticity, at 20 and 30 min post injection ($p = 0.024$ and 0.036, respectively).

DISCUSSION

Brain-derived neurotrophic factor is a prominent neurotrophic factor with a long history and a variety of effects. Such effects include the modulation of cell survival (ranging from protection to cell death), the enhancement of neurite outgrowth and axonal regeneration, the promotion of myelination, as well as the modulation of synaptic transmission, the post-injury immune

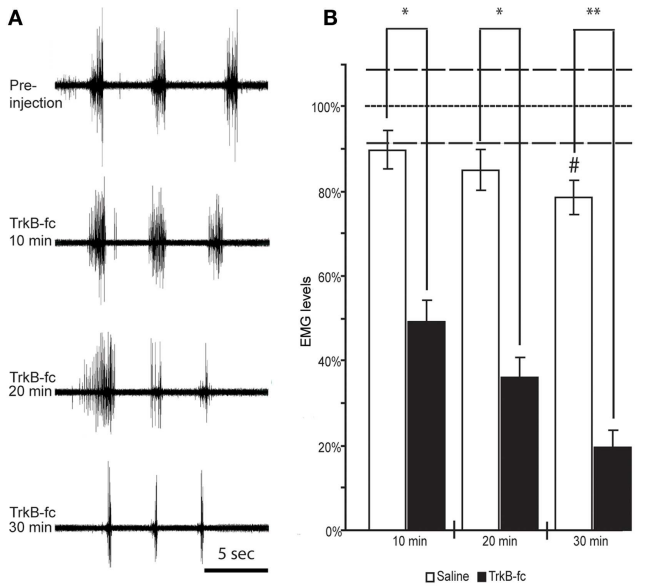

FIGURE 5 | Spinal BDNF antagonist reduces resistance to overcome wrist flexion in animals that exhibit spasticity-like symptoms. EMG recordings from wrist flexor muscles of the forelimb ipsilateral to the lesion before and at three different time points following TrkB-Fc injection show a gradual decline in stretch-evoked EMG responses. Note the relative inactivity in the recording before and in between the stretch-evoked bursts **(A)**. When quantifying the EMG response and normalizing it to the values found in control animals under anesthesia only (did not receive Saline or TrkB-Fc injections; dotted line) we found a significant drop in EMG response at 10 min, and a continuous decline over the next 20 min when TrkB-Fc infusion was compared to saline injections **(B)**. #Indicates a significant reduction of EMG response at 30 min post saline injection when compared to control animals that were under anesthesia only (dotted line; $p = 0.0286$). White columns represent rats that received saline, black columns represent rats that received the BDNF antagonist. Bars show means ± SEM; $*p < 0.05$. $**p < 0.005$. Dashed lines indicate the SEM for control anesthesia (dotted line).

response and neuronal excitability [reviewed in Ref. (8)]. Not surprisingly, BDNF also frequently makes it onto the list of potential SCI treatments [e.g., Ref. (11, 24–30)]. Considering the broad effects of BDNF, it is also not surprising that the mechanisms by which BDNF influences functional outcomes is sometimes difficult to discern. Understanding the mechanism by which a treatment influences functional recovery (or decline) is more challenging when several treatments are combined. A good example of this can be found in a recent report by Lu and colleagues (11), where a combined treatment not only resulted in enhanced plasticity of various descending fiber systems (e.g., serotonergic fibers) and axonal regeneration, but also in an undesired effect (i.e., increased occurrence of spasticity). Obviously it is of utmost importance to explore whether the increased spasticity was due to treatment-mediated augmentation of neurite growth and plasticity and/or possibly through an acute neuro-excitatory effect of BDNF (31, 32).

Another challenge of using BDNF for treating SCI is its well-established role in affecting sensory and nociceptive pathways in the spinal cord (21) and its ability to modulate glutamate receptors and sensitization (33, 34). Thus, a possible link between the observed spasticity-like symptoms found in the present study and neuropathic pain could be suggested. Yet, in this study no consistent change was found in thermo-sensitivity and it is unlikely that the signs of spasticity are related to increased pain. For example, it could be assumed that when assessing the grip strength, increased pain sensitivity would result in a withdrawal rather than an increase in grip strength.

Another potential mechanism involved in promoting spasticity could be aberrant serotonergic sprouting and the subsequent increased levels of serotonin, which could increase neuronal excitability (35, 36). In the current study, we did not find significant differences in the serotonergic innervation between the groups and demonstrate that a BDNF antagonist reduces spasticity within minutes. This indicates that an acute neuro-excitatory effect of permanent BDNF expression underlies the increase in signs of spasticity. The relatively fast effect of the TrkB antagonist also indicates that, although baseline activity in the flexed muscle was very low, a contracture [shortening of the muscle; Ref. (37)] was not involved in the observed spasticity-like symptoms.

It has to be kept in mind that spasticity-like symptoms were also observed in a small percentage of rats with spinal hemisection that received only GFP expressing viral vectors. It appears as though the continuous BDNF over-expression exacerbated this naturally occurring process by decreasing the threshold for the development of spasticity. Curiously, the application of the BDNF antagonist also reduced the spasticity in one control treated rat, indicating that BDNF signaling might also be involved in post-injury regulation of neuronal excitability. Indeed, increases in BDNF expression after SCI have been reported (38–40), which can contribute to increased neuronal excitability by reducing the amount of current required to reach threshold [i.e., rheobase; Ref. (41, 42)].

There are many possible mechanisms by which BDNF can exert its excitatory effects. For example, BDNF may increase calcium and sodium influx through TrkB signaling (43). Furthermore, activation of the TrkB-PLC pathway may raise the membrane potential through calcium-mediated channel opening (44). BDNF has also been reported to down-regulate the potassium-chloride co-transporter KCC2 (45), which down-regulates the inhibitory influence of gamma-aminobutyric acid (GABA) receptors. The most rapid and potent excitatory effects of BDNF, however, have been shown to involve Nav1.9, on a time scale similar to the action of glutamate (32).

Considering its neuro-excitatory properties, it is not surprising that over-expression of BDNF can also promote beneficial effects without promoting neuronal survival or regeneration. For example, Boyce et al. (41), showed that the injection of BDNF expressing vectors improved locomotor function in rats with complete spinal cord transections.

Effects of BDNF on synaptic connectivity have been described in dissociated cultures of early embryonic hippocampal neurons, where it increased the number of functional synaptic connections (46). At the same time, studies of long-term effects of BDNF in dissociated cortical cultures have suggested that BDNF decreases neuronal firing rate by reducing the strength of all excitatory inputs onto a given neuron (47). Bolton et al. (48) found that actions of

BDNF underlying the increase in activity, involved enhancement of both excitatory and inhibitory synaptic transmission in parallel, but via distinct cellular mechanisms.

In the current study, it can only be speculated which neuronal population(s) responded to the BDNF antagonist, since the stretch induced spasticity-like reflex was decreased. From earlier studies (31, 49) it is likely that motoneurons play a major role in the development of spasticity. Generally speaking, following the administration of the BDNF antagonist we found a decline in EMG amplitude (see **Figure 5** at 20 min) and a decline in burst duration in parallel to the decline in spasticity-like symptoms. This is in line with the idea that after SCI spasticity is related to the re-occurrence of plateau potentials in motoneurons allowing activity beyond the time of depolarization (49, 50).

In conclusion, although the permanent expression of BDNF using viral vectors may well promote certain aspects of recovery through neuro-excitatory mechanisms, the potential side effects would caution such application and point to the use of regulated expression systems to control BDNF expression (14). The general involvement of the TrkB receptor in the development of spasticity warrants further exploration.

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Reaching and grasping in autism spectrum disorder: a review of recent literature

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Impairments in motor functioning, which, until recently, have rarely been a primary focus in autism spectrum disorder (ASD) research, may play a key role in the early expression of biological vulnerability and be associated with key social-communication deficits. This review summarizes current knowledge of motor behavior in ASD, focusing specifically on reaching and grasping. Convergent data across the lifespan indicate that impairments to reaching and grasping emerge early in life, affect the planning and execution of motor programs, and may be impacted by additional impairments to sensory control of motor behavior. The relationship between motor impairments and diagnostic outcomes will be discussed.

Keywords: reaching, reach-to-grasp, motor planning, motor execution, movement, autism spectrum disorder, review

INTRODUCTION

Autism spectrum disorder (ASD) is a developmental disorder characterized by impairments in social communication and the presence of repetitive or restricted behaviors (1). ASD is one of the most prevalent forms of developmental disability internationally, with current estimates at 1 in 88 (2, 3). In his original case series, Kanner (4) described ASD primarily in relation to severe impairment in social–emotional and communication ability but also commented on several aspects of motor development: motor milestones were generally *within normal limits* and fine motor coordination was "*very skillful*," although some patients had gross motor deficits. However, more recent reports suggest that gross and fine motor deficits are prevalent in ASD (5–10) and include impairments in basic motor control (11–13), difficulty performing skilled motor gestures (14, 15), abnormal patterns of motor learning (16, 17), and disturbances in the reach-to-grasp movement (18, 19). To date, these motor abnormalities are categorized as "associated (as opposed to core) symptoms" (8) and are thought to interfere with the development of adaptive skills (15, 20–22). Motor impairments may have primary effects on achieving independence in activities of daily living (such as holding a spoon), but also secondary effects on social functioning, by interfering with children's ability to participate in age-appropriate activities with peers (such as team sports).

The embodied theory of cognition posits that we should consider cognition in terms of its function in serving adaptive behavior (23). It follows therefore that complex adaptive behaviors, such as those of the hands, should be more closely related to cognitive functions than simple adaptive behaviors, such as those of walking. Reaching and grasping is a complex and fundamental motor activity that serves as a vital mode of exploration for children as they learn about the physical world (24). The ability to plan, execute, and monitor ongoing movement is an important aspect of completing an action toward a goal that is integrated in the environment (25). As such, disturbances in grasping patterns may impact how children play, explore, use tools, and engage socially. This review is aimed at providing a detailed summary of current knowledge of skilled use of the hands in ASD, focusing specifically on reaching and grasping.

Systematic searches were performed in four computerized bibliographic databases (PUBMED, ISI WEB of Science, PsycINFO, ScienceDirect) to identify existing literature on reaching and grasping in ASD. The search terms included "reaching" and/or "grasping" with "autism." Additional articles of interest were located in the reference sections of the articles from the systematic search. To be included in the review, a paper had to: (1) examine hand movements during reaching and/or grasping tasks in children with ASD, (2) include a comparison group of typically developing (TD) children (without a family history of ASD), but it could also include other groups for comparison, such as children with other forms of developmental disability (DD), and (3) confirm the ASD diagnosis using a multidisciplinary approach. Twenty-five articles met inclusion criteria and were included in the body of the review. The results of the search are presented below; beginning with a description of how manual-motor behavior develops in the first years of life in infants at-risk for, or diagnosed with, ASD. The remainder of the review is organized around the framework of a motor episode; describing how ASD affects motor planning and motor execution, as well as how ASD affects ongoing motor adjustment and knowledge across the lifespan (**Figure 1**). The review ends with a discussion of the implications of impairments to motor behavior, and how they relate to diagnosing ASD.

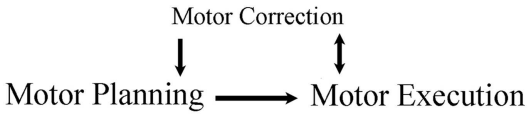

FIGURE 1 | Framework of review. A movement is planned and then executed. The executed movement is monitored, as online corrections aide ongoing movements and offline corrections aide the planning of subsequent movements.

REVIEW FINDINGS

EARLY MOTOR DEVELOPMENT

The analysis of early object manipulation may yield information on atypical development even before the onset of more core symptoms of ASD. During typical development, infants grasp objects and manipulate them using their oral, tactile, and visual senses to explore object characteristics (26). These sensorimotor skills are strongly associated with hand and finger sophistication in later development (27, 28). For example, after grasping a block, infants will bring it to their face to look at it, will rub their fingers along it to feel its texture, and will place it in their mouth to taste it. Atypical object exploration has been reported for infants as young as 12 months of age, who are later diagnosed with ASD. Compared with TD peers, infants who later received a diagnosis of ASD demonstrated more spinning and rotating of objects, as well as unusual visual exploration of objects (29). Retrospective parent reports of oral- and manual-motor skills from primary caregivers of children with ASD ($n = 172$) and TD children ($n = 44$) suggest that impaired oral-motor abilities (e.g., blowing a raspberry, sticking out tongue, and making animal sounds) and manual-motor abilities (e.g., grabbing dangling toys, block play) were able to distinguish ASD from TD children during infancy and toddlerhood (with sensitivity at 83% and specificity at 93% for oral-motor abilities and sensitivity at 89% and specificity at 86% for manual-motor skills in children later diagnosed with ASD). Surprisingly, correlational analyses revealed that oral- and manual-motor abilities of infants with ASD were better able to distinguish children with ASD from their TD peers than delays in the prototypical milestones of crawling or responding to name (30). A comparison of ASD and DD is necessary to separate the ASD-specific impairments from general delay when examining the associations between oral- and manual-motor abilities and social-communication outcomes. Nevertheless, oral- and manual-motor skills are not purely a "motor problem" and children with high verbal skills likely have better comprehension as well as expression, although such tasks do not require much verbal instruction.

Accordingly, several groups have examined whether oral, manual, and motor skills are related to diagnostic outcomes in infants at high-risk (HR) for ASD (for example, younger siblings of a child with ASD). Bhat et al. (31) examined the relationship between early gross motor behavior, as measured by the Alberta Infant Motor Scale [AIMS; (32)] at 3 and 6 months of age, and communication outcomes, as measured by the Mullen Scale of Early Learning [MSEL; (33)] at 18 months of age in HR ($n = 24$; 12 male) and TD infants ($n = 24$; 9 male). Compared to TD controls, HR siblings displayed the delayed motor performance on the AIMS at 3 and 6 months of age, but more importantly, all HR siblings who met criteria for a communication delay at 18 months of age exhibited a motor delay at 3 months of age. Mulligan and White (34) prospectively examined the relationship between sensory and motor behaviors in HR infants ($n = 13$; mean age 12.6 months; 5 males; 4 of the 13 were diagnosed with ASD at 30 month follow-up) and their TD peers ($n = 12$; mean age 12.1 months; 5 males) by asking infants and caregivers to participate in a 10-min play session and a 5-min eating session. Their behaviors were video-recorded and coded for the presence or absence of mouthing objects, object manipulation, hand to mouth with spoon, and plays with food. HR and TD infants showed a similar performance across the two sessions, although the HR infants moved around less and manipulated objects in their hands less frequently than the TD controls.

The relationship between poor motor ability and ASD continues into childhood. Using Part I (oral-motor assessment) of the Kaufman Speech Praxis Test for Children (35), Adams (36) compared oral-motor abilities and simple and complex phonemic production in children with ASD ($n = 4$; mean age 8.5 years) against a TD control group ($n = 4$; mean age 9.0 years). Children were asked to execute non-speech motor movements (e.g., pucker lips), produce simple phonemes (e.g., vowel-to-vowel movements), and produce complex phonemes (complex consonant production, polysyllabic synthesis). Children with ASD were impaired on performance of oral movements, particularly those involving in the tongue and lips, and these impairments impacted their ability to perform complex phonemic production and sound blending. In accordance with these results, Gernsbacher et al. (30) found that performance of oral- and manual-motor behaviors in ASD differed depending on level of verbal fluency. Minimally fluent ($n = 20$; mean age 7.4 years) and highly fluent children with ASD ($n = 20$; mean age 8.3 years) completed Part I of the Kaufman Speech Praxis Test for Children (35) and were coded as "able" or "unable" to complete tasks of "control saliva," "protrude tongue," "produce vocalizations," and "pucker lips," etc. Overall, the minimally fluent children were less able to complete oral–manual skills than the highly verbal children, showing impairment on tasks such as "open mouth," "spread lips," and any tasks involving with the tongue. Results such as these highlight the important relationship between non-vocal oral abilities and vocal production. An understanding of these impairments is important when assessing social and communication ability in HR infants, as well as older children with ASD, as impairments in oral- and manual-motor ability can confound the assessment of both verbal and non-verbal language, extending into the ability to engage socially with peers. That said, it is important to acknowledge that many factors contribute to communication functioning other than oral–motor skills. Moreover, difficulties comprehending instructions may confound assessment of motor skills in children with ASD who have receptive language delays, which may need to be taken into account in interpreting other findings summarized in this review.

MOTOR PLANNING

The analysis of motor planning may yield early information concerning impairments in cognitive processing in ASD (37). Before completing a motor act, such as reaching for a block to build a tower, a motor plan first needs to be developed. Motor planning

involves the sequence of motor commands that convert the current state of one's body into the desired state. Thus, when building a tower, a person must formulate a plan that consists of lifting his/her hand, extending it toward a block, shaping his/her digits to grasp the block, and then transporting the block to the table to begin construction.

Reaction time tasks

Recording reaction time is the simplest way to measure motor planning, as it provides a basic measure of the time taken to formulate a motor plan. The majority of studies report that participants with ASD typically show longer reaction times than their TD peers (18, 38–41). However, when presented with simple tasks, such as drawing a line between the two targets, children with ASD and TD perform similarly. Dowd et al. (42) investigated motor planning and motor execution in young children with ASD ($N = 11$; mean age = 6.2 years) and TD children ($N = 12$; mean age = 6.6 years) using a point-to-point movement task, in which participants were required to use a stylus to move between two points on a digital screen. Overall, ASD and TD groups did not differ on any measures examined, but the ASD group did have more variable reaction times. In a similar experiment, Papadopoulos et al. (43) presented adolescents with Asperger's disorder ($N = 20$; mean age 9.6 years), high-functioning ASD ($N = 19$; mean age 9.8 years), and TD children ($N = 18$; mean age 9.8 years) with visual stimuli on a tablet; two small or large yellow circles were positioned on a horizontal plane from left to right and were separated by a space of 12–25 cm. The participants were asked to draw a line between the two targets as fast and accurately as possible. Kinematic analysis showed that time to complete the movement did not differ between the three groups; however, the high-functioning ASD group had more variable end-points when compared to the TD group, suggesting the lack of a well-formed movement plan following a series of repetitions. It is interesting that more variable reaction times are typical of children with Attention Deficit Hyperactivity Disorder [ADHD; (44)], and given that a substantial proportion of children with ASD also show signs of ADHD (45), it may be important to determine the specificity of the finding of high variability to ASD (i.e., examine children with ASD who do and do not show signs of ADHD).

When presented with a more complex task, group differences begin to emerge in relation to planning a movement. Glazebrook et al. (46) asked the participants with ASD ($n = 9$; mean age 26.9 years) and their TD peers ($n = 9$; mean age 25.1 years) to move their index finger as quickly as possible to an illuminated circular target after a starting cue. During the trials, the size of the targets as well as the distance between the targets varied. As reported with simpler tasks, adults with ASD had more variable performance than the TD controls, but they also required more time to prepare and execute their movements, and reached lower peak acceleration and velocity than TD controls. In a follow-up experiment, Glazebrook et al. (38) used a more complex experimental set-up consisting of a black box with 10 switches, 2 of which served as a start position for the index finger of each hand. Adults with ASD ($n = 18$; mean age 23.7 years; 17 male) and TD controls ($n = 18$; mean age 20.6 years; 12 male) were presented with a valid precue to indicate either hand required (left/right) or distance of the target to grasp (near/far). Following illumination of the target, the ASD participants took longer to respond and complete the movement, and again were more variable in responding than the TD controls. When performing the same task, but receiving an invalid precue, Nazarali et al. (40) found that adults with ASD ($n = 12$; mean age 26.2 years; 12 male) take longer to reprogram and complete their movement (as indicated by increased reaction and execution times) than their TD peers ($n = 12$; mean age 22.8 years; 10 male). The effect was even more pronounced for invalid "hand" cues than invalid "direction" cues. These results are of particular importance for planning deficits in ASD. That is, when presented with an invalid "hand" precue, additional sequences of movements must be included in the new plan (i.e., put down left hand, lift right hand, reach to left space), than if presented with an invalid "direction" cue (i.e., move left hand to left space instead of right space). It follows therefore that if ASD is indeed associated with a planning deficit, it would not be surprising that the ASD group would be more affected than their TD peers. In accordance, the complex tasks presented above require multi-level processing; seeing a cue, formulating a plan, and initiating a motor response. As such, it is possible that observed impairments on such tasks may not be purely related to motor skills *per se*, but rather from an incoordination between cognitive processing and motor output.

Reach and grasp tasks

That individuals with ASD take longer to respond to an invalid cue may lend further weight to findings from sequential motor tasks, which indicate that children with ASD may be less responsive to visual information when planning a sequential task. Using a reach, grasp, and place paradigm, Fabbri-Destro et al. (47) examined how children with high-functioning ASD ($n = 12$; mean age = 10 years) and sex and age-matched controls execute motor plans by manipulating the size of the container into which a grasped object is to be placed. While TD participants adjust the temporal characteristics of the reach and grasp components of the sequence based on the size of the final placement container, children with ASD did not alter how the movements were executed. The authors suggested that children with ASD program sequential movements in independent steps, rather than as a cohesive pattern and do not utilize the visual feedback of end-point target when planning their overall movement. Thus, it could be argued that the delayed response following the presentation of an invalid cue may not be due to planning deficits *per se*, but rather an impairment in registering and responding to visual feedback. Indeed, evidence from functional imaging of connective networking in the brain suggests that individuals with ASD have impaired communication between brain networks, and thus may have trouble coordinating a movement in response to a visual cue (48).

Hughes (17) examined motor planning in children with ASD by employing a reach-to-grasp task that encouraged a particular hand posture. Hughes also included a group of children with DD as a comparison group to help identify ASD-specific impairments to planning ability. Children with ASD ($n = 36$; 12–14 years), DD ($n = 24$; 10–12 years), and TD ($n = 28$; 3–5 years) were asked to pick up a rod that had one end painted black and the other end painted white and place one of the colored ends into one of two

disks so that the rod stood upright. By varying the starting position of the bar, it is possible to encourage the participants to produce an overhand or underhand grip, leading to either comfortable or awkward final posture depending on their planning abilities (see **Figure 2**). The criterion for a correct response, and thus appropriate motor planning, was an appropriate hand action on the underhand trials, in which the person begins with an uncomfortable grasp to end with a comfortable grasp. There were no group differences on the overhand trials, which required no special planning (grasp horizontal bar and supinate wrist to place end closest to pinky finger into ring). For the underhand (uncomfortable) condition, however, the ASD group made fewer correct initial postures than the DD group, and both groups together performed more poorly than the TD group. Hughes (17) suggested that performance of the ASD group resulted from a fundamental deficit in motor planning leading to inability to plan a series of movements that would result in a comfortable end-grasp posture. However, a similar experiment using an end-state comfort task by van Swieten et al. (49) failed to detect motor planning differences between ASD and TD groups. Children with ASD ($n = 20$; age range 9–14 years), developmental coordination disorder (DCD; $n = 11$; age range 9–13 years), and TD peers ($n = 44$; age range 9–14 years) were presented with a wooden dowel attached to a rotating platform. One end of the dowel was painted red and the participants were told to place their thumb on the red end of the dowel as the start position, and rotate their wrist to move the dowel 180° to the end position. The children had to choose between performing either the minimum amount of rotation or end-state comfort (on 50% of the trials, these coincided). Interestingly, the ASD and TD groups performed similarly on the task, choosing end-state comfort on approximately 75% of trials; however, both groups differed from the DCD group, who more often chose minimal rotation over end-state comfort (approximately 60% of trials). The discrepancy between the findings from Hughes (17) and van Swieten et al. (49) may be due to the complexity of the plan required to complete the tasks. The Hughes (17) task parameters required the processing of three sequential aspects of the reaching motion; that is, participants needed to choose between an overhand and underhand grasp, lift the object, and either supinate or pronate their wrist to place the object in a hole. In contrast, the task of van Swieten et al. (49) only required the child to process one aspect of the motion (either supinate or rotate their wrist), begging the question of whether the motor impairments seen on the Hughes (17) task may be due to problems processing multiple pieces of information to formulate a succinct motor plan.

To summarize, analysis of motor planning in ASD has suggested increased variability in movement onset and offset (42, 43), increased reaction time to valid cueing (38, 39), delays in reinitiating and completing a movement following invalid cueing (38, 40), and impairments when planning a comfortable end-grasp posture, depending on the complexity of the plan required (17, 49). When taken together, the results of motor planning literature suggest that individuals with ASD have trouble in formulating a motor plan when asked to process multiple pieces of information (i.e., complex task), which may be cognitively taxing and thus interferes with motor output.

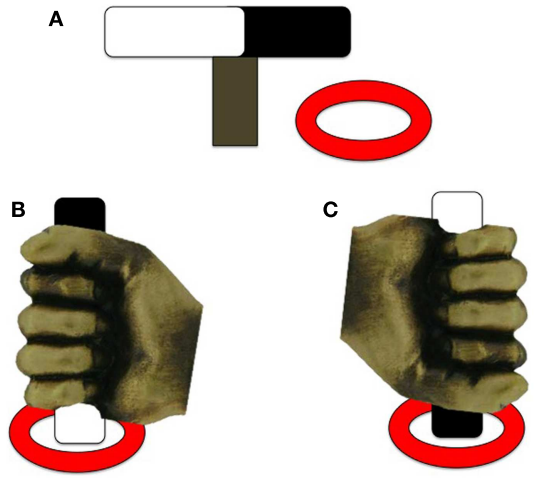

FIGURE 2 | Experimental design of the underhand grasps used in Hughes (17). **(A)** The rod and ring set-up; **(B)** example of a *comfortable* end-state underhand grasp; **(C)** example of an *uncomfortable* end-state underhand grasp. Note that the rod is positioned the same for each trial, only the color of the end of the rod to be placed in the ring differs between trials [adapted from Ref. (17)].

MOTOR EXECUTION

Analyses of motor execution (that is, acts of carrying out planned movements) provide the opportunity to understand the neurological underpinnings of cognitive processing that precede such movements. Commands from the motor cortex are sent to the corresponding nerves and muscles to carry out the motor act. For example, after planning to grasp a block with the right hand, a person must then specify the particular muscle contractions to move the limb in the correct direction and shape the digits appropriately for grasping. Due to the reciprocal interactions between motor cortex, sensory input, and motor output, there are ample opportunities for errors to occur when executing a motor plan. Here, we review motor execution in children with ASD.

Grasping tasks

Using a grasp and place task, Forti et al. (50) found that the movement duration of participants with ASD is nearly twice as long as those of controls. Participants with ASD ($n = 12$; mean age 3.5 years; nine males) and age and sex-matched TD controls were instructed to transport a rubber ball from a start location and drop it into a hole, located 30 cm away, while wearing kinematic markers (markers placed on the body that allow the online/offline tracking of body segments). In addition to taking longer to complete the movement, children with ASD had higher velocities at movement terminus. Although the ASD group was able to accurately transport the ball and drop it into the hole, every member of the ASD group made corrections *at least once* after entering the area of the hole, whereas *fewer than half* the TD controls made corrections. Interestingly, there were no differences observed for the initial movement phases, which should reflect motor planning processes. In a related study, Stoit et al. (51) examined feed-forward motor control in children with ASD

($n = 31$; mean age 11.6 years) and TD children ($n = 29$; mean age 10.5 years) using a precision versus power grasp task. Feedforward movements rely on internal models for accuracy and do not require the online use of sensory feedback evolving during the action (52). Participants were seated behind a table and presented with two cylinders, a small cylinder affording precision grasping and a large cylinder affording power grasping. For each trial, participants received a cue, administered by a human hand, to indicate the location (left/right) or grip-type (precision/power) of the target to be grasped (see **Figure 3**). As in the previous study, movement times were significantly longer in the ASD group, although there were no differences in initiation errors or time to respond following start cue between the two groups. Using a similar reach-to-grasp task, Mari et al. (18) report that reaching and grasping kinematics are largely uncoupled and executed in a successive non-overlapping manner in children with ASD. Children with ASD and their TD peers ($n = 20$ per group; 7–13 years) grasped wooden blocks of varying sizes and distances and specific kinematic measures were recorded, including time to reach peak velocity, deceleration time, as well as the coordination of the reach and grasp components. Because the reach component is controlled by the proximal musculature of the shoulder and elbow and the grasp component is controlled by distal musculature of the forearm and hand, it is possible that the ASD group might show an impairment of coordination. Overall, the children with ASD performed the movement quite well, and did not differ from their TD peers. Exploring the results further, the performance of the ASD group was contrasted by IQ. An identified "lower functioning" group (IQ range 70–79) showed evidence of desynchronization between the reach and grasp components, whereas the identified "higher functioning" group (IQ range 80–109) demonstrated a closely integrated and overlapping movement. These results highlight the importance of including IQ and/or developmental matched controls to determine specificity of findings to ASD.

The results of Cattaneo et al. (53) also support the incoordination of motor components of a reaching-to-grasp movement in ASD. Electromyography (EMG) recorded muscle activity related to mouth opening during an eating task in children with ASD and age-matched TD controls ($n = 8$; mean age = 6.5 years for both groups) showed that EMG activity started before the hand even grasped the object for the TD group. In contrast, EMG activity in the children with ASD started much later, when the hand was bringing the food to the mouth. A recent report by Pascolo and Cattarinussi (54) critically evaluated the results of Cattaneo et al. (53) and failed to replicate their finding of impaired synchronization between grasping and eating. Pascolo et al. employed the same methodology as Cattaneo et al. but applied increased control over the experimental set-up. For example, the supplementary information that accompanied the original article by Cattaneo et al. acknowledged that the distance between the child and the food varied across trials and there were extra personnel in the room when the experiment was conducted (which could be distracting). To examine the effect of these limitations on mouth activation, Pascolo et al. varied the distance of target (near, far, and comfortable distance) and had the children reach for food in a quiet room without extra personnel. Pascolo et al. (54) did not find any differences between the performance of the ASD group ($n = 7$; mean age = 7.3 years) and their TD peers ($n = 12$; mean age = 7.7 years), as both groups opened their mouth after the food had been grasped. Interestingly, when looking at the effect of distance on mouth opening, Pascolo et al. found that the further the target was away from the body, the later the onset of mouth opening. The lack of replication between Cattaneo et al. and Pascolo et al. likely relates to differences in experimental methodology employed. Pascolo et al. carefully controlled for two extraneous influences on the performance of children with and without ASD, by having them repeat the same movement numerous times in a quiet setting. Cattaneo et al. had children with and without ASD perform a grasping and eating movement in a more naturalistic setting, with variance in food location and extraneous persons present. The difference in set-up between these two experiments emphasizes the importance of task boundaries when considering experimental results. When presented with a quiet environment in which one movement is repeated, ASD children perform similarly to TD children. When they are presented with a more naturalistic environment, in which variance occurs between trials, and extraneous personnel are present, the cognitive system of children with ASD becomes taxed, resulting in impaired motor performance. This is in accordance with results from motor planning, which suggest that motor performance of individuals with ASD similarly become impaired when asked to process multiple pieces of information (17, 38, 47).

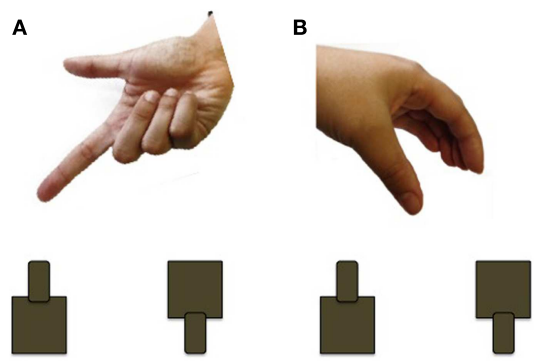

FIGURE 3 | Example of the cueing used by Stoit et al. (51). **(A)** Pointing cue to indicate cylinder to be grasped (left or right); **(B)** hand shape cue to indicate cylinder to be grasped (precision or power).

MOTOR CORRECTION

The analysis of motor corrections can provide information on an individuals' ability to understand and respond to ongoing stimuli in the environment. Ongoing adjustment during movement execution is termed *online control*. The internal representation of the movement is compared to the executed movement, and adjustments to the movement are made based on visual and proprioceptive feedback (55). For example, when lifting a block to build a tower, somatosensory feedback guides the application of differential force to blocks made of foam versus those made of wood.

Load-lifting tasks

In a unique experiment, Schmitz et al. (25) investigated motor adjustment during a bimanual load-lifting task in children with ASD ($n = 8$; mean age 7.9 years, six males) and their TD peers ($n = 16$; mean age 6.0 years; seven males). Participants wore a bracelet on their right hand equipped with a strain gage that supported a platform on which a load could be placed. Motor adjustment was measured from the angular displacement of the forearm along the elbow joint, and activity of bicep and tricep muscles were recorded using surface electrodes. The response of the arm was measured when the load was removed by the experimenter or by the participants' left hand. The results indicated that the maximal angular amplitude of the elbow did not differ between ASD and TD children in either the experimenter- or self-unload conditions, although the latency for bicep inhibition took longer in the ASD group. The delay in bicep response of the ASD group suggests a lack of anticipation of the unloading force, and as such, they respond only after receiving sensory feedback that the load had been lifted from the platform.

A recent experiment by David et al. (24) examining motor adjustment included a comparison group of children diagnosed with DD to help distinguish between impairments due to general delay versus those that were ASD specific. Grip and load force were measured in children with ASD, DD, and TD peers ($n = 21$ per group; 2–6 years) during a grasp and place task. Grip force was measured from the digits on the grasping hand and load force was measured from the proximal musculature of the reaching arm and shoulder. Within the TD group, age was inversely related to grip-to-load force onset latency and time to peak grip force; however, there were no similar age-related decreases between grip and load force for either the ASD or DD groups, suggesting that the impairments to motor adjustment on this task reflect a maturational delay, rather than an ASD-specific delay. In an earlier report, David et al. (56) examined grip and load force adjustments in a group of older, high-functioning children with ASD ($n = 13$; mean age 11.2 years). The adolescents were instructed to lift a target from a start position on load cell and place it on a target platform, approximately 6″ away. On this task, the ASD group had longer grip-to-load force onset latencies, greater grip force at movement onset, and more variable performance than TD controls. However, peak grip force and time to peak grip force did not differ between the ASD and TD groups. The children with ASD consistently did not respond until the load was removed, suggesting they were unable to use ongoing experience to anticipate upcoming unload force.

Adaptation tasks

Motor adaptation is the modification of a voluntary movement based on error feedback between repeated trials (57). To be considered "adaptation," the movement must change in respect to one or more parameters (e.g., force or direction), the change must occur gradually (i.e., over minutes to hours), and once these changes have occurred, the person must show "after-effects" and "de-adapt" the movement in a similar manner to return back to the original state (58). To understand the role of visual and proprioceptive feedback in motor adaptation in children with ASD, Masterton and Biederman (59) trained children with ASD ($n = 11$; mean age 10.4 years) and intellectual disability (ID; $n = 11$; mean age 11.6 years), as well as younger ($n = 11$; mean age 9.1 years) and older TD children ($n = 11$; mean age 14.1 years) to place a wooden block onto a target while viewing the target apparatus through a prism lens that displaced vision of their environment. Overall, the ASD and ID groups took longer to adjust their movements under the adaptation task, requiring almost double the amount of time to adapt to reaching with the prism glasses than both TD groups. Interestingly, transfer of motor adaptation of the reaching hand to the non-adapted (non-reaching) hand was found only for the ASD group. The authors suggest that the transfer of adaptation to the non-reaching hand is a clear indication that ASD children rely on proprioceptive, rather than visual information to complete the target-reaching task. It is possible that difficulty with processing sequential visual information may account for the ASD participants' motor execution impairments and consequent reliance on proprioceptive input.

Other experiments examining motor adaptation have not reported differences in adaptation rates between ASD and TD groups. Gidley Larson et al. (60) had high-functioning ASD ($n = 20$; mean age 10.9 years; 17 males) and TD ($n = 16$; mean age 10.8 years; 11 males) participants complete a ball-throwing task at baseline without prisms (pre-adaptation), while wearing prism goggles (adaptation), and again without prism glasses (post-adaptation). In contrast to the findings of Masterton and Biederman (59), the ASD and TD groups showed similar adaptation rates and adaptation effects on movement performance. With a sub-set of the same participants, Gidley Larson et al. (60) further explored adaption in ASD by asking participants to grasp the handle of a robot tool to move a cursor onto a target, which was presented on a screen. The view of the hand controlling the robot tool was blocked throughout the task. On some of the trials, a perturbation (force or visual) was given to assess for participants ability to plan alternate strategies. All children exhibited clear indications of adaptation and reached similar rates of adaptation to the force and visual perturbations, with no significant group differences on any of the measures. The discrepancy in findings may result from the simpler adaptation tasks in Gidley Larson et al. (60) (i.e., throwing a ball and moving a robot tool), compared to those of Masterton and Biederman (59), which required the grasping and placement of small blocks, a more cognitively taxing task.

Motor knowledge

The ability to calibrate our body to perform motor actions is referred to as affordance perceptions. When shaping our digits to grasp, we use a smaller aperture for a block to be obtained with a pincer grasp and a larger aperture for a block to be obtained with a power grasp when building our tower. Affordance perception contributes to successful performance of many motor and non-verbal social capabilities. For example, when participating in team sports, such as badminton, one needs to be able to calibrate his/her body to hit the shuttlecock lightly, compared to tennis, in which the ball needs to be hit with more force. Being able to adjust one's body allows for successful motor performance on both tasks. Linkenauger et al. (61) determined that adolescents with ASD poorly estimate their motor affordances when presented with a perceptual-motor integration task. Youth ($n = 12$; mean

age 11.1 years; all male) and adults with ASD ($n = 8$; mean age 22.4 years; all male), and age- and sex-matched TD controls were asked to estimate the maximum extension of their reaching arm (i.e., how far they could reach), as well as maximum digit aperture (i.e., the largest foam block their digits were able to grasp). Following their estimates, participants completed a reach distance task and grasping task to determine their maximal actual values. The ASD groups made drastically larger errors (17–20% for youth; 14–26% for adults) than the TD groups (3–5% for youth; 5–7% for adults), suggesting they overestimated their motor affordance. These findings raise the possibility that motor deficits in ASD could originate in the inability to use the motor system to determine action capabilities and utilize prior knowledge of our own capabilities to aid in planning and executing the task at hand.

To examine the relationship between action understanding and ASD, Cossu et al. (62) presented high-functioning ASD children ($n = 15$; mean age 8.1 years; 13 males) and two TD samples, one matched for chronological age (mean age was 8.7 years) and a second, for younger chronological age (mean age 4.9 years), with three tasks. The children watched a video clip and were asked to imitate actions (conventional or non-conventional actions on objects), produce pantomimes of actions (e.g., shown a tool and required to pantomime the correct action of the tool), or understand a pantomimed action (e.g., watch an actor mime an action without an object and point to the object "used" in the pantomimed action). The authors found that the children with ASD were significantly worse at imitating conventional actions on objects, imitating finger posturing, and imitating oral–facial gestures than both the younger and age-matched controls. The children with ASD performed similarly to the younger control group when identifying tools used in pantomimed actions, but both groups performed worse than the older TD group. The simultaneous impairment of action imitation, production, and comprehension of pantomime action suggests that the process of constructing an action motor representation is impaired in children with ASD. Critically however, is that language ability was not controlled for in these studies. It has been reported that the ability to imitate familiar gestures (such as conventional actions on objects) is correlated with language comprehension (63). Without controlling for language ability, one cannot rule out that the lack of imitation may be the result of reduced comprehension of the task requirements (64).

In summary, individuals with ASD appear to be impaired in both the online [i.e., use of ongoing sensory feedback; (25)] and offline control of movement [i.e., using memory from previous trials; (24, 56)], as well as in estimating their motor abilities (61). That is, they are unable to use both ongoing visual feedback, as well as information from a previous movement, to plan subsequent movements more effectively [also noted by Khan et al. (65)]. These impairments may result from deficits in the visual control of movement in ASD, resulting in an increased reliance on proprioceptive feedback to complete movements [as supported by adaptation transferring to the non-adapted hand for ASD only; (59)].

DISCUSSION

There is now robust evidence from early motor development, motor planning and execution, as well as motor correction that movement is impaired in ASD. Very young children display abnormal play with toys (e.g., spinning, flicking), less toy play, and atypically visually explore objects (29, 30). As they get older, children with ASD show impairments in motor planning, including delays in movement initiation and impairments when planning complex sequences of movements resulting in a comfortable end-grasp posture (17, 38, 42, 43), and impairments in motor execution, such as increased movement duration, end-point corrections at movement terminus, and desynchronization between components of a reaching movement (38–40, 50, 53). Impairments to online and offline corrections are also evident, as they are unable to use both ongoing visual feedback, as well as information from a previous movement to plan subsequent movements more effectively (24, 25, 56). One might postulate that abnormal toy play, including abnormal sensory control, in very young infants could interfere with subsequent opportunities for motor learning and may also impact social communication. For example, if a child has trouble in grasping an object, and continues to stare at it as he or she spins the object in his or her hands [as per Ref. (29)], the child in turn may spend less time showing the object to a parent or friend and engaging in other joint attention behaviors.

Are motor impairments and cognitive outcomes in ASD related? Findings linking motor ability to outcomes in individuals with ASD have been replicated numerously in the literature (66–68). For example, the transition to independent sitting is associated with greater variations in babbling (69), motor delays at 18 months of age are highly predictive of a diagnosis of ASD at 3 years of age in HR toddlers (70), and better motor performance in newly diagnosed 2-year-olds with ASD is associated with better future outcomes at 4 years of age (71). Although delays in motor and communication development may represent co-existing but relatively independent aspects of the ASD phenotype, there may be consequences of motor delays that impact on opportunities for developing and practicing social-communication skills. For example, if a child is delayed in sitting, and spends most of his or her time on the tummy, then he or she would have less time with the hands free to engage in reaching and grasping for objects, showing objects, and requesting objects than an infant who has matured to a sitting position. As such, the onset of these "social" behaviors may also be delayed. This is consistent with the findings of Libertus and Needham (72), who found that TD infants who engage in active, self-produced reaching movements also engage in spontaneous orienting to faces, whereas infants who engage in passive toy play (watching others play with objects) showed less spontaneous orienting to faces. Clearly, there are other factors that influence social-communication development, as well as examples of neurological conditions associated with severe motor impairments yet relatively preserved social skills [e.g., early onset neuromuscular disorders; (73)]. However, there is evidence that motor- and social-communication skills are correlated in ASD, both in the school age years (74) and in infancy (31). Moreover, gross and fine motor delays may be among the earliest identifiable signs distinguishing infants with ASD from their TD peers (75–77).

Impairment in object manipulation may also impact how others' actions are understood (51, 78–81). Evidence for this comes from findings that, during action observation, mu rhythm desynchronization is less evident in ASD. Mu rhythm is a pattern of

electrical activity that comes from the area of the brain that controls voluntary movement (primary motor cortex) when at rest. When large number of neurons synchronize in preparation for a movement, or when viewing an actor making a movement, the mu rhythm is described as "desynchronized" (82). Bernier et al. (83) found reduced mu rhythm desynchronization during movement observation in ASD, and reduced desynchronization was associated with poorer imitation skills. Similarly, Oberman et al. (84) report that, although individuals with ASD exhibit desynchronization of mu rhythm during voluntary movements, mu desynchronization is absent when observing an actor perform the same movement. Interestingly, the degree of mu desynchronization in ASD is sensitive to level of familiarity, only responding when individuals can identify with the stimuli in a personal way (85). The lack of a mu desynchronization response when observing an actor may result from an impaired mirror neuron mechanism (MNM) in ASD (62). Mirror neurons are involved in imitation of simple movements (86), learning of complex skills (87), in the perception of communicative actions (88), and in the detection of basic action intentions (89). Parietal mirror neurons code the goal of both an executed and observed motor act, such as grasping an object, and also code the overall intention of the action, whether the actor intends to bring the grasped object to the mouth or place it in a container (90–93). Deficits in the MNM have been reported during movement execution and observation for children with ASD [Ref. (94, 95); see review by Rizzolatti and Fabbri-Destro(96)]. As mentioned previously, Cattaneo et al. (53) employed EMG to record muscle activity related to mouth opening during an eating task in ASD. When observing an actor pick up a food item and transport it to the mouth, EMG increases in mouth muscles were found for the TD controls, but not for the ASD group. These results suggest that children with ASD have impaired mu desynchronization that may translate to a dysfunctional MNM. Such impairments may impact motor learning and action understanding, which may ultimately lead to misinterpretation of others' actions.

Although mirror neurons play an important role in action execution and observation (97, 98), they are unlikely to fully account for the myriad of motor impairments displayed by individuals with ASD. Pathological studies consistently report abnormalities in brain regions known to mediate motor function, including the cerebellum and subcortical white matter (99–106). The cerebellum is one of the key structures required to form accurate internal models of motor acts, making reciprocal connections with motor areas of the cortex to carry out planned corrections during movement execution (107, 108). As such, it is likely that cerebellar abnormalities play a role in movement correction impairments seen in ASD, as well as impairments to eye movements [such as prolonged staring; for a recent review of the role of the cerebellum in ASD, see Ref. (109)]. In addition, abnormal connectivity between adjacent primary sensory and motor areas has been reported in ASD (48, 51), and may account for impairments seen during the online control of movement (110). Moreover, reduced connectivity between more distal areas of the motor system, such as between visual and motor regions sub-serving action, may be responsible for impairments in motor planning and motor execution in individuals with ASD (48, 51).

How do motor impairments relate to social impairments? Typically, a child has a full repertoire of movement that he or she can use to engage in social interactions. With respect to the current review, the ability to properly manipulate objects is important for activities of daily living (such as brushing teeth), engaging in solo play activities (such as assembling a puzzle), and participating in team sports (such as baseball). Yet, many children with ASD have impaired motor behavior, detectable as early as 3 months of age (31). Being able to participate in peer play would require a child to respond in a timely manner (catch a ball before it hits you or the ground), perform skilled motor tasks (hitting a ball with a baseball bat), engage in eye contact (to understand and show action intention), and respond to social cues (understanding when it is appropriate to steal a base). Many of these behaviors are those that are impaired by ASD. Not surprisingly, Leary and Hill (20) propose that motor ability might have a significant impact on the core characteristics of ASD. The idea is, when a person is unable to respond to another's action in a timely fashion, he/she will miss the positive reinforcement associated with interpersonal interactions. A child's experiences throughout development may be drastically altered if, at an early age, he/she is unable to remain involved in social interaction, and as a result, may withdrawal from social activities [reported in Ref. (20)]. This "motor cognition" perspective does not imply that social impairments are a direct result of motor impairments, but rather that impaired movements may interfere with opportunities for positive social experiences and thus, social learning. Conversely, reduced social interaction opportunities may also contribute to poor action understanding. Thus, the relationship between social and motor competencies/impairments may be reciprocal in ASD, a hypothesis that remains to be explored in future longitudinal research.

There are common methodological limitations present in the literature reviewed here. First, many of the articles have relatively small sample sizes; Adams (36) sample consisted of only 4 children with ASD, Glazebrook et al. (46) recruited only 9 children with ASD, and sample sizes of individuals with ASD in the other studies ranged from as few as 8 (53) to as many as 36 (17). With the small sample sizes in several of the studies, there is a risk of participation bias in oversampling individuals with ASD who present with motor difficulties. Second, there is quite an age range in several of the experiments. The developmental course of motor development in individuals with ASD is not well understood, particularly when considering the timespan from toddlerhood to adulthood. Because of this, it is difficult to compare the results from one age group (i.e., young childhood) to another (i.e., adulthood). Third, there is a general lack in appropriate controls. When determining the ASD-specific deficits in movement, many studies report the use of TD control only. However, the results of Hughes (17) and David et al. (24) highlight the importance of including a control for intellectual or developmental level. Similarly, the results of Mari et al. (18) demonstrate the importance of stratifying intelligence when interpreting experimental results. Fourth, importantly, the severity of ASD symptomology varies across the studies reviewed here, and as such, the comparability of study conclusions might be constrained by the methodological limitations present in the literature.

Overall, there has been much research examining the relationship between social communication and motor behavior in ASD. To fully engage in social interaction, a child has a full movement repertoire of functional actions for use in communication and for understanding the communicative nature of others' movements (111). A shift in focus to this "motor cognition" perspective suggests that interventions for children with ASD should include both a motor and a social component, as there is ample evidence that impairments in cognitive function are associated with impairments in movement (70, 76, 112–115). Many activities that promote social skills, such as cooperative board games or card play that involve turn taking, require the use of fine motor skills (e.g., grasping small game pieces, shuffling cards). As such, incorporating motor training into intervention programs could boost confidence in action capabilities and promote socialization and communication.

AUTHORS CONTRIBUTION

Lori-Ann R. Sacrey made substantial contributions to conception and design of the review, collected and reviews the papers, prepared the first draft of the paper, and approved the final draft. Tamara Germani contributed to the conception of the review, provided a critical review of the manuscript, and approved the final draft. Susan E. Bryson contributed to the conception of the review, provided a critical review of the manuscript, and approved the final draft. Lonnie Zwaigenbaum contributed to the conception of the review, provided a critical review of the manuscript, and approved the final draft.

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Timing training in three children with diplegic cerebral palsy: short- and long-term effects on upper-limb movement organization and functioning

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Despite the great need of interventions to maintain and improve motor functions in children with diplegic cerebral palsy (DCP), scientific evaluations of existing training methods are rare. This study aimed to explore individual effects of synchronized metronome training (SMT) on motor timing, spatio-temporal movement organization, and subjective experiences of changes in upper-limb functions in three children with DCP. All children participated in an individualized 4-week/12 session SMT training regime. Measurements before training (Pre), after training (Post1), and at 6 months post completed training (Post2) were made by the applied SMT training equipment, optoelectronic registrations of goal-directed upper-limb movements, and a questionnaire assessing subjective experiences of changes in upper-limb functions and usability. In general, the training regime was shown to have little effect on motor timing. However, some positive changes in spatio-temporal movement organization were found. Two children also reported substantial long-lasting positive changes in subjective experiences of hand/arm functionality in terms of increased movement control and reduced muscle tone. For these children, parallel kinematic findings also indicated smoother and faster movement trajectories that remained at Post2. Although highly individualized, the shown improvements in upper-limb kinematics and subjective experiences of improved functionality of the hands/arms for two of the cases warrant further explorations of SMT outcomes in children with DCP.

Keywords: diplegic cerebral palsy, intervention, synchronized metronome training, motor control, kinematic, motor coordination, children

INTRODUCTION

The characterization of cerebral palsy (CP) has moved from mainly describing deficits in motor functioning to implicating multiple modalities including sensory, perceptual, and motor problems (1). In addition, therapeutic approaches to CP are beginning to move from an exclusive focus of limiting lower-level motor constraint to also addressing improvements of higher-level derived deficits such as problems with motor planning ability (2, 3). Accordingly, increased attention is being directed toward training methods that encompass improvements in action planning and connections between multiple modalities in children with CP, for example, timing and rhythmicity training or motion interactive games (4, 5).

Assuming that motor performance is mediated by an internal timing mechanism (6, 7), enhanced motor timing is expected to positively affect the performance and planning of motor actions. In line with this notion, repetitive and rhythmic movements have been shown to improve arm paresis following a stroke (8) and induce reorganization of motor networks within the central nervous system (9). Further, interventions based on rhythm perception and production and/or timing and rhythmicity training, such as rhythmic auditory stimulation (RAS), music intonation therapy (MIT), and the Interactive Metronome® (IM), have been reported to improve motor functions in a variety of clinical populations and functions [e.g., Ref. (10–13)]. For example, RAS has been found to re-establish healthy gait dynamics in patients with Parkinson's disease (14). Of particular relevance to the present study, two recent case studies involving chronic stroke patients (15) and children with hemiplegic CP (HCP) (5) observed positive effects of timing and rhythmicity training in terms of reduced arm impairment, increased functional ability, and better organized goal-directed upper-body movements. Taken together, these reports suggest that timing and rhythmicity training may contribute to increased brain communication, efficiency, and synchrony between brain regions related to motor functions, which leads to improved motor functions and better coordination of movements (16).

The IM is a synchronized metronome training (SMT) device thought to improve the execution of motor programs (17). To this end, the IM apparatus employs a metronome beat to set a rhythm that the participant uses to time motor tasks. A computerized guidance system provides auditory and/or visual feedback to the participant to illustrate the accuracy of synchronization between his/her motor performance and the cueing beat. IM training involves reducing the mean negative synchronization error during normal tracking of a regularly occurring auditory tone metronome beat. Thus, the IM method is targeted at practicing

motor planning and timing for enhanced temporal synchronization of movements. As such, the method appears favorable as an intervention for children with CP.

Although the IM and other training regimes seem theoretically promising, there are few studies to date that have used sensitive measurements to evaluate the potential effects of these training methods on performance and/or possible transfer effects to different functions. In this effort, kinematic analysis has been shown to be a promising tool (4) and has been used previously to identify positive short- and long-term effects of IM training in children with relatively mild HCP (5). The aim of the present study was to continue the latter exploration in three children with diplegic CP (DCP), at a more severe level of disability, to investigate whether a similar pattern regarding improved timing ability and potential long-term retention of effects in spatio-temporal movement organization could be observed in these cases following 4 weeks of IM training. Further, a questionnaire aimed at detecting subjective experiences of possible changes in the arms and hands with regard to muscle tone and functionality in daily living activities was administered.

MATERIALS AND METHODS

PARTICIPANTS

Participants included three children with DCP recruited locally through registration records at Kolbäcken Child Rehabilitation Centre in Umeå, Sweden (**Table 1**). Two participants (case II and III) received upper-limb botulinum toxin treatment but not in close occurrence to their respective individual training and testing period. One participant (case III) received post-surgery (lower limb) physical therapy training in parallel with participation. Informed parental and child consent was obtained, the study was approved by the Umeå Regional Ethical Board and conducted in accordance with the Declaration of Helsinki. In addition, kinematic data from one typically developing (TD) child (girl, 12 years) performing the goal-directed task at one measurement session were collected in order to provide the readers with an example of the task performance differences between DCP and TD shown in **Figure 2**.

APPARATUS AND PROCEDURE

A detailed description of the study design and methods can be found elsewhere (5). All participants underwent 4 weeks of individually adjusted IM training (12 sessions, ~30 min/session), supervised by a trained IM instructor (case I–II: AMJ, case III: assisting physical therapist). Due to the immobility of the legs of the participants, training only involved bilateral and unilateral rhythmic movements of the upper-limbs, with instant auditory feedback (guide sounds) of timing synchronization. Two baseline assessments (2 min clapping to a pre-set beat of 54 bpm with or without guide sounds) were executed at the start of each session and at Pre, Post1 (1 week), and Post2 (6 months). These assessments were used as a measure of individual changes in self-paced and auditory guided timing [deviation in milliseconds (ms) to the auditory signal] and rhythmic performance (variability of motor responses). Variability was measured as the mean deviation from an exact synchronization (regardless of the clap being late or early). Only registered sensor presses (i.e., successful claps) were used in the variability measure. Case II had nearly complete paresis of the right arm/hand, thus, training and baseline assessments were tailored to activate primarily the more functional left side. In addition, case II exhibited severe hypersensitivity toward the auditory presented guide sounds, resulting in spasticity and inability to perform the timing training. Consequently, guide sounds were not introduced until the later stages of the training when case II showed greater audio tolerance. Due to the varying abilities of the children they successfully completed different numbers of repetitions within their individualized IM training. In total, case I completed 13 011, case II 7 746, and case III 6 692 repetitions within their 12 sessions of IM training.

Three-dimensional (3D) kinematic recordings (six-camera, ProReflex, Qualisys Inc., Gothenburg, Sweden) of goal-directed upper-limb movements (pressing three light-switch buttons in a sequential order with a clenched fist) were made before (Pre) and at two occasions after the 4-week training period; Post1 ~1 week after concluded training and Post2 at 6 months after concluded training. For case I, markers were fixated with skin-friendly adhesive tape to the left and right shoulders (diameter: 29 mm), elbows (diameter: 19 mm), and wrists (diameter: 12 mm). For case II and III, markers were only attached to the preferred side (shoulder, elbow, wrist) and non-active shoulder (see **Figures 1A,B**, for the experiment condition, full marker set-up, and a matching 3D recording).

The data were sampled at a frequency of 120 Hz/s and the pre-set recording time was individually adjusted based on individual pre-practice and instruction trials that, if possible, were made with both hands. In the unimanual condition, the test paradigm involved performance with either the non-preferred (more affected) or preferred arm–hand (less affected) and with both arms–hands simultaneously in the bimanual condition, corresponding to a total of 36 trials. The participants were instructed to press the light-switch buttons in a sequential order starting on an auditory computer-generated signal. The sequential order was determined by a contra-balanced block design, where the children started from a specific point and pressed the three light-switch buttons starting from the bottom (and moving to the top, "extension"), top (and moving to the bottom, "flexion"), side (and moving inward, "adduction"), or center (and moving outward, "abduction") with either the right, left, or both hands (see **Figure 1A**). Due to a severely affected non-preferred side, case II

Table 1 | Participant demographics, hand and gross motor function, and comorbidities.

Case	Age (years)	Sex	MACS	GMFCS	Other diagnoses
I	12	F	II	III	ID, autism, epilepsy, visual deficit
II	16	M	IV	IV	ID, dysarthrosis, strabismus, visual deficit
III	13	M	III	IV	ID, CVI, partial epilepsy, strabismus, asthma, scoliosis

MACS, manual ability classification system; GMFCS, gross motor function classification system; ID, intellectual disability; CVI, cortical visual impairment.

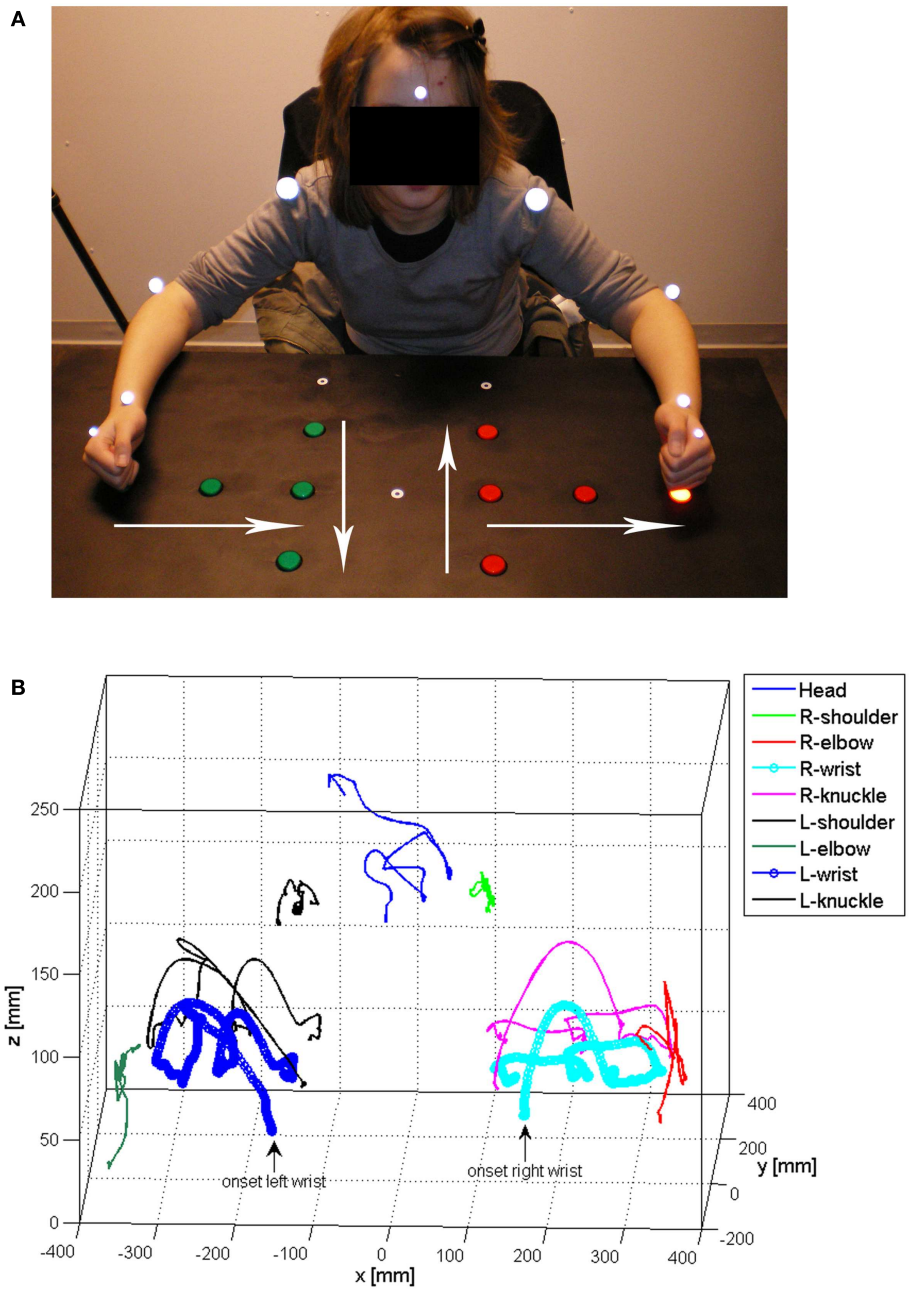

FIGURE 1 | Photo illustrating the experiment condition and the marker set-up (A), and an example of 3D movement registration (displacement of the corresponding markers) during (bimanual) light-switch task performance (pressing three light-switch buttons in a sequential, side-to-center order) (B). Starting positions of the hands are denoted by the small white circles at the lower end of the table in 1A and the white arrows denote the four directions studied (bottom–top, top–bottom, side–center, center–side).

and III only performed the task with the less affected side during testing (12 trials each in total) using the thumb (case II) or index finger (case III) as they were unable to form a clenched fist. The onset and offset of each trial were identified from the 3D movement trajectories (*X*, *Y*, and *Z* plane) and the tangential velocity of the wrist marker (see **Figures 2A,B**). On- and off-set were further verified by 2D video recordings that were synchronized with the optoelectronic recording system. The onset of the movement was determined as the frame when the wrist marker had a velocity of 20 mm/s and increased during the following five frames. The offset was defined as the frame when the wrist marker had a velocity of 100 mm/s and increased after the last successful light-switch button press.

Subjective experiences of IM training effects on upper-limb function with regard to muscle tone and functional ability in daily living were collected by means of a questionnaire before

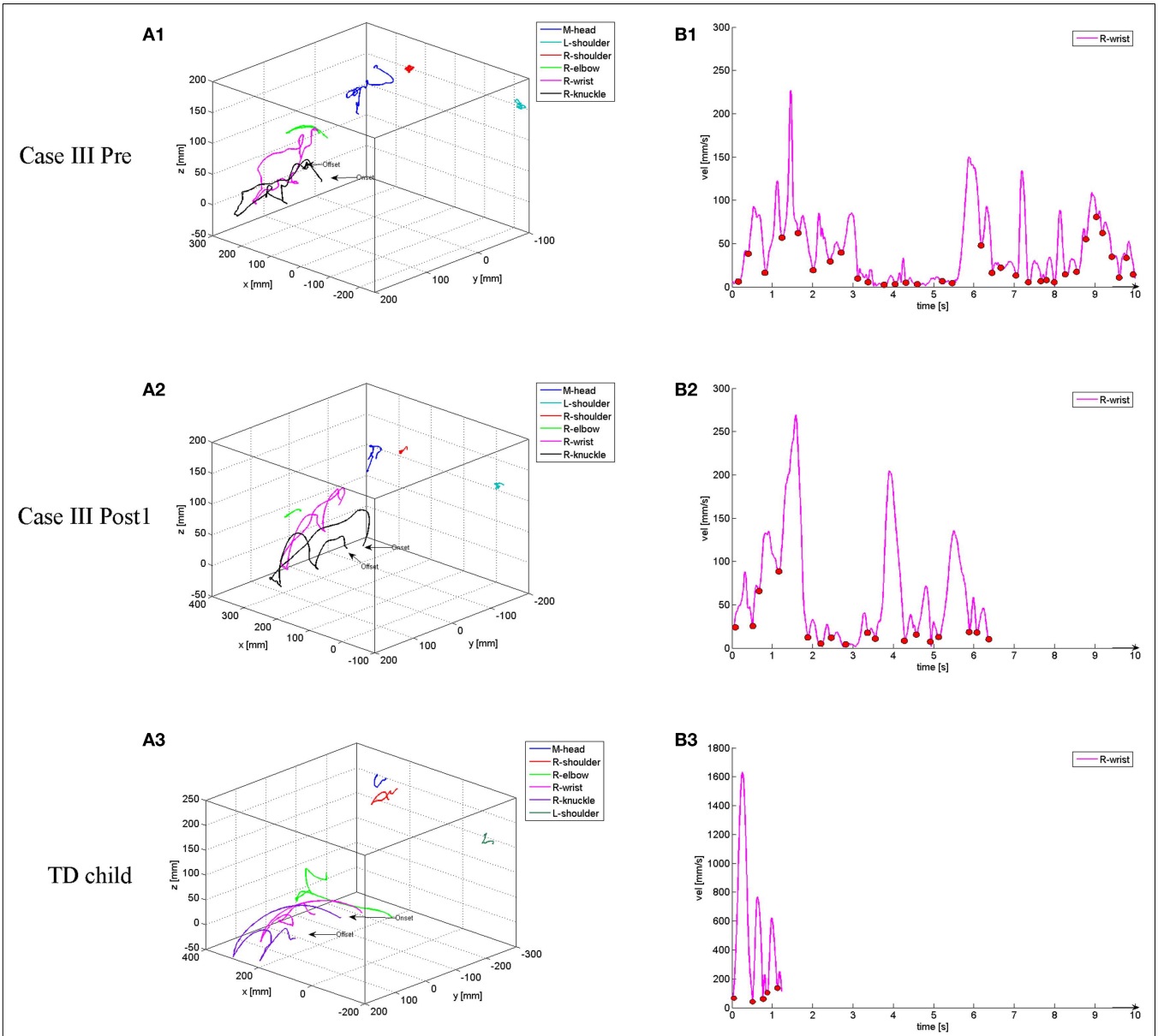

FIGURE 2 | Examples of 3D movement displacement during unimanual light-switch task performance (starting from resting and sequentially moving to the top button, the middle button, and the bottom button) with corresponding velocity profiles from the wrist marker for Case III at the Pre (A1,B1) and Post1 (A2,B2) occasion, and for a 12-year-old, typically developing (TD) comparison child (A3,B3). The small circles in the velocity profiles denote the start/stop of a movement unit (MU) corresponding to 31 MUs/10 s duration in B1, 16 MUs/6.4 s duration in B2, and 4 MUs/1.3 s duration in B3.

training commenced; directly after, and at 3 and 6 months after completed training. Participants were asked to judge changes as; (1) substantially positive, (2) somewhat positive, (3) unchanged, (4) somewhat negative, or (5) substantially negative, with possibility to give open-ended descriptions and examples of any changes in experience.

KINEMATIC DATA ANALYSIS

Prior to analyses, the kinematic data were smoothed using a second order 12 Hz Butterworth filter. Extracted parameters from the markers were the cumulative (3D) distance (accumulated movement distance) and the number of movement units (MUs, segmentation of movement trajectories) by use of customized MATLAB (The Mathworks Inc., Boston, MA, USA) scripts. A MU was defined as an acceleration phase followed by a deceleration phase with an accumulated increase or decrease in velocity of at least 20 mm/s and an acceleration or deceleration exceeding 5 mm/s^2 (18), exemplified in **Figure 2B**. Further, the duration of each individual task performance was identified and extracted (see **Figure 2B**). Before statistical analyses, all data were mean valued to

one light-switch button press as the number of successful presses varied between trials.

STATISTICAL ANALYSIS

Wilcoxon matched pairs tests with an alpha value of 0.025 were used to analyze differences in kinematic outcomes (based on trial level data) between Pre and Post1, and Pre and Post2. Effect sizes were derived using Pearson's correlation coefficient for significant results. For case I, no analyses by side were conducted due to an inadequate number of data points (report is thus based on data including both sides). Only significant test statistics and effect sizes of these results are presented. All mean values (M), standard deviations (SD), and significant effects are presented in **Table 2**.

RESULTS

CASE I

Training outcomes

Case I showed a modest improvement in self-paced timing ability (mean timing deviation from exact synchronization without guide sounds) from Pre to Post1 and a more pronounced improvement when guide sounds were included. The variability was lower with guide sounds (millisecond variability; Pre = 151; Post1 = 94; Post2 = 135) than without (millisecond variability; Pre = 542; Post1 = 284; Post2 = 375). The timing ability was not substantially changed from Post1 to Post2 (see **Figure 3A**).

Unimanual condition

A twofold increase in the number of MUs of the head at Post2 compared with Pre ($T = 3$, $p < 0.01$, $r = 0.10$) was shown. The 3D distance of the shoulder increased significantly between the Pre and Post2 occasion ($T = 4$, $p < 0.01$, $r = 0.51$). Similarly, the 3D distance of the elbow increased between Pre and Post2 ($T = 4$, $p < 0.01$, $r = 0.38$). Further, the 3D distance of the head increased from Pre to Post1 ($T = 23$, $p = 0.01$, $r = 0.12$) and Pre and Post2 ($T = 0$, $p < 0.01$, $r = 0.38$). See **Table 2**.

Bimanual condition

As in the unimanual condition, the number of MUs of the head increased significantly between Pre and Post2 ($T = 31.5$, $p < 0.01$, $r = -0.04$). The 3D distance of the shoulder ($T = 6$, $p < 0.01$, $r = 0.44$) and elbow ($T = 12$, $p < 0.01$, $r = 0.08$) increased significantly between Pre and Post1. Further, the increase in distance of the elbow remained at Post2 ($T = 23$, $p < 0.01$, $r = 0.04$). See **Table 2**.

Subjective experience of changes in arm and hand function

Case I reported no changes in muscle tone or the functionality of the arms and hands after completing IM training.

Case I summary

Although with large variability, case I showed a modest improvement in timing ability without guide sounds and a more substantial improvement with guide sounds. Although large, the variability in the timing responses did decrease between Pre and Post1, both in the self-paced and auditory feedback conditions. Some significant changes in kinematics were however shown, mainly from the Pre to Post2 tests. Generally, in both the uni- and bimanual condition the 3D distance increased with decreased variability.

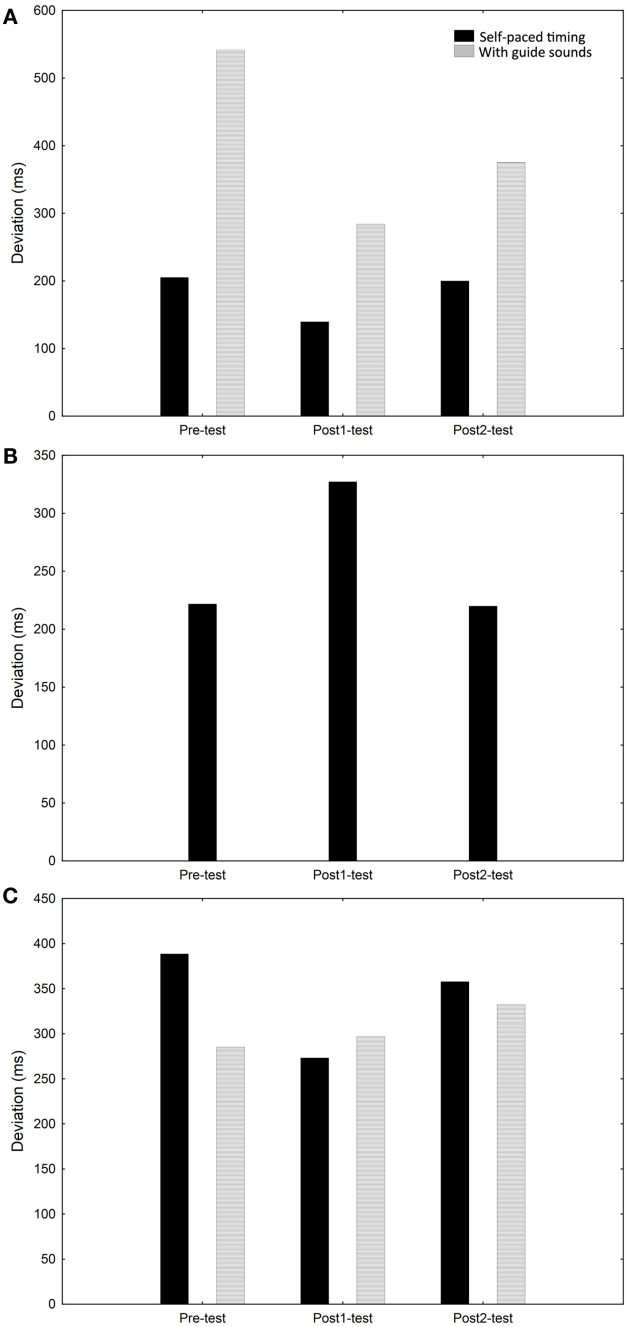

FIGURE 3 | Rhythmic and timing performance (self-paced and with guide sounds) for case I (A), case II (B), and case III (C) at the Pre, Post1, and Post2 occasions.

Specifically, the 3D distance of the head increased, as did the number of MUs in the unimanual condition. Case I reported no effects of the IM training on daily functionality or muscle tonus.

CASE II

Training outcomes

Case II showed no improvement in self-paced timing ability and displayed large variability (millisecond variability; Pre = 150;

Table 2 | Durations, MUs, and 3D distances for the cases presented by occasion.

	Unimanual								Bimanual							
	Pre		Post1			Post2			Pre		Post1			Post2		
	M	SD	D	*M*	SD	D	*M*	SD	*M*	SD	D	*M*	SD	D	*M*	SD
CASE I																
Duration (s)	1.4	0.3	+	1.5	0.3	+	1.6	0.7	2.3	0.4	−	2.3	0.4	+	2.4	0.6
MUs (*n*): shoulder	7.7	2.9	+	10.7	6.2	−	7.2	3.8	14.8	4.3	+	17.7	6.4	−	12.1	3.7
MUs (*n*): elbow	5.4	1.8	+	7.4	3.4	−	5.3	2.3	11.2	3.2	+	12.1	3.3	−	9.9	3.1
MUs (*n*): wrist	6.1	2.0	+	6.4	3.3	−	5.4	2.3	11.1	3.6	+	13.0	3.9	−	10.5	2.6
MUs (*n*): head	5.2	2.5	+	7.3	5.3	+	**10.5**	**4.3****	8.8	1.9	+	9.8	2.3	+	**12.0**	**4.9****
3D distance: shoulder	73	25	+	103	63	+	**77**	**22****	108	38	+	**170**	**45****	+	118	34
3D distance: elbow	152	57	+	200	134	+	**182**	**48****	184	43	+	**238**	**39****	+	**235**	**42****
3D distance: wrist	205	47	+	248	87	+	232	89	268	52	+	276	70	+	307	54
3D distance: head	58	23	+	**104**	**70****	+	**105**	**71****	164	67	+	198	43	+	195	46
CASE II																
Duration (s)	5.3	1.4	−	4.4	0.4	−	**3.4**	**1.3***								
MUs (*n*): shoulder	30.0	10.5	−	28.1	6.0	−	**17.8**	**9.8****								
MUs (*n*): elbow	32.7	12.7	−	31.7	12.4	−	**18.0**	**11.7***								
MUs (*n*): wrist	25.0	7.6	−	22.5	7.1	−	**14.3**	**9.3****								
MUs (*n*): head	19.7	7.5	+	22.4	5.6	+	22.8	17.5								
3D distance: shoulder	96	16	+	118	52	−	92	49								
3D distance: elbow	234	49	+	328	177	−	187	107								
3D distance: wrist	328	57	+	392	140	−	290	181								
3D distance: head	373	96	−	245	178	−	**154**	**90***								
CASE III																
Duration (s)	5.5	0.8	−	3.2	0.4	−	**3.1**	**0.6***								
MUs (*n*): shoulder	58.8	31.7	−	**23.1**	**8.4****	−	**22.7**	**6.2****								
MUs (*n*): elbow	47.9	27.8	−	**24.8**	**9.3***	−	**19.7**	**4.7***								
MUs (*n*): wrist	31.5	14.4	−	**13.0**	**5.5****	−	**12.4**	**2.8****								
MUs (*n*): head	49.1	19.8	−	**18.5**	**7.9****	−	**23.0**	**4.7****								
3D distance: shoulder	76	29	−	73	23	−	42	17								
3D distance: elbow	103	21	+	114	83	−	76	22								
3D distance: wrist	152	41	+	184	29	+	161	61								
3D distance: head	108	29	−	103	42	−	90	50								

M, mean; SD, standard deviation; D, direction of change relative to the pre-test (+, increase; −, reduction); s, seconds; MU, mean number of movement units. Significant differences relative to the pre-test occasion are indicated in bold.

**p < 0.025, **p < 0.01.*

Post1 = 163; Post2 = 161). The self-paced timing ability was slightly worse at Post1 versus Pre but improved again slightly at Post2 (see **Figure 3B**).

Unimanual condition

As shown in **Table 2**, a significant reduction in movement duration was apparent between Pre and Post2 ($T = 3$, $p < 0.025$, $r = 0.16$). The number of MUs of the shoulder ($T = 3$, $p < 0.01$, $r = 0.14$), elbow ($T = 5$, $p < 0.025$, $r = 0.21$), and wrist ($T = 11$, $p < 0.01$, $r = 0.23$) was also reduced significantly from Pre to Post2. Regarding the 3D distance of the head, a reduction between Pre and Post2 was apparent ($T = 0$, $p < 0.025$, $r = 0.17$).

Subjective experience of changes in arm and hand function

Directly after completing IM training, case II reported a substantial improvement with regard to movement ability of the less affected arm and hand (the trained side). Further, the more affected arm and hand were perceived as having substantially less muscle tone, a somewhat improved usability in leisure activities, and a substantial improvement in movement ability. These changes generally remained at the 3- and 6-month follow-up.

Case II summary

Case II showed some variability in timing performance during training and at the Pre, Post1, and Post2 test occasions, while no clear improvement in timing ability was shown. The movement duration decreased and some significant changes in terms of kinematics were also shown for this participant, all emerging at Post2. MUs of the shoulder, elbow, and wrist decreased, as did the relative 3D distance of the head. Interestingly, although no real improvements were shown in timing and the effects on movement kinematics did not emerge until the Post2 test occasion, case II

reported substantial improvements in movement ability and muscle tone directly after the training period and, although slightly less substantial, after 6 months. More importantly, these changes allowed case II to engage more in leisure activities and to use the less affected arm in daily living situations.

CASE III

Training outcomes

Case III showed marginal improvements in self-paced timing ability but not with guide sounds. The variability was stable over the occasions both with (millisecond variability; Pre = 159; Post1 = 181; Post2 = 174) and without (millisecond variability; Pre = 187; Post1 = 183; Post2 = 169) guide sounds. The changes in timing ability from Pre to Post1 were not maintained at Post2 (see **Figure 3C**).

Unimanual condition

The movement duration was significantly reduced between Pre and Post2 ($T = 0$, $p < 0.025$, $r = 0.71$). The number of MUs was reduced between Pre and Post1 of the shoulder ($T = 1$, $p < 0.01$, $r = 0.29$), elbow ($T = 3$, $p < 0.025$, $r = 0.59$), wrist ($T = 1$, $p < 0.01$, $r = 0.39$), and head ($T = 1$, $p < 0.01$, $r = 0.21$). These reductions in movement segmentation remained at Post2 (shoulder: $T = 1$, $p < 0.01$, $r = -0.09$; elbow: $T = 3$, $p < 0.025$, $r = -0.49$; wrist: $T = 1$, $p < 0.01$, $r = -0.30$; head: $T = 1$, $p < 0.01$, $r = -0.06$). See **Table 2**.

Subjective experience of changes in arm and hand function

Case III reported that the less affected arm and hand had somewhat less tone, somewhat improved usability in dressing, feeding, and leisure activities, and somewhat improved movement ability directly after the IM training had concluded. The largest changes, as reported in the open-ended questions, related to movement control, speed, and motivation to activate the hand and arm. The changes that were reported to remain at the 3- and 6-month follow-up were also mainly related to motivation, movement control, and speed.

Case III summary

Case III showed a marginal improvement in timing ability with large millisecond deviation from exact synchronization with the metronome and large variability. In the kinematic task, movement duration decreased gradually between Pre and Post2. In terms of kinematic outcomes, a substantial reduction in the number of movement segmentations between Pre and Post1 was apparent and these remained at Post2. The kinematic results suggest large effects on temporal aspects of movement trajectory while subtle and variable changes could be noted on spatial parameters. Case III experienced some meaningful changes in muscle tone and movement ability, which improved elements of daily living ability. The most persistent changes were reported to be related to motivation, movement control, and speed.

DISCUSSION

The IM training regime aims at facilitating underlying neural processing capacities to improve the execution of motor programs (17). This case study was aimed at exploring the effects of 4 weeks of IM training on timing and rhythmic ability with the arms and hands, planning and spatio-temporal organization of goal-directed upper-limb movements, and the subjective experience of effects on muscle tone and functional ability in daily living in three children with a DCP diagnosis. Of interest was also to investigate the existence of long-term retention of possible effects. Using the same study design and methods as in the present study, we have previously shown that two children with HCP displayed long-lasting motor learning as manifested by remaining timing ability and significant Pre–Post advances in spatio-temporal movement organization following IM training (5). The interpretations of the results from the current study are, however, less straightforward.

SYNCHRONIZED METRONOME TRAINING OUTCOMES

In the present study, all cases showed relatively poor initial timing ability with high variability and either modest, marginal, or no convincing improvements at Post1 and Post2. However, an indication of motor learning was apparent for case I who showed some reduction in variability at Post1, both with and without guide sounds, where also a reduction in the millisecond deviation from exact synchronization with the metronome was apparent. Although these effects did not remain at Post2, the results can be regarded as an indication of motor learning during the active phase of training. Perhaps, a longer training period would be preferable for this case. It is also possible that an increased amount of training that included more repeated activations would have improved the timing ability for case II and III. In our previous study including two children with HCP, a relatively good initial timing ability and an evident and stable improvement in timing, both self-paced and with guide sounds, were shown (5). Other studies have shown similar results at a group level in children with ADHD (19), mixed attentional and motor coordination disorders (12), children with no known disability (10), and in skilled golfers (20). Another possible explanation for the present findings is thus that the IM equipment may fail to detect changes in timing and rhythmicity in cases with severe biomechanical constraints.

In general, the present findings suggest that the efficacy of IM training is dependent upon the severity of the child's condition and the specific constraints that this imposes. Specific constraints may be located in the interpretation and amount of information derived from sensory input as well as in proprioception and the control over muscle groups needed for successful performance (21). A specific constraint may be found in the emergent timing properties of trajectory control that are requested by the SMT method applied. This is relevant because most movements in IM training should be smooth, continuous, and circular in fashion (e.g., hand clapping with circular motions). In such conditions, timing is a by-product that emerges from the dynamics of trajectory control (22) where inability to produce movements with emergent timing elements may be a specific limitation that might account for the relatively poor timing performance of the participants. Further, sources of constraints may be found in the presence of ID, visual deficits, and the diagnosis of autism, which of course poses special consideration of training and outcomes. As such, the training equipment used in this study may not have an optimal design to meet the specific individual needs in order to maximize its accessibility for the participants.

SPATIAL AND TEMPORAL KINEMATIC PROPERTIES

Although limited improvements in timing ability were shown for all cases, some significant changes in movement kinematics were found. Most kinematic studies of upper-body motor functions in children with CP have focused on HCP (2, 3, 23–29) and investigations into these abilities in children with DCP are sparse. In the current study, substantial reductions in movement segmentations (MUs) were shown for both case II and III. Smoothness of movement trajectory has been shown to have high test–retest reliability when investigating a reach-and-grasp task in children with varying degrees of CP (30) and can be used as a measure of both biomechanical functionality and motor planning ability (27). For case II and III, little change was detected on spatial properties of the movements, suggesting that the increase in smoothness of the movement trajectories has a more temporal character. An accompanying increase in movement speed was also apparent. Taken together, these findings suggest that case II and III showed improvements in motor control and/or planning ability after the IM training. The effects emerged at Post2 for case II and at Post1 for case III, with remaining effects at Post2 for case III, thus indicating a possible reorganization of movement representations in the motor cortex as an effect of SMT. Case I on the other hand showed pre- to post-test increases in MUs of the head in both the bi- and unimanual condition. This finding could be interpreted as an expression of compensatory strategies by means of increased looking. It could, however, alternatively indicate difficulty attending to the task. At the same time, the 3D distances of the (proximal) shoulders and elbows increased, whereas no significant changes over test occasions were detected for the (distal) wrists regarding both 3D distance and MUs. Thus, augmented head and proximal movements did not seem to affect the more distal reaching strategy in case I, suggesting no alternation of the underlying movement representations (and planning) related to the end-motion trajectories as an effect of SMT.

SUBJECTIVE EXPERIENCE OF TRAINING OUTCOME

Despite that no convincing changes were detected in synchronization ability, substantial subjective immediate and long-term improvements were reported in relation to muscle tone, arm/hand functionality, and usability by case II and III. These effects are in line with the more considerable improvements shown in terms of movement organization for these cases.

SUITABILITY OF IM TRAINING IN CHILDREN WITH DCP

Given the results shown in this case study, the IM training regime appears to be a feasible method for upper-limb timing training in children with DCP. However, it is a poor instrument for detecting changes in rhythmic ability and its accessibility seems to be somewhat limited for children with more severe types of CP. For the children participating in the present study, it is plausible that the repeated activation element, rather than the training of synchronization embedded in IM training, was driving the changes detected. Further, the individual biomechanical constraints and co-occurring sensory–motor, cognitive, and neuropsychiatric diagnoses likely reduced the accessibility to the IM training regime. Thus, when considering timing training for children with DCP, it is recommended that special attention should be given to individual needs and abilities and efforts should be made to improve accessibility. Previous studies reporting positive effects of timing training have mainly investigated clinical cases with unilateral brain lesions such as individuals with chronic hemiparetic stroke (8, 15) and HCP (5). On a speculative note, it is possible that this is due to bimanual timing training facilitating effects from the non-paretic to the paretic side. In the case of bilateral brain lesions (involved in DCP), effects may not be underpinned by a similar bilateral transfer of skill.

LIMITATIONS OF THE PRESENT STUDY

Apart from obvious limitations, such as the cases being few and heterogeneous, there are some additional limitations to the present study that need to be addressed. Firstly, the use of two different instructors for the training sessions could have affected the outcome of the training. Given that the training was carried out in accordance with the manual provided by the IM, however, the effect of the instructor should be minimal. Secondly, the IM device does not allow extraction of data other than overall mean values. Unfortunately, this makes the quantification of deviation from the beat in the timing task less optimal. Thirdly, although the participants had to struggle with the simple light pressing task due to the severity of their CP and performed relatively few trials, there is a possibility that improvements at the post-intervention sessions could be related to increased familiarity/practice with the task itself. With this in mind, the use of multiple pre-tests would have strengthened the present design. Alternatively, to control for such potential learning effects in more functionally adept individuals, cases could be habituated to the task prior to the start of the intervention.

CONCLUDING REMARKS

While the effects of IM training on motor timing were unconvincing, several promising changes in kinematic outcomes and functionality could be observed for two of the cases. This case study highlights the importance of developing accessible and individualized training methods that can accommodate the complexity of function that is always associated with early brain lesions that cause CP. Further, the kinematic outcomes pinpoint the importance of developing sensitive measures that are adjustable to the individual competencies of the child with CP. By adopting such an approach, more refined and systematic evaluations of training programs can be made, allowing a better scientific justification of different therapeutic interventions. Based on the current findings, further research investigating the effects of SMT methods in children with DCP are warranted. In future studies, it would be advisable to use larger samples with a case–control design and a dose–response SMT paradigm to maximize individual effects. Additionally, it would be relevant to study the effects of introducing SMT training at an earlier age.

AUTHOR CONTRIBUTIONS

Anna-Maria Johansson contributed in conceptualizing and designing the study, was in charge of the IM training and collected the data, carried out the data analyses and interpreted them, prepared the first draft of the paper, and approved the final draft as submitted. Erik Domellöf contributed in conceptualizing and

designing the study, took part in and supervised the data collection, participated in the data analyses and their interpretation, co-wrote, reviewed and revised the manuscript, and approved the final draft as submitted. Louise Rönnqvist contributed in conceptualizing and designing the study, took part in and supervised the data collection, participated in the data analyses and their interpretation, co-wrote, reviewed and revised the manuscript, and approved the final draft as submitted.

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"Left neglected," but only in far space: spatial biases in healthy participants revealed in a visually guided grasping task

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Hemispatial neglect is a common outcome of stroke that is characterized by the inability to orient toward, and attend to stimuli in contralesional space. It is established that hemispatial neglect has a perceptual component, however, the presence and severity of motor impairments is controversial. Establishing the nature of space use and spatial biases during visually guided actions amongst healthy individuals is critical to understanding the presence of visuomotor deficits in patients with neglect. Accordingly, three experiments were conducted to investigate the effect of object spatial location on patterns of grasping. *Experiment 1* required right-handed participants to reach and grasp for blocks in order to construct 3D models. The blocks were scattered on a tabletop divided into equal size quadrants: left near, left far, right near, and right far. Identical sets of building blocks were available in each quadrant. Space use was dynamic, with participants initially grasping blocks from right near space and tending to "neglect" left far space until the final stages of the task. *Experiment 2* repeated the protocol with left-handed participants. Remarkably, left-handed participants displayed a similar pattern of space use to right-handed participants. In *Experiment 3* eye movements were examined to investigate whether "neglect" for grasping in left far reachable space had its origins in attentional biases. It was found that patterns of eye movements mirrored patterns of reach-to-grasp movements. We conclude that there are spatial biases during visually guided grasping, specifically, a tendency to neglect left far reachable space, and that this "neglect" is attentional in origin. The results raise the possibility that visuomotor impairments reported among patients with right hemisphere lesions when working in contralesional space may result in part from this inherent tendency to "neglect" left far space irrespective of the presence of unilateral visuospatial neglect.

Keywords: pseudoneglect, visuospatial neglect, attention, human, peripersonal space, reach-to-grasp, handedness

INTRODUCTION

Successful action and interaction with the environment are dependent on correctly perceiving the space around us as well as the objects within that space. In our daily lives we interact with and manipulate objects which are nearby; for example, picking up a glass of water at the dinner table. We also interact with objects which are further away by moving to the target, changing posture, or using a tool to bring the object within working space. Accordingly, space is typically behaviorally differentiated into peripersonal and extrapersonal space. Peripersonal space is commonly defined as the space immediately surrounding the body in which hand and arm actions on objects can be performed most effectively (1). In contrast, extrapersonal space refers to the space beyond peripersonal space (2). Interactions with an object in extrapersonal space would require a person to physically move toward the object, or the object would need to be moved toward the person. Impairments of spatial perception can have a devastating effect on our functional independence and quality of life.

A relatively common acquired disorder of spatial perception is hemispatial neglect which is characterized by deficits in the ability to respond to, orient toward, and attend to stimuli presented in contralesional (typically the left side of) space despite intact basic motor and sensory functions (3). A number of clinical tests are commonly used to assess the presence, severity, and progression of neglect, including line bisection tasks, target cancelation, target detection, and drawing and copying tasks. These tasks are normally completed in peripersonal space with neglect patients (following right hemisphere stroke) typically displaying a rightward bias of veridical midpoint in the line bisection task, decreasing target detection from right to left in the target cancelation and detection tasks, and a drawing which is incomplete on the left hand side in the copying task (4–10). Double dissociations of neglect symptoms have, however, been reported between contralesional peripersonal and extrapersonal space for the line bisection and task cancelation tasks. In some patients the rightward bias is present only in peripersonal space and is attenuated or extinguished in extrapersonal space, conversely other patients show more severe neglect in extrapersonal space than in peripersonal space (11–17). Dissociation between peripersonal and extrapersonal space has also been observed amongst neurologically intact adults when using

the line bisection task. In contrast to neglect patients, however, healthy adults typically display a systematic leftward displacement of the line midpoint from true center when completing the task in peripersonal space, a phenomenon which is commonly referred to as *pseudoneglect* (18, 19). While some studies have failed to find an effect of distance (i.e., peripersonal vs. extrapersonal space) in *pseudoneglect* (14, 20) other studies have reported that when working in extrapersonal space, healthy participants display similar rightward shifts of bisection as patients with neglect (21–25). Collectively, these observations suggest a functional and neural dissociation between the coding of near and far space in humans.

Despite the apparent simplicity of the target cancelation and line bisection tasks, they are both complex activities in which perceptual and motor factors are generally implicated. While the severe perceptual deficits experienced by people living with hemispatial neglect have been extensively studied and well documented [see Ref. (26–28) for review], the presence, direction, and severity of visuomotor impairments, particularly in contralesional space, is less clear. Numerous studies have reported visuomotor difficulties that parallel the perceptual impairments of neglect patients (29–35), while other studies have shown normal (or near normal) visuomotor performance in reaching and grasping tasks on both sides of space amongst neglect patients (36–39). Methodological considerations including the common omission of a patient group with right hemisphere lesions but without neglect continue to contribute to the ongoing controversy surrounding visuomotor performance amongst individuals with neglect (40); however, thus far, an inimitable explanation for the divergence in the literature has yet to be determined. It is necessary to first establish the nature of space use and potential spatial biases during goal-directed visually guided actions amongst healthy individuals before we can fully understand the presence, severity, and ultimately the rehabilitation and treatment of visuomotor deficits in patients with neglect.

Accordingly, the purpose of the present series of studies was to characterize space use during an ecologically valid visually guided grasping task in healthy adults. The task involved reaching for and grasping building blocks scattered on a tabletop in order to replicate a series of 3D models (41, 42). The tabletop was notionally divided into equal size quadrants differentiated into left and right hemispace and near and far reachable space (left near, left far, right near, and right far). The blocks necessary to build each model were available in each of the quadrants (i.e., equivalent characteristics for each quadrant). The grasping task was conducted amongst right-handed (*Experiment 1*) and left-handed (*Experiment 2*) participants, allowing us to determine whether handedness plays a role in the patterns of space use. The experiment was subsequently repeated amongst right-handed participants fitted with eye tracking glasses (*Experiment 3*) allowing us to investigate whether spatial biases observed during the grasping task were attentional in origin.

EXPERIMENT 1: RIGHT-HANDERS

MATERIALS AND METHODS

Participants

Sixteen self-reported right-handed participants were recruited from the University of Lethbridge student population to take part in *Experiment 1* (six males; 18–35 years). Participant gender was not balanced, as gender differences have not been reported in earlier studies involving a similar task (43). The study was performed with approval by the University of Lethbridge Human Subject Research Committee. Written informed consent was provided prior to the initiation of the study. Participants were naïve to the purposes of the study.

Apparatus and stimuli

Handedness questionnaire. Participants completed a modified version of the Edinburgh (44) and Waterloo (45) handedness questionnaires upon completion of the building block task. This modified handedness questionnaire included questions on hand preference for 22 different activities, with participants identifying which hand they prefer to use for each activity [see Ref. (42) for complete description].

Block building task. Participants were instructed to construct a total of eight models; four using MEGA BLOKS® and four using LEGO® blocks (ranging in size from <0.7 L $\times$ 0.7 W $\times$ 1.0 cm H to 6.3 L $\times$ 3.1 W $\times$ 2.0 cm H). Each model was constructed from 10 blocks, which varied in color, size, and/or shape (for a total of 40 blocks per set of 4 models). The blocks for one set of four models were distributed within the workspace (70 L $\times$ 122 W $\times$ 74 cm H) which was notionally divided into equal sized quadrants demarcated by left (LEFT) and right (RIGHT) hemispace, as well as near and far reachable space. Near reachable space (NEAR) was defined as the space within reach of either hand without trunk flexion (approximately 0–35 cm), whereas far reachable space (FAR) was the workspace beyond the limits of actable space without trunk flexion (approximately 35–70 cm). These limits were adjusted for each participant to account for body/arm length. Each participant sat on the chair in front of the table and was asked to fully extend his/her arms (without trunk flexion). The point on the table at which the tip of the fingers reached was considered the limit of NEAR and the beginning of the FAR reachable space. The outer boundary of FAR space was such that it represented the furthest reachable space with trunk flexion and full arm extension (approximately 70 cm). There were no visible demarcations in the workspace that would cue participants that space use was the variable of interest. One set of the same 10 blocks necessary to complete a single model was randomly distributed into each quadrant of the workspace (**Figure 1A**); participants were unaware of this manipulation.

Procedures

Participants were seated centrally in front of the table (122 L $\times$ 122 W $\times$ 74 cm H) at a normalized distance such that when the arms were fully extended the fingertips would reach the notional division between NEAR and FAR reachable space. Consequently, a change in posture (i.e., trunk flexion) was necessary in order to grasp the blocks in FAR (reachable) space. The first model to be replicated was placed on a base plate located centrally at the far junction between left and right space (**Figure 1A**). Participants were requested to replicate the displayed model as quickly and accurately as possible on a second base plate (19 L $\times$ 19 W cm) located centrally immediately in front of the participant (at the intersection of right and left space; **Figure 1A**) from the blocks distributed on the tabletop. No further instructions were provided.

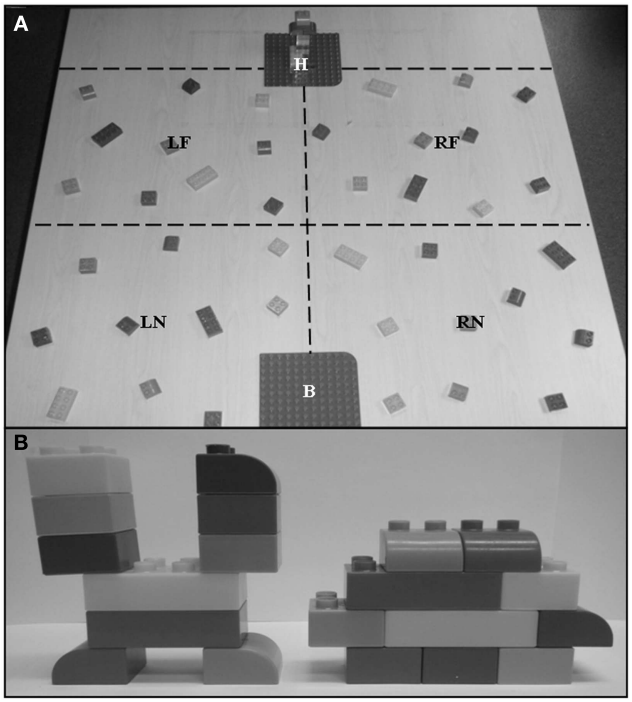

FIGURE 1 | Experimental set-up. **(A)** Example of workspace prior to first trial. Red dashed lines notionally divide the workspace into quadrants; left near (LN), left far (LF), right near (RN), and right far (RF) reachable space. Model to be replicated is located on far base plate "H" positioned between LF and RF quadrants. Model to be constructed on near base plate "B" positioned between LN and RN quadrants. **(B)** Examples of 10-piece models. Workspace set-up and models are illustrated with MEGA BLOKS®.

As such, participants were free to use either or both hands to grasp blocks, construct, and stabilize the model. Prior research (42) using the task has highlighted the bimanual nature of the task, however, no specific instruction as to hand use were provided. Following replication of the sample model, both models were removed from the table and a new model to be replicated was provided, this process was repeated until a set of four models had been completed. Building blocks were not replaced between trials, but were replaced between each set of four models. The same eight models were used for each participant (see **Figure 1B** for examples); model order was randomized between participants.

The total time taken to complete each trial (i.e., search and construction) was recorded using a stopwatch. In addition, model construction was recorded for subsequent analysis using a digital video camera (JVC HD Everio®) placed directly in front of the participants (approximately 160 cm away from participant) with a clear view of the workspace, building blocks, and participants' hands.

Data processing and analysis

All video recordings were analyzed offline. Each grasp was scored manually as a left- or right-handed grasp to ipsilateral or contralateral space. The total number of grasps was also calculated to allow the determination of the percentage of right hand use [(number of grasps with right hand/total number of grasps) × 100]. In addition, the videos of the construction of the first and fourth model in each model set were manually scored to provide the number of building blocks removed from each quadrant for each model. Model 1 provides information on space use when there is equal opportunity to grasp blocks from any quadrant of space while Model 4 offers data on the space attended to (i.e., grasped from) last. To provide a more detailed indication of space use, participant grasps were numbered in the order of occurrence (1–40) and that number was allocated to the appropriate quadrant. Each set of four models yielded a sum grasp total of 820 for the 40 blocks. The minimum possible grasp total for a quadrant was 55, which would indicate that all 10 blocks for the first model (grasps 1–10; $1+2+3+4+5+6+7+8+9+10=55$) were selected from the same quadrant. The highest possible grasp total for a quadrant was 355, indicating that all 10 blocks for the fourth model (grasps 31–40; $31+32+33+34+35+36+37+38+39=355$) were selected from the same quadrant. Within a quadrant, a grasp total between 55 and 355 would indicate that the blocks in that quadrant were selected over the course of more than one model. Lower numbers indicate that blocks from that quadrant were grasped earlier in the construction of the model set; higher numbers indicate that blocks were generally grasped later in model construction. Data were averaged across model sets.

Data were analyzed using SPSS Statistics 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $\alpha = 0.05$ unless otherwise stated. Effect size (ES) was reported as η^2 values. Handedness questionnaire and hand use data were summarized descriptively. The percentage of contralateral grasps made with each hand over the course of model set construction was assessed using paired samples *t*-tests. Trial times were entered into a one-way repeated measures analyses of variance (RM ANOVA), with Models 1–4 as a within-subject factor. When statistical significance was reported Bonferroni corrected pairwise comparisons were performed between all model pairs ($p \leq 0.008$). Space use data for the first and fourth models (based on blocks used) were entered into separate two-factor (hemispace × distance) RM ANOVAs, with hemispace (LEFT, RIGHT), and distance (NEAR, FAR) as within-subject factors. Bonferroni corrected pairwise comparisons were performed between the near space quadrants (LN and RN) and the far space quadrants (LF and RF) pairs ($p \leq 0.025$) when statistical significance was established. Similarly, overall space use as determined by grasp total scores was analyzed using a two-factor (hemispace × distance) RM ANOVA. Subsequently, Bonferroni corrected planned pairwise comparisons were performed between left far (LF) space and left near (LN), right near (RN), and right far (RF) space ($p \leq 0.017$).

RESULTS

Handedness questionnaire

All participants self-reported as right-handed; this was confirmed by the handedness questionnaire score. The average handedness questionnaire score was $+34.8 \pm 4.9$ (scores ranging from +22 to +41) where +44 would indicate exclusive right hand use for the identified activities (−44 would indicate exclusive left hand use).

Trial times

Trial times were significantly affected by the model being constructed [$F(3,45) = 4.922$, $p = 0.005$, ES = 0.247], with participants completing the final model (Model 4) significantly faster than the first [$t(15) = 4.724$, $p < 0.001$] and third [$t(15) = 3.653$, $p = 0.002$] models.

Hand use for grasping

Overall, participants used their dominant right hand for 69.5 ± 13.9% of all grasps. Analysis of contralateral grasps showed that participants used their left hand significantly less than their right hand [$t(14) = 5.488$, $p < 0.001$] when grasping in contralateral space (right hand = 21.9 ± 12.8%; left hand = 2.4 ± 1.73%).

Space use

First model. Participants grasped 6.5 ± 1.5 blocks from right and 3.5 ± 1.5 blocks from left hemispace to construct the first model, resulting in a significant main effect of hemispace [$F(1,15) = 20.932$, $p < 0.001$, ES = 0.583; **Figure 2A**]. A significant main effect of distance [$F(1,15) = 21.867$, $p < 0.001$, ES = 0.593; **Figure 2A**] revealed that when constructing the first model participants grasped more blocks from near reachable space than from far reachable space (NEAR = 6.5 ± 1.7 blocks; FAR = 3.5 ± 1.7 blocks). The interaction between hemispace and distance was not significant ($p > 0.05$).

Fourth model. When constructing the fourth model, participants grasped more blocks from left space when compared with right space as indicated by a significant main effect of hemispace [$F(1,15) = 17.790$, $p = 0.001$, ES = 0.543; LEFT = 6.3 ± 1.9 blocks, RIGHT = 3.7 ± 1.9 blocks; **Figure 2A**]. In addition, a significant main effect of distance [$F(1,15) = 12.023$, $p = 0.003$, ES = 0.445; **Figure 2A**] revealed that participants

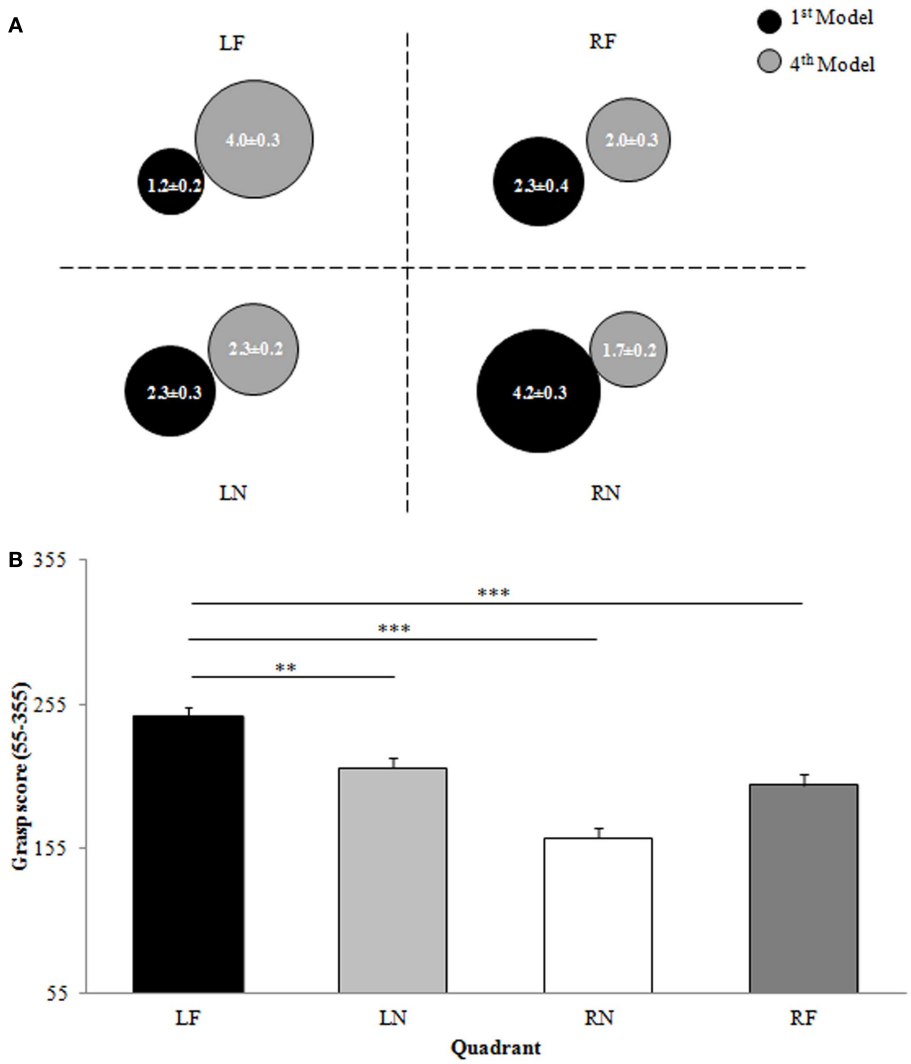

FIGURE 2 | Experiment 1: right-handers. **(A)** Representation of the proportion of blocks (0–10) used from each quadrant for the construction of the first and fourth models in the model set. Data are means and standard errors. **(B)** Overall grasp score for each quadrant of space. Data are means and standard errors. LF, left far; LN, left near; RN, right near; RF, right far. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ with respect to LF.

grasped more blocks from far reachable space when compared with near reachable space (NEAR = 4.0 ± 1.6 blocks, FAR = 6.0 ± 1.6 blocks). Moreover, a significant hemispace by distance interaction [$F(1,15) = 4.747$, $p = 0.046$, ES = 0.240; **Figure 2A**] indicated that participants differentially grasped blocks from left or right hemispace depending upon whether they were grasping in near or far reachable space. Participants displayed a tendency to grasp more blocks from left space than right space when grasping in near reachable space [LN–RN, $t(15) = 2.663$, $p = 0.018$]. This pattern of preference for blocks from left hemispace was further exacerbated when participants were reaching in far reachable space [LF–RF, $t(15) = 3.626$, $p = 0.002$].

Overall. When investigating the overall patterns of space use for grasping during model set construction a significant main effect of hemispace [$F(1,15) = 28.011$, $p < 0.001$, ES = 0.651; **Figure 2B**] was observed. On average, participants grasped blocks from left space later in model set construction when compared with right space (LEFT = 462.3 ± 46.9, RIGHT = 357.7 ± 46.9). Participants also grasped blocks from far reachable space on average later in model set construction when compared to near reachable space as confirmed by a significant main effect of distance [$F(1,15) = 14.973$, $p = 0.002$, ES = 0.500; NEAR = 372.9 ± 49.5, FAR = 447.1 ± 49.5; **Figure 2B**]. Although there was not a significant interaction between factors ($p > 0.05$), comparisons between the overall grasp score for the LF quadrant and the overall grasp scores for each of the other three quadrants did reveal that participants grasped blocks from LF space significantly later in model set construction than from LN [$t(15) = 4.015$, $p = 0.001$], RN [$t(15) = 6.352$, $p < 0.001$], or RF [$t(15) = 4.663$, $p < 0.001$] space (**Figure 2B**).

DISCUSSION

The purpose of *Experiment 1* was to describe space use during an ecologically valid bimanual visually guided grasping task amongst right-handed participants. The results demonstrated that space use for grasping varied according to hemispace and spatial proximity to the participant. More specifically, when participants had the opportunity to grasp building blocks from any quadrant of space (i.e., Model 1) they preferentially selected blocks from right space; moreover, the majority of blocks were selected from near reachable space. In contrast, participants largely ignored (or, "neglected") the blocks in LF space until later in model set construction.

It is possible that this pattern of space use may have been influenced by hand dominance and associated biomechanical constraints. One could argue that participants chose to grasp in right hemispace first because that space is closer to their dominant right hand. To examine this possibility, the protocol used in *Experiment 1* was repeated in a group of left-handed participants for *Experiment 2*. If the pattern of grasping observed in *Experiment 1* was a consequence of handedness, it was expected that left-handed participants would display the reverse behavior; that is, participants would choose to grasp from left hemispace first, and would "neglect" right rather than left far space.

EXPERIMENT 2: LEFT-HANDERS

In *Experiment 2* the conditions of *Experiment 1* were repeated with left-handed participants.

MATERIALS AND METHODS

Participants

Sixteen self-declared left-handed participants from the University of Lethbridge took part in *Experiment 2* (nine males; 18–35 years). The study was performed with approval by the University of Lethbridge Human Subject Research Committee. Written informed consent was provided prior to the initiation of the study. Participants were naïve to the purposes of the study.

Apparatus and stimuli

Apparatus and stimuli were identical to those used in *Experiment 1*.

Procedures

Procedures were the same as in *Experiment 1*.

Data processing and analysis

The data processing and analysis techniques used in *Experiment 1* were repeated for *Experiment 2*.

RESULTS

Handedness questionnaire

Participants had an average handedness questionnaire score of −14.8 ± 11.3. The range of scores was from +9 to −29, two participants reported using their right hand on average more than their left in the selection of activities targeted by the questionnaire. All participants, however, self-identified as being left-handed and all participants used their left hand to fill the questionnaire and sign the consent form.

Trial times

A significant main effect of model [$F(3,45) = 4.203$, $p = 0.011$, ES = 0.219] indicated that trial times were significantly affected by the model being constructed. More specifically participants completed the final model (Model 4) significantly faster than the first [$t(15) = 3.142$, $p = 0.007$] model.

Hand use for grasping

Left-handed participants used their non-dominant right hand for 45.6 ± 10.0% of all grasps. Interestingly, there was not a significant difference between hands when analyzing contralateral grasps ($p > 0.05$) with participants using their right hand for 4.0 ± 3.7% of grasps to left hemispace and their left hand for 8.4 ± 8.6% of grasps to right hemispace.

Space use

First model. A significant main effect of distance [$F(1,15) = 111.667$, $p < 0.001$, ES = 0.917; **Figure 3A**] revealed that participants grasped significantly more blocks from near compared to far reachable space (NEAR = 7.5 ± 1.3 blocks; FAR = 2.5 ± 1.3 blocks) when constructing the first model. There was not a significant main effect of hemispace or a significant hemispace by distance interaction ($p > 0.05$).

Fourth model. Participants grasped more blocks from left space compared to right space (LEFT = 5.9 ± 1.9 blocks, RIGHT = 4.1 ± 1.9 blocks) when constructing the fourth model as indicated by a significant main effect of hemispace [$F(1,15) = 10.970$, $p = 0.005$, ES = 0.422; **Figure 3A**].

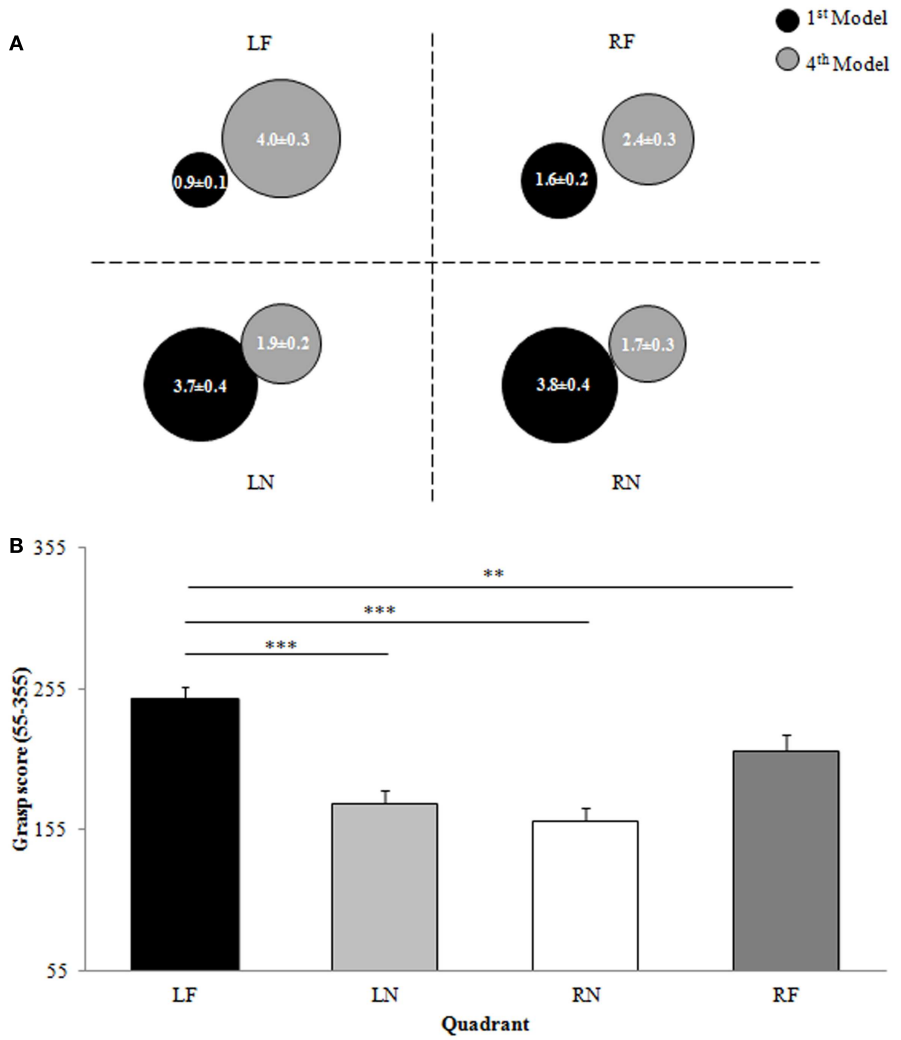

FIGURE 3 | Experiment 2: left-handers. **(A)** Representation of the proportion of blocks (0–10) used from each quadrant for the construction of the first and fourth models in the model set. Data are means and standard errors. **(B)** Overall grasp score for each quadrant of reachable space. Data are means and standard errors. LF, left far; LN, left near; RN, right near; RF, right far. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ with respect to LF.

In addition, left-handed participants grasped significantly more blocks from far reachable space than near reachable space [$F(1,15) = 14.195$, $p = 0.002$, ES = 0.486; NEAR = 3.6 ± 1.9, FAR = 6.4 ± 1.9; **Figure 3A**]. Moreover, block selection from near and far reachable space was differentially affected by whether the block was being grasped in left or right space, as indicated by a significant hemispace by distance interaction [$F(1,15) = 5.400$, $p = 0.035$, ES = 0.265; **Figure 3A**]. When reaching to near space participants did not differentially grasp blocks from left or right hemispace (LN–RN, $p > 0.025$); however, when reaching to far space participants grasped more blocks from left space when compared to right space [LF–RF, $t(15) = 3.014$, $p = 0.009$].

Overall. A significant main effect of hemispace [$F(1,15) = 5.807$, $p = 0.029$, ES = 0.279; **Figure 3B**] revealed that participants grasped blocks from left hemispace on average later in model set construction than blocks located in right hemispace (LEFT = 436.2 ± 54.6, RIGHT = 383.8 ± 54.6). In addition, a significant main effect of distance [$F(1,15) = 60.472$, $p < 0.001$, ES = 0.801; **Figure 3B**] indicated that participants grasped blocks from far reachable space on average later in model set construction than those located in near reachable space (NEAR = 347.1 ± 45.7, FAR = 372.9 ± 45.7). Moreover, a hemispace by distance interaction that approached significance [$F(1,15) = 4.123$, $p = 0.060$, ES = 0.216; **Figure 3B**] suggested that participants differentially grasped blocks from near and far reachable space depending on whether the blocks were being grasped from left or right hemispace. More specifically, the overall grasp score was significantly higher in LF space than LN [$t(15) = 7.588$, $p < 0.001$], RN [$t(15) = 7.015$, $p < 0.001$], and RF [$t(15) = 3.093$, $p = 0.007$] space, indicating that on average participants grasped blocks from left far space later in model set construction than the blocks elsewhere in space (**Figure 3B**) just as right-handers did.

Comparison between right- and left-handed participants

A three-factor RM ANOVA was conducted to assess the effect of hand dominance on space use as determined by the overall grasp score. Hemispace (LEFT, RIGHT) and distance (NEAR, FAR) were within-subjects factors, *Experiments 1* and *2* were the between subject factor. A significant distance by experiment interaction [$F(1,30) = 4.246, p = 0.048, \text{ES} = 0.124$] indicated that the experiment (and consequently the participants handedness) influenced space use with respect to whether the participant was grasping in NEAR or FAR reachable space. Specifically, participants in *Experiment 2* (left-handed) grasped blocks from NEAR reachable space on average earlier in model set construction than participants in *Experiment 1* (right-handed). The interactions between hemispace and experiment, and distance, hemispace, and experiment were not significant ($p > 0.05$) implying that the "neglect" of LF space was not a product of hand dominance.

Correlation analysis for Experiments 1 and 2

To further investigate whether the tendency to neglect the LF space was a product of using the left hand less often for grasping, a bivariate correlation analysis was conducted between the overall grasp score for space use in LF space and the average left hand use for grasping on the data from all participants (right- and left-handed). Right-handed participants grasped with their left hands $29.8 \pm 13.8\%$ of the time, in contrast with left-handers who used their left hands for $54.2 \pm 9.9\%$ of grasps. The overall correlation between left hand use and LF space was not significant ($p > 0.05$), suggesting that the neglect of LF space is not related to hand use. In addition, a correlation analysis between the overall grasp score for LF space and the handedness questionnaire score was not significant ($p > 0.05$).

DISCUSSION

The second experiment was designed to investigate whether "neglect" of LF reachable space was a consequence of handedness. In other words, because the participants in *Experiment 1* were all right-handed, one could argue that this hand preference was the cause of the observed LF neglect. Surprisingly, left-handed participants behaved much as right-handers. Both right- and left-handed participants delayed grasping in LF space generally toward the end of the task. This finding (and the fact that there was no correlation between overall LF space use and left hand use for grasping or handedness questionnaire score) suggests that the observed spatial biases were not simply a product of biomechanical constraints resulting from hand dominance.

The phenomenon that young healthy adults display an inherent tendency to neglect LF reachable space during grasping expands our current knowledge of visuospatial processing in general, but also has implications for our understanding of visuomotor deficits in a variety of patient populations. In the first instance it is necessary to elucidate the basis of these spatial biases. One possibility worthy of investigation was that the pattern of space use was a consequence of attentional biases. Accordingly, the experimental protocol was repeated with participants wearing eye tracking glasses (coupled with a scene camera) to provide an inference of the direction of visual attention during the reaching-to-grasp task. We hypothesized that if the neglect of LF space during grasping was a consequence of inattention, then the patterns of gaze would closely mirror those of grasping (i.e., participants would not direct visual attention to left far space until later in model set construction).

EXPERIMENT 3: EYE TRACKING

In *Experiment 3* the protocol used in *Experiments 1* and *2* was repeated in a population of right-handed participants fitted with eye tracking glasses to provide information on gaze position.

MATERIALS AND METHODS

Participants

Twelve self-reported right-handed participants were recruited for this study (three males; 18–35 years). The University of Lethbridge Human Subject Committee approved the study. All participants provided written informed consent prior to participation in the study. Participants were naïve to the nature of the study.

Apparatus and stimuli

Handedness questionnaire. The handedness questionnaire used was the same as that used in *Experiments 1* and *2*.

Building block task. The building block task that was used was the same as that used in *Experiments 1* and *2* with the exception that participants constructed only one set of four models (using MEGA BLOKS®) in the task.

Eye tracker. Participants were fitted with head-mounted eye tracking glasses (Eyelink II®; SR Research, Osgoode, ON, Canada) with a scene camera mounted anteriorly near the center of the headband. The eye tracking glasses allow 3D eye tracking whilst the addition of the scene camera enables the overlay of gaze position onto the outward scene video (collected at 30 Hz).

Procedures

The procedures were the same as those used in *Experiments 1* and *2* with the exceptions that the workspace consisted of a tabletop (70 L × 120 W × 74 cm H) surrounded on three sides by black partitions and walls. The eye tracker was fitted to the participant and calibrated according to manufacturer recommendations. Following calibration participants were requested to close their eyes while the building blocks were distributed appropriately on the tabletop. The first model to be replicated was placed on a base plate located centrally at the far border of the workspace between left and right space (**Figure 1A**). Once data recording was initiated, participants were instructed to open their eyes and use the available blocks to replicate the displayed model as quickly and accurately as possible on a second base plate (19 L × 19 W cm) located centrally immediately in front of them (at the intersection of right and left space; **Figure 1A**). Following completion of the model, participants were asked to close their eyes while both models were removed and a new model to be replicated was provided. Each participant constructed the same four models; model order was randomized between participants.

Gaze position and model construction were recorded for subsequent analysis using the eye tracker and associated scene camera.

Data processing and analysis

All recordings were analyzed offline. The videos were cropped into individual trials (i.e., Models 1–4) using the events of the eyes opening and the final release of the constructed model with both hands. The scene videos for the first and fourth model were manually scored to provide the number of blocks (0–10 blocks) grasped from each quadrant for these models. In addition, a more detailed indication of space use was provided by numbering each grasp (1–40) as described in *Experiment 1*.

Each frame of gaze position data was also manually scored as being allocated to a particular quadrant of space, the "home" model and plate, or the "build" model and plate to provide an inference of overt visual attention. The initial gaze position (not directed toward the home or build models) during construction of Model 1 was recorded for each participant. The relative proportion of the trial during which gaze was directed to each of the quadrants was calculated [(frames with gaze located in a specific quadrant/overall frames that gaze position was located in any of the four quadrants) × 100] for each of the four models. Gaze directed to the home and build models and plates was excluded from the analysis.

Data analysis procedures were the same as those used in *Experiments 1* and *2* with the exceptions that the initial gaze position data (for Model 1) was summarized descriptively and gaze position data for the first and fourth models and over the course of the complete model set were entered into separate two-factor (hemispace × distance) RM ANOVAs, with hemispace (LEFT, RIGHT), and distance (NEAR, FAR) as within-subject factors.

RESULTS

Handedness questionnaire

Participant handedness was determined by the modified handedness questionnaire, with the average score of $+32.3 \pm 5.2$ (scores ranging from +20 to +38) confirming that all participants were right-handed.

Trial times

A main effect of model approaching significance [$F(3,33) = 2.783$, $p = 0.056$, ES $= 0.202$] suggested that trial time was affected by the model being constructed, follow-up comparisons however, failed to reach significance ($p > 0.017$).

Hand use for grasping

Participants used their right hand for $60.0 \pm 11.9\%$ of all grasps. The analysis of contralateral grasps revealed that participants made significantly more contralateral grasps with the right hand to left hemispace ($11.9 \pm 10.1\%$) when compared to grasps made with the left hand to right hemispace [$1.9 \pm 2.6\%$; $t(11) = 2.907$, $p = 0.014$].

Space use

First model. When constructing the first model in the set, participants grasped more blocks from right space than left space (LEFT $= 3.5 \pm 1.2$ blocks, RIGHT $= 6.5 \pm 1.2$ blocks) as confirmed by a significant main effect of hemispace [$F(1,11) = 17.471$, $p = 0.002$, ES $= 0.614$; **Figure 4A**]. In addition, a main effect of distance [$F(1,11) = 7.694$, $p = 0.018$, ES $= 0.412$; **Figure 4A**] indicated that participants grasped more blocks from near space when compared to far space (NEAR $= 6.8 \pm 2.3$ blocks, FAR $= 3.2 \pm 2.3$ blocks). The hemispace by distance interaction was not significant ($p > 0.05$).

Fourth model. A significant main effect of hemispace [$F(1,11) = 11.957$, $p = 0.005$, ES $= 0.521$; **Figure 4A**] revealed that participants grasped significantly more blocks from left hemispace than from right hemispace (LEFT $= 6.7 \pm 1.7$ blocks, RIGHT $= 3.3 \pm 1.7$ blocks) to construct the fourth model in the model set. Space use for grasping during the construction of the fourth model was not, however, significantly influenced by distance ($p > 0.05$). Participants grasped 4.3 ± 2.1 blocks from near space and the remaining 5.7 ± 2.1 blocks from far space. In addition, the hemispace by distance interaction was not significant ($p > 0.05$).

Overall. When analyzing space use for grasping across the construction of all four models it was found that participants grasped blocks from left hemispace on average later in model set construction than blocks in right hemispace, as indicated by a significant main effect of hemispace [$F(1,11) = 13.128$, $p = 0.004$, ES $= 0.544$; LEFT $= 457.2 \pm 44.8$, RIGHT $= 362.8 \pm 44.8$; **Figure 4B**]. A significant main effect of distance [$F(1,11) = 6.374$, $p = 0.028$, ES $= 0.367$; **Figure 4B**] revealed that participants grasped blocks in far space on average later than those in near space (NEAR $= 359 \pm 70.6$, FAR $= 461 \pm 70.6$). The hemispace by distance interaction failed to reach significance ($p > 0.05$), however, planned comparisons between the overall grasp score for LF space and the overall grasp score for each of the other three quadrants of space (as per *Experiments 1* and *2*) revealed that participants grasped blocks from LF space on average later in model set construction than from LN space [$t(11) = 2.882$, $p = 0.015$], RN space [$t(11) = 4.063$, $p = 0.002$], or RF space [$t(11) = 3.169$, $p = 0.009$; **Figure 4B**].

Gaze position

Gaze position data for one participant was discarded due to equipment failure. An example of gaze position data during construction of the fourth model for one participant is provided in **Figure 5A**.

First model. Gaze position for the remaining 11 participants was not significantly affected by hemispace or distance ($p > 0.05$) during the construction of the first model. Furthermore, the hemispace by distance interaction was not significant ($p > 0.05$).

Fourth model. A significant main effect of hemispace [$F(1,10) = 33.588$, $p < 0.001$, ES $= 0.771$; **Figure 5B**] was observed during construction of the fourth model in the set, with participants spending a higher proportion of model construction time with gaze positioned in left hemispace as compared to right hemispace (LEFT $= 69.9 \pm 11.4\%$, RIGHT $= 30.1 \pm 11.4\%$). In addition, when constructing the fourth model participants spent more time with gaze positioned in far space as compared to near space (NEAR $= 27.0 \pm 20.3\%$, FAR $= 73.0 \pm 20.3\%$) as indicated by a significant main effect of distance [$F(1,10) = 14.149$, $p = 0.004$, ES $= 0.586$; **Figure 5B**]. The hemispace by distance interaction was not significant ($p > 0.05$).

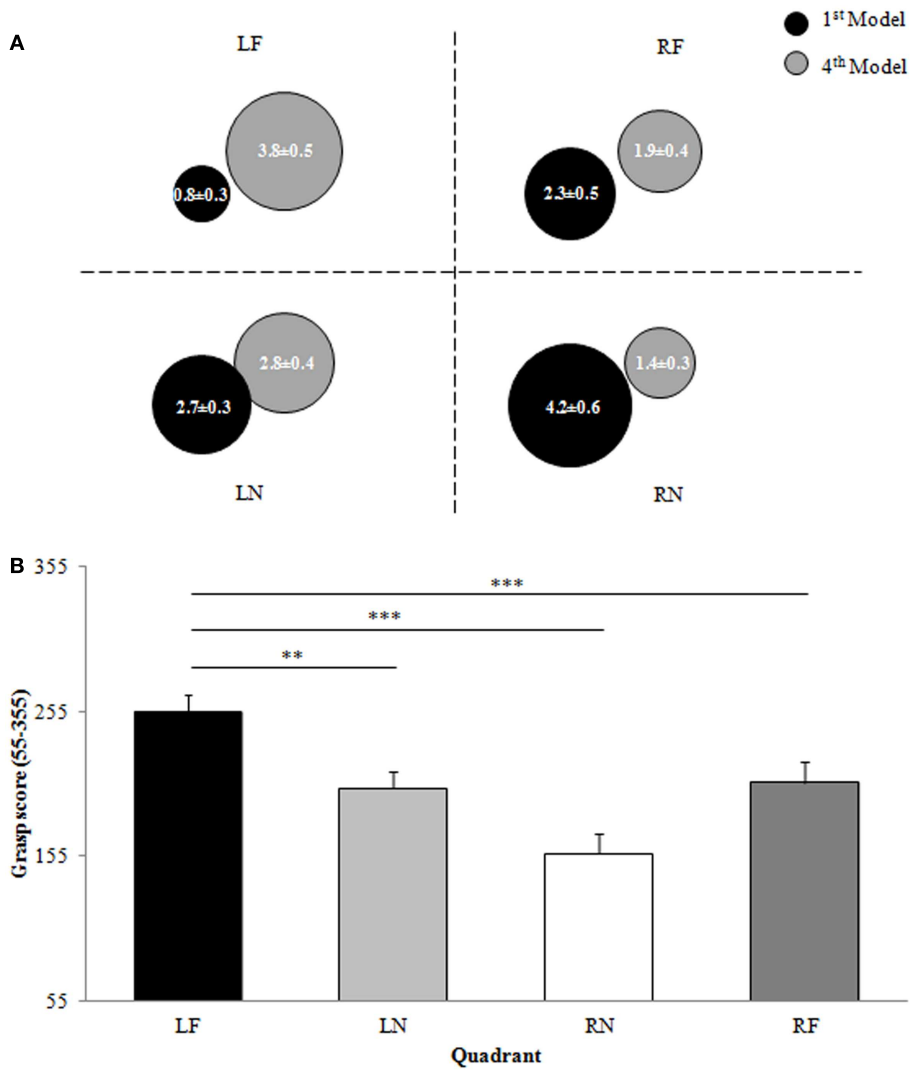

FIGURE 4 | Experiment 3: eye tracking. Space use: **(A)** Representation of the proportion of blocks (0–10) used from each quadrant for the construction of the first and fourth models in the model set. Data are means and standard errors. **(B)** Overall grasp score for each quadrant of space. Data are means and standard errors. LF, left far; LN, left near; RN, right near; RF, right far. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ with respect to LF.

Overall. A significant main effect of hemispace [$F(1,10) = 4.741$, $p = 0.054$, ES = 0.322; **Figure 5C**] revealed that on average participants directed gaze more toward left hemispace than right hemispace (LEFT = 55.8 ± 8.9%, RIGHT = 44.2 ± 8.9%) across the construction of all four models. Furthermore, a significant main effect of distance [$F(1,10) = 17.180$, $p = 0.002$, ES = 0.632; **Figure 5C**] was reported with gaze position being directed toward near space for 35.2 ± 11.9% of model set construction and far space for the remaining 64.8 ± 11.9%. The hemispace by distance interaction was not significant ($p > 0.05$). The fact that gaze was predominantly directed toward left space across the construction of the model set suggests that during construction of the second and/or third models participants must have allocated overt attention predominantly to left hemispace. Analysis using a 2 (hemispace) × 2 (distance) × 4 (Model) RM ANOVA revealed a significant hemispace by model interaction [$F(3,30) = 11.338$, $p < 0.001$, ES = 0.531]. More specifically, during construction of the first two models participants allocated gaze equally to left and right hemispace ($p > 0.05$), however, for construction of the third and fourth models, participants spent significantly more time with gaze allocated to left hemispace [Model 3, $t(10) = 3.721$, $p = 0.004$; Model 4, $t(10) = 5.795$, $p < 0.001$]. Finally, to investigate whether the tendency to direct gaze to left hemispace resulted from participants directing gaze to LF space planned pairwise comparisons between LF space and LN, RN, and RF space were conducted. Results indicated that participants directed their gaze to the LF quadrant for a significantly larger proportion of model construction than LN space [$t(10) = 3.096$, $p = 0.011$] and RN space [$t(10) = 4.210$, $p = 0.002$]. Participants displayed a tendency to direct gaze toward LF space more than RF space, however, this

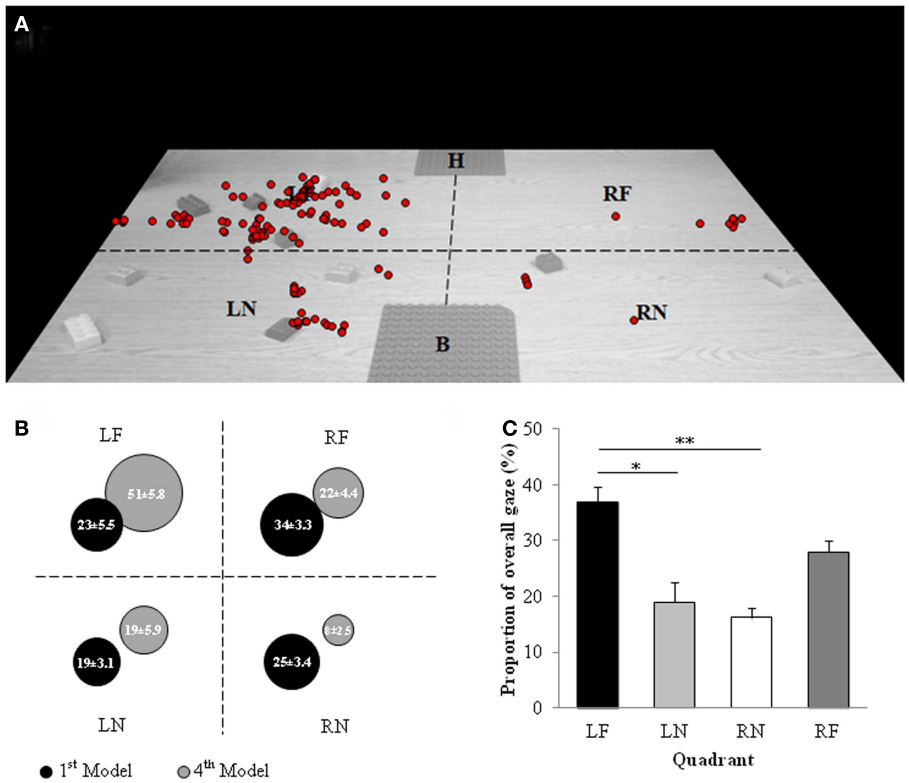

FIGURE 5 | Experiment 3: eye tracking. Gaze position: **(A)** Example of gaze position data during construction of the fourth model for a single participant. Each red circle represents gaze position for a single frame of video. Dashed black lines notionally divide workspace into quadrants; left near (LN), left far (LF), right near (RN), and right far (RF). The model to be replicated is located at the far "home" base plate "H" and model being constructed is located at the near "build" base plate "B." **(B)** Representation of the proportion of gaze (%) directed toward each quadrant of reachable space during construction of the first and fourth models in the model set. Data are means and standard errors. **(C)** Overall proportion of gaze directed to each quadrant of space. Data are means and standard errors. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ with respect to LF.

difference failed to reach significance [$t(10) = 1.924$, $p = 0.083$; **Figure 5C**].

Initial gaze position

Initial gaze position was located in LN space for 18.2% of participants, LF space for 27.3% of participants, RN space for 9.1% of participants, and the RF quadrant for the remaining 45.5% of participants. To gain an inference of whether gaze was directed to the quadrant where the first block would be grasped from or rather whether the participants surveyed their options prior to initiating grasping, a Cramer's V test between initial gaze position and initial grasp location was completed. The Cramer's V analysis was not significant ($p > 0.05$), suggesting participants did not initially locate their gaze to the quadrant from which they would grasp the first block.

DISCUSSION

Overall patterns of space use were very similar between *Experiments 1* and *3*, with right-handed participants grasping blocks from LF space on average later in model set construction than blocks in the other three quadrants. As anticipated, the overall pattern of gaze position paralleled the pattern of grasping, particularly during construction of the fourth model with gaze being directed to task relevant locations. More specifically, visual attention appeared to be fairly evenly distributed between quadrants early in model set construction when participants were presumably surveying the workspace assessing their options, however, by the fourth model participants' dedicated considerable visual attention to LF space. Whilst this finding might not be surprising, as the majority of the remaining blocks were located in the LF quadrant what was perhaps surprising were the results from the initial gaze analysis. Previous studies have shown that grasping is preceded by eye movements toward the object to be grasped (46–51). The results of the initial gaze analysis however, showed no relationship between initial gaze position and initial grasp location. This finding suggests a dissociation between gaze and grasp and will be discussed in more detail in the general discussion. The findings from *Experiment 3* do provide support for the notion that the spatial biases, specifically the neglect of LF space, observed amongst right- and left-handed participants during the reaching-to-grasp task may be attentional in origin; this possibility will be further discussed in the following section.

GENERAL DISCUSSION

We assessed space use for grasping during a bimanual visually guided reach-to-grasp task amongst healthy adults. In addition,

we characterized the patterns of gaze position throughout the grasping task to provide an inference of overt visual attention. Participants were required to locate, grasp, and orient to specific building blocks available on a tabletop in order to replicate a series of complex 3D models. The tabletop was notionally divided into equal sized quadrants differentiated by left and right hemispace, as well as near and far reachable space. The building blocks necessary to construct each model were available in each of the quadrants. Participants displayed a tendency to "neglect" left far space until the final stages of the task. Moreover, similar patterns of space use were observed for both right- and left-handed participants suggesting that the patterns of space use for grasping were not simply a result of hand dominance and associated biomechanical constraints. Despite a dissociation between initial gaze position and initial grasp location, the overall gaze position data largely corresponded with patterns of grasp (i.e., participants overt attention was directed toward the space from which participants were grasping building blocks) which highlights the possibility that the observed "neglect" of left far space may partially be a result of inherent attentional biases.

To our knowledge, this is the first study to characterize space use during a natural visually guided bimanual grasping task in healthy adults. The findings of this study expand our understanding of spatial cognition in humans. This knowledge has thus far been largely garnered from studies on non-human species or alternatively from studies that have utilized standardized paper-and-pencil or computerized assessments to test specific aspects of spatial attention and perception, spatial memory and/or mental imagery (52, 53). Though these common assessments have provided a wealth of knowledge on spatial biases in healthy, aging, and patient populations, the tasks are typically presented in two-dimensional space (i.e., computer monitor) and/or are unimanual and are therefore not truly representative of the bimanual object interactions that we complete hundreds of times each day. As such, these standardized tasks do not address the question of whether equivalent spatial biases are present in real-world grasping tasks. By developing an ecologically valid bimanual task that has well characterized motor and perceptual demands (i.e., replicable visuomotor requirements, visuospatial complexity) it has been possible to add to our understanding of the spatial biases that occur in complex, multi-factorial tasks typically encountered in the everyday environment.

We found that when constructing the first model in the model set (i.e., when there was equal opportunity to grasp blocks from all quadrants) right-handed participants preferentially grasped blocks from right hemispace. Left-handed participants also selected marginally more pieces from right hemispace when compared to left hemispace (5.4 blocks from right hemispace, 4.6 blocks from left hemispace); however, it should be noted that this differential pattern of lateral space use was not significant. All participants grasped the majority of blocks from near reachable space (i.e., reachable without movement of the trunk). Whilst the spatial biases observed relating to distance likely reflect the biomechanical efficiency and comfort of grasping targets in closer proximity to the body (i.e., shorter movement trajectory) we suggest that the lateral spatial biases may be influenced by hand preference. It has previously been reported that right-handed individuals use their dominant hand almost exclusively when grasping objects in ipsilateral space (i.e., right hemispace) or at the body midline (54–56). Furthermore, a strong right hand preference remains when right hand dominant individuals reach to contralateral space. In contrast, left-handed individuals have a tendency to use their dominant and non-dominant hands more equally, normally using the hand ipsilateral to the object for grasping (41, 57–59). In agreement with these earlier studies, the participants in *Experiment 1* grasped approximately $71 \pm 14\%$ of the blocks for the first model with their dominant right hands. In contrast, the left-handed participants in *Experiment 2* grasped around $46 \pm 10\%$ of the blocks with their non-dominant hands, moreover, 91% of these grasps with the right hand were in right hemispace. Although it remains possible that the "neglect" in LF space is due exclusively to biomechanical constraints, it is unlikely. First, assuming that left-handers by definition are more skilled with their left hand, one would have expected this group to show the opposite pattern of space use to right-handers and therefore neglect RF space. This was not the case, however, with left-handers showing a similar pattern of neglect of LF space to right-handers. Second, there was no correlation between left hand use and the overall grasp score (space use) in the LF quadrant. This finding strongly suggests that hand preference for grasping did not influence participants' space use with respect to the LF quadrant. Finally, investigations of kinematics of left- and right-handed reach-to-grasp movements have revealed, at most, minimal differences between hands (60–63) suggestive that the preference to use the right hand (particularly in right space) is not driven by a kinematic advantage.

In stark contrast to the pattern of space use observed during construction of the first model, when constructing the fourth and final model in the series participants grasped the majority of blocks from the far left quadrant where the majority of the remaining blocks were located. The finding that LF space was largely "neglected" until alternative spatial locations had been exhausted was confirmed by the overall grasp score data. This "neglect" of LF space until later in model set construction would appear to be somewhat intuitive for right-handed participants based upon the biomechanical inefficiency associated with the longer movement trajectory to make grasps to far contralateral space with the dominant hand, or alternatively the necessity of using the non-dominant ipsilateral hand. Indeed, this would be consistent with the literature (64–66) suggesting that contralateral movements are computationally more complex and therefore presumably more effortful for the participant. Again, however, the pattern of space use for grasping was largely consistent between right- and left-handed participants. As left-handers typically reach to left far space with their dominant hand it appears that biomechanical inefficiencies cannot fully explain the "neglect" observed. In contrast, the spatial biases for grasping seen in the current studies are consistent with numerous studies that have found that neurologically healthy adults tend to display a rightward bias in bisection performance when viewing lines in extrapersonal space (21–25). Gamberini et al. (22) for example, presented participants with lines at four viewing distances (two in peripersonal space, two in extrapersonal space) in both real and virtual environments. Participants displayed an abrupt left-to-right shift of bisection upon transitioning from peripersonal to extrapersonal space in both environments.

Despite the entirety of the current task being completed in reachable space, it should be noted that in our experiments a leftward bias, characteristic of the *pseudoneglect* exhibited by healthy adults (18, 19) was not observed when participants were grasping in near reachable space.

The gaze position data collected during the same reaching-to-grasp task provides additional insight into the spatial biases observed amongst the right- and left-handed participants and suggests the possibility that the patterns of space use for grasping may have their origins in visual attentional biases. During construction of the first model participants' gaze appeared to be fairly evenly distributed in all quadrants. We speculate that the lack of spatial bias during the construction of the first model results from the novelty of the workspace and task. This postulation was further supported by inspection of the initial gaze position data, which indicated a dissociation between gaze position and initial grasp location. Initially, gaze was predominantly directed to RF space whilst the participants' initial grasp tended to be located in LN space. In agreement with the literature (67–70) the participants appeared to scan the workspace to locate the salient blocks prior to initiating construction rather than use memory of spatial location. Furthermore, participants did not limit their search to the favored area of grasping (i.e., RN space). During the construction of subsequent models, however, as may be expected the spatial distribution of the gaze position data largely mirrored that observed in the grasping behavior with gaze being directed toward the task relevant locations (46–51). Specifically, when constructing the fourth model, participants directed overt visual attention predominantly toward left hemispace and far space, this corresponds with the location of the majority of the blocks remaining in the array (as well as the spatial biases observed during grasping). Interestingly, the overall gaze position data (i.e., across all four models) indicated that on average participants dedicated a greater proportion of overt visual attention to left hemispace and far space.

A possible explanation for the increased visual attention to left hemispace in the right-handed participants is that when grasping blocks from left space participants would either be using their dominant right hand to reach and grasp in contralateral space, or alternatively would be using their non-dominant hand to grasp the block in ipsilateral space. It is conceivable that both of these scenarios would be more attentionally demanding for the participant than using the dominant hand to reach in ipsilateral space. Therefore, we may expect that participants would allocate more attentional resources (i.e., gaze) to effectively and efficiently grasp blocks in left hemispace. Further inspection of the gaze position data presented the possibility that the participants' overt visual attention may have been drawn toward far space by the placement of the "home" model. This postulation is in agreement with prior work (67–70) suggesting that one of the two major functions of the eyes during everyday actions is to gather information on objects with which we are interacting (*locating* and *checking*). In the case of the model building task this would necessitate frequently checking the "home" model to identify the next block to be located as well as to ensure the accuracy of the replica model. Despite the exclusion of gaze directed to the "home" model or base plate from our analyses, the "home" model may have attracted the participants gaze to far space. We intend to examine these possibilities in future studies to elucidate the basis of the observed spatial biases described here. Despite the reported natural propensity for gaze to be drawn toward left far space the finding that the general pattern of gaze followed that of grasping provides support for the postulation that the observed patterns of space use for grasping result from attentional biases.

Our findings have implications for our understanding of visuomotor deficits in a variety of patient populations, particularly those with hemispatial neglect. The data suggests that neurologically intact individuals physically neglect left far space, potentially as a consequence of inattention to this spatial location. This raises the possibility that the spatial biases observed among individuals with left hemispace neglect (i.e., bias toward right hemispace) may not purely be a result of syndrome specific neglect but may reflect in part an exacerbation of an inherent tendency to neglect left far peripersonal space. Alternatively, it is possible that the findings could be explained by hemispheric specialization for visually guided grasping. Neuroimaging studies have revealed several brain areas implicated in the planning and execution of human [i.e., superior parieto-occipital cortex (SPOC); (71, 72)] and primate [i.e., V6A; (73, 74)] visually guided grasping. Furthermore these studies have highlighted the unique role of the posterior parietal cortex in coding reachable vs. unreachable space (71, 72, 75). For example, Gallivan et al. (72) found that SPOC was selectively activated for objects within reachable space. Interestingly, this activation was found in the left hemisphere for both right- and left-handers. If SPOC in the left hemisphere turns out to be specialized for distinguishing object within reach then, one might expect objects within right hemispace to be preferentially discriminated. This bias could account for the late use of LF space for grasping. Future research should aim to elucidate the basis of the neglect of left far space with respect attentional biases and/or hemispheric specialization. In addition, the contribution of this inherent neglect of left far space to the visuomotor deficits observed in patients with right hemisphere lesions with and without unilateral visuospatial neglect warrants further investigation.

AUTHOR CONTRIBUTIONS

Conception and design of study: Claudia L. R. Gonzalez and Devon C. Bryant. Provision of study materials and analysis tools: Claudia L. R. Gonzalez. Collection of Data: Devon C. Bryant and Natalie de Bruin. Analysis and interpretation of data: Natalie de Bruin and Claudia L. R. Gonzalez. Drafting and revision of manuscript: Natalie de Bruin and Claudia L. R. Gonzalez.

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Different evolutionary origins for the Reach and the Grasp: an explanation for dual visuomotor channels in primate parietofrontal cortex

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The Dual Visuomotor Channel Theory proposes that manual prehension consists of two temporally integrated movements, each subserved by distinct visuomotor pathways in occipitoparietofrontal cortex. The Reach is mediated by a dorsomedial pathway and transports the hand in relation to the target's extrinsic properties (i.e., location and orientation). The Grasp is mediated by a dorsolateral pathway and opens, preshapes, and closes the hand in relation to the target's intrinsic properties (i.e., size and shape). Here, neuropsychological, developmental, and comparative evidence is reviewed to show that the Reach and the Grasp have different evolutionary origins. First, the removal or degradation of vision causes prehension to decompose into its constituent Reach and Grasp components, which are then executed in sequence or isolation. Similar decomposition occurs in optic ataxic patients following cortical injury to the Reach and the Grasp pathways and after corticospinal tract lesions in non-human primates. Second, early non-visual PreReach and PreGrasp movements develop into mature Reach and Grasp movements but are only integrated under visual control after a prolonged developmental period. Third, comparative studies reveal many similarities between stepping movements and the Reach and between food handling movements and the Grasp, suggesting that the Reach and the Grasp are derived from different evolutionary antecedents. The evidence is discussed in relation to the ideas that dual visuomotor channels in primate parietofrontal cortex emerged as a result of distinct evolutionary origins for the Reach and the Grasp; that foveated vision in primates serves to integrate the Reach and the Grasp into a single prehensile act; and, that flexible recombination of discrete Reach and Grasp movements under various forms of sensory and cognitive control can produce adaptive behavior.

Keywords: prehension, Reach, Grasp, Jeannerod, dual visuomotor channels, parietofrontal cortex, visually guided grasping, haptically guided grasping

INTRODUCTION

Prehension, the act of reaching to grasp an object, is used for many everyday functions, the most common of which is to retrieve a food item and place it in the mouth for eating. Prehension is performed with little conscious effort and appears as a seamless act. Thus, it is not surprising that it is sometimes considered a single movement in experimental research (1–4) or that it is proposed to have a single evolutionary origin, possibly derived from walking (5), climbing through tree branches (6, 7), digging (8), or capturing prey (9).

Nonetheless, distinctive changes in prehension have been reported after brain injury as some patients display curious impairments in hand preshaping for grasping despite being able to accurately transport the hand to the location of a visual target. To explain this phenomena Jeannerod (10) proposes that prehension actually consists of two distinct but temporally integrated movements, a Reach and a Grasp, each mediated by different neural pathways which project from visual to motor cortex via the parietal lobe. The Dual Visuomotor Channel Theory (10, 11) has since received support from electrophysiological, neuroanatomical, and brain imaging studies while also generating insight into the biomechanics of prehension [for reviews see Ref. (12–15)]. Nonetheless, it does raise new questions concerning the evolutionary origins of prehension. Specifically, how did the Reach and the Grasp come to be mediated by different neural substrates? Indeed, the theory seems to suggest that prehension has not one, but two, evolutionary origins.

Various animal species display a wide range of Reach and Grasp specializations using the tongue, mouth, neck, tail, trunk, or hand, each of which can be guided by various sensory modalities including olfaction, audition, somatosensation, and vision (16, 17). Thus, evolutionary pressures favoring either the Reach or the Grasp could explain differences in forelimb specialization in different phylogenetic lineages. As an extreme example, the third digit is specialized for stepping in the horse (18) and specialized for foraging and prey capture in the aye-aye (19, 20). In primates, the Reach and the Grasp appear to have co-evolved and are put to integrated use in the many movements that comprise

prehension. Nevertheless, distinct functional, biomechanical, and neuroanatomical features of the Reach and the Grasp suggest that each has its own evolutionary history.

This review re-examines the origins of primate prehension with the aim of identifying evolutionary antecedents for the Reach and the Grasp. The Dual Visuomotor Channel Theory is described first, followed by behavioral, neuropsychological, and developmental evidence that without vision prehension decomposes into discrete Reach and Grasp components. Comparative evidence is then presented to show that Reach and Grasp movements are not only identifiable in the forelimb movements of primates, but also in many non-primate species. Collectively, the evidence suggests that the Reach and the Grasp are derived from different evolutionary origins and were only recently, in phylogenetic terms, integrated together under visual control in primates.

THE DUAL VISUOMOTOR CHANNEL THEORY

The Dual Visuomotor Channel Theory has its origins in the proposal that pointing has two phases. A ballistic movement brings the forelimb to the general location of a target and then a visually guided corrective movement positions the hand on the target (21). Indeed, dual phase guidance may be a general feature of animal movement (22). The distinctive contribution of the Dual Visuomotor Channel Theory is that it describes prehension in ethological terms: the Reach serves to bring the hand into contact with the target by transporting it to the appropriate location whereas the Grasp serves to shape the hand for target purchase. As distinct behaviors, the Reach and the Grasp may be subject to different evolutionary pressures and adaptive specializations that can be analyzed by comparative methods.

Distinctive features of the Reach and the Grasp are summarized in **Table 1**. The Reach transports the hand to the location of the target so that the digits align with appropriate contact points on the target. It is produced largely by proximal musculature of the upper arm, is guided by the extrinsic properties of the target (location and orientation), and is coded in egocentric coordinates relative to the reacher. The Grasp preshapes the digits by first opening them to a peak aperture that scales to target size, then gradually closes them on approach to the target, and finally closes them completely for target purchase. The Grasp is produced mainly by distal musculature of the hand and digits, is guided by the intrinsic properties of the target (size and shape), and can be coded in spatial coordinates intrinsic to the hand irrespective of the hand's location relative to the body (23).

The Reach and the Grasp are subserved by largely segregated visuomotor pathways in occipitoparietofrontal cortex (**Figure 1**). The dorsomedial Reach pathway projects through the superior parietal lobule via the parietal reach region (PRR), which includes the superior parieto-occipital cortex (SPOC/V6A), medial intraparietal sulcus (mIPS), and anterior precuneous (aPCu). It then projects to dorsal premotor cortex (PMd), and finally to primary motor cortex (M1). The dorsolateral Grasp pathway projects through the anterior intraparietal sulcus (aIPS) to ventral premotor cortex (PMv) and from there to M1 (13, 14, 24–27). Long-train intracortical microstimulation of the dorsomedial pathway elicits reaching movements in awake and anesthetized monkeys, whereas microstimulation of the dorsolateral pathway elicits grasping and/or manipulatory movements (28–30).

Table 1 | Reach and Grasp components of the DVC theory.

	Reach	Grasp
1. Musculature	Proximal (upper arm)	Distal (lower arm and hand)
2. Function	Transport hand to target	Shape hand for target purchase
3. Spatial properties	Extrinsic (location and orientation)	Intrinsic (size and shape)
4. Spatial coordinates	Egocentric	Non-Ego and Egocentric
5. Visuomotor channel	Dorsomedial parietofrontal cortex	Dorsolateral parietofrontal cortex

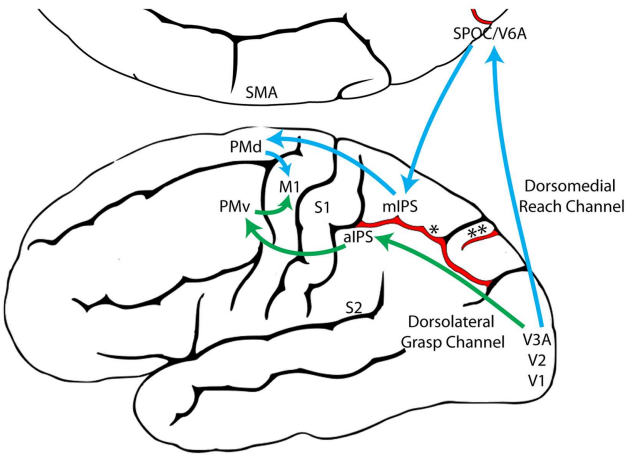

FIGURE 1 |The dorsomedial Reach pathway (Blue) and the dorsolateral Grasp pathway (Green), adapted from Grafton (31). (aIPS, anterior intraparietal sulcus; M1, primary motor cortex; mIPS, medial intraparietal sulcus; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; SMA, supplementary motor area; SPOC, superior parieto-occipital cortex; V1, primary visual cortex; V2, secondary visual cortex; V3A, visual area 3A; V6A, visual area 6A; *, intraparietal sulcus; **, parieto-occipital sulcus).

The Dual Visuomotor Channel Theory posits that concurrent visual inputs to the dorsomedial and dorsolateral pathways allow the Reach and the Grasp to be simultaneously executed as a single integrated act (**Figure 2A**). The preeminent role of vision is illustrated by the act of foviating the target from movement onset until target contact (32). This visual attention is essential for identifying the terminal point of the Reach, i.e., contact locations on the target, and also for coordinating closure of the hand on approach to the target. Nevertheless, non-visual and cognitive inputs may act through the visuomotor Reach and Grasp pathways in order to acquire targets in the absence of visual guidance (33), to produce pantomime Reach and Grasp movements (34, 35), and also to produce spontaneous Reach and Grasp gestures associated with speech (36).

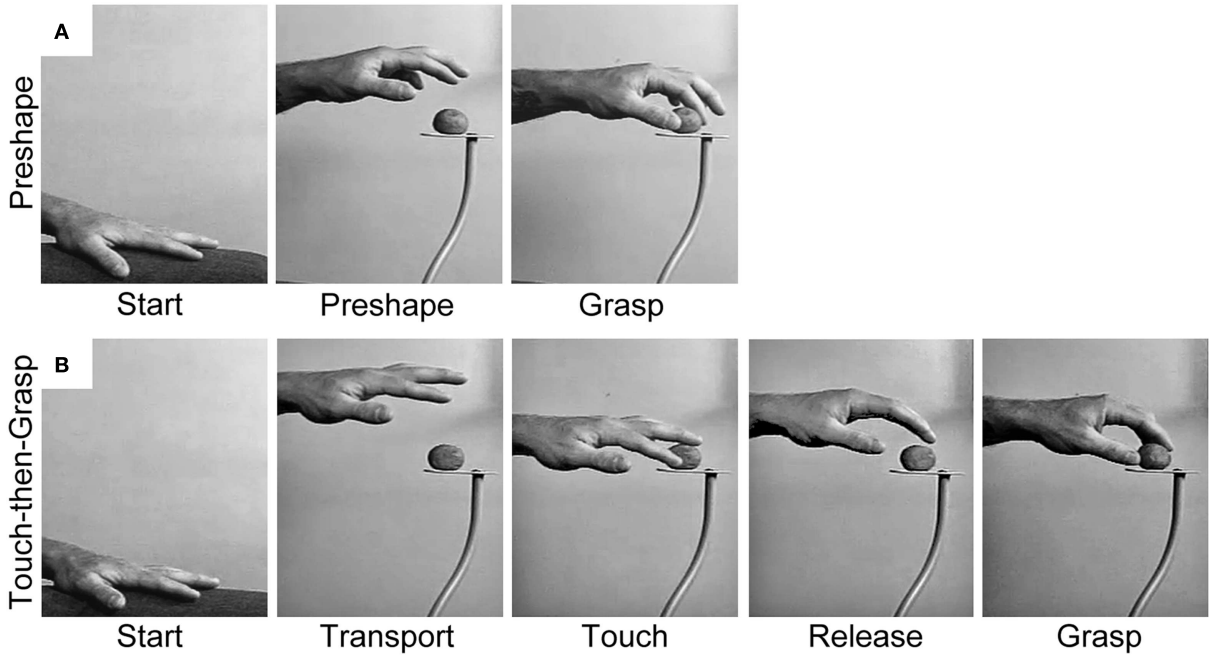

FIGURE 2 | Representative still frames illustrating (A) the Preshape strategy used to acquire a visible target and (B) the Touch-then-Grasp strategy used to acquire an unseen and unknown or uncertain target. Note: for the Preshape strategy the Reach and the Grasp are temporally integrated such that the hand preshapes and orients to the intrinsic properties of the target before touching it. For the Touch-then-Grasp strategy the Reach and the Grasp are temporally dissociated such that the hand does not shape to the intrinsic properties of the target until after touching it.

VISUAL OCCLUSION DISSOCIATES THE REACH AND THE GRASP IN HEALTHY ADULTS

One approach to dissociating the Reach and the Grasp is to manipulate the relative extrinsic or intrinsic properties of a single visual target, but this manipulation has produced ambiguous results. For example, Jeannerod (10) finds that when the size of a visible target is changed unexpectedly, the Grasp is altered but the Reach is not. In contrast, Jakobson and Goodale (37) find that both the Reach and the Grasp are altered. The difficulty in dissociating the Reach and the Grasp with this approach is that when the shape or location of the visual target is changed, both extrinsic and intrinsic target properties are altered resulting in concurrent adjustments in both the Reach and the Grasp.

An alternative way to dissociate the Reach and the Grasp is to remove vision, such that the extrinsic and intrinsic properties of the target must be determined non-visually. Karl et al. (38) asked blindfolded participants to reach for targets of varying size: a blueberry, donut ball, and orange slice. Targets were randomly presented, one at a time, on a pedestal in front of the participants so that they would not know which target they were reaching for on any given trial. In performing the task, participants advanced an open hand above and then down onto the target, often palpitating in the region of the target until touching it. The dorsal trajectory and open digits appeared to enhance the chances of target contact. After touching the target, the participants used haptic cues to shape the digits for grasping. Sometimes the hand released contact with the target before the digits preshaped and closed to Grasp. At other times, the target was stabilized or manipulated by some digits while the remaining digits shaped to Grasp. Hand scaling after target contact was equal to that of visually guided hand preshaping. Thus, when the extrinsic and intrinsic properties of the target cannot be visually determined, the prehensile act decomposes into sequential Reach and Grasp movements, each guided by somatosensation. The Reach, likely mediated by proprioception, is performed first and serves to locate the target by touching it. Only after contact do the hand and digits shape to haptic cues in order to Grasp the target. This two-staged act is termed a Touch-then-Grasp strategy and is illustrated in **Figure 2B**.

A variation of this experiment had participants learn about the extrinsic and intrinsic properties of the target through repeated non-visual experience (39). Blindfolded participants reached 50 times for a donut ball. Although initially unknown, both the location and size of the target could be learned through repetition. As was found in the unknown target experiment, participants persisted in using a dorsal Reach trajectory, in which the hand approached the target from above and an open hand and digits were used to locate the target by touching it. Nevertheless, within a few trials the participants began to preshape hand aperture to the size of the target before touching it. Scaling of hand aperture became indistinguishable from that of sighted participants. Thus, previous non-visual experience had differential effects on the Reach and the Grasp such that a dorsal Reach trajectory became coupled with a preshaped Grasp. Another experimental variation had participants perform the task using peripheral vision, a manipulation that provided enough visual information to identify each target while still degrading information about target size and

location (Hall et al., unpublished). Similar results were obtained, participants maintained a dorsal Reach trajectory but could scale hand aperture to target size before touching it albeit, less accurately than under foveal vision.

The finding that previous somatosensory experience can instruct accurate hand preshaping for the Grasp raises the question of whether online haptic inputs could produce fully integrated Reach and Grasp movements similar to that of visually guided prehension. Online haptic feedback is known to be available in acts such as reaching for a part of the body or objects on the body. Thus, participants were asked to reach for one of three different sized food targets that were randomly placed in their mouth by the experimenter (40). When reaching to grasp the target, participants preshaped and oriented the hand prior to target contact, closed the digits in anticipation of target contact, and successfully grasped the target on the first attempt. Scaling of hand aperture was as accurate, and for some food items, more accurate, than that of visually guided grasping. Thus, online haptic information from a target held in the mouth is as informative as online vision for guiding integrated Reach and Grasp movements.

The behaviors called upon in these studies resemble many everyday actions in which people reach for and manipulate objects under degraded visual conditions. Such acts include reaching for objects in the dark, reaching for objects contacting the body (41, 42), or sequential reaching acts in which one object is grasped while visual attention is directed to a subsequent target. Collectively, these studies support the idea that somatosensation and vision both have access to the Reach and Grasp pathways (43–47). As will be discussed below, this conclusion further suggests that somatosensation may have been formative in the evolution of distinct Reach and Grasp movements and their underlying neural substrates.

VISUAL IMPAIRMENT DISSOCIATES THE REACH AND THE GRASP AFTER BRAIN INJURY

The Reach and the Grasp are also dissociated after localized brain injury that disrupts visual input to one or both of the visuomotor pathways. Patients with such injury display optic ataxia; an impairment in visually guided hand movements despite normal visual perception (48, 49). Recent work with optic ataxic patients support the postulate of the Dual Visuomotor Channel Theory that the visuomotor pathways of the Reach and the Grasp are subject to a double dissociation.

A number of patients with damaged visual inputs to the Grasp, but not the Reach, pathway have been described (50, 51). These patients have no problem reaching to the location of a visual target and consistently touch it on the first attempt; however, they use an open hand to do so and only close their digits to grasp the target after touching it. Thus, these patients seemingly adopt a modified Touch-then-Grasp strategy. They use vision to determine the target's extrinsic properties (location) but are unable to use vision to determine the target's intrinsic properties (size and shape) and thus cannot preshape the hand to Grasp prior to target contact. Instead they rely on haptic cues after target contact to shape their digits to the contours of the target in order to Grasp it.

Cavina-Pratesi and colleagues (52) describe the reverse condition, in which a patient cannot perform a visually guided Reach but can perform a visually guided Grasp. The patient, M.H., suffered an anoxic episode, disrupting visual inputs to the Reach but not the Grasp pathway. M.H. accurately opens, preshapes, and closes his hand to Grasp a visual target, but only if the target is located adjacent to his hand; i.e., if he doesn't have to Reach for it. If he does have to Reach for it, he must first locate it by touch before shaping his hand to Grasp it: "Presumably M.H., wittingly or unwittingly, compensates for the direction and distance errors resulting from his damaged visual reaching network, by habitually opening his hand widely: the wider the hand aperture, the higher the probability of successfully acquiring the object." M.H.'s visually guided Reach movements are inaccurate regardless of whether the movement is directed inward (toward his body) or outward (away from his body), indicating that his deficit is related to visual guidance of the Reach and not the location of the target within egocentric space. Thus, M.H. can use vision to guide his hand in relation to the intrinsic (size and shape) but not extrinsic (location) properties of a target.

The neural substrates that integrate the Reach and the Grasp under visual control may extend beyond the cortex into the spinal cord. Karl and Whishaw (53) re-examined the Reach and the Grasp movements of monkeys with bilateral corticospinal tract (CST) lesions, first described by Lawrence and Kuypers (54). The analysis suggests that these monkeys may also use a Touch-then-Grasp strategy to acquire visual targets. They Reach toward the target using an open and extended hand and often miss the target on the first attempt. They then palpitate the hand in the vicinity of the target until they touch it. After initial contact, the hand releases contact with the target, re-shapes, re-orients, and finally closes to Grasp the target (**Figure 3**). Similar impairments in hand preshaping have also been reported following more selective CST lesions in monkeys (55–57).

Taken together these lesion studies suggest that the visuomotor pathways of the Reach and the Grasp are separate. They also suggest that if brain injury deprives a subject of visual information, somatosensory mediated Reach and Grasp movements are adopted. Finally, it is possible that direct corticomotoneurons in primates mediate the motor output for visual control of the Reach and the Grasp pathways. It is instructive that direct corticomotoneurons and the dorsal visual stream evolved concurrently in the primate lineage.

DISSOCIATION OF THE REACH AND THE GRASP IN EARLY INFANCY

At about 5 months of age human infants begin to haphazardly reach for visual targets, gradually becoming more accurate at bringing a single hand to the target, and finally developing precision grips to grasp (58–63). We have re-examined the development of infant reaching in order to determine whether the Reach and the Grasp have different developmental profiles. The results show that the Reach and the Grasp emerge independently as PreReach and PreGrasp movements in early development and require a significant length of time to become fully integrated under visual control.

Young infants produce a variety of PreReach movements before they can direct a single hand to the location of a visual target. From birth infants can orient the eyes and head to a visual target (64, 65). Soon after, they reach for the target with the mouth

by thrusting the head forward and flexing the abdominals [Ref. (66); Video S1 in Supplementary Material], eventually they use a fisted hand to swipe and wave at the target (67). Consummation of these PreReach movements into a targeted, visually guided Reach only emerges at about 5 months of age. Initially an open hand advances along a jerky trajectory to make imprecise contact with the target (61). This ability develops equally whether the infant has sight of their hand or not and successful contact with the target is signaled by haptic rather than visual feedback (68, 69). However, by 7–9 months, visual control of the Reach improves significantly such that the location and orientation of the open hand accurately reflect the extrinsic properties of the target at the moment of target contact (53, 70, 71).

Young infants also produce a variety of PreGrasp movements before they can preshape the hand and digits to match the contours of a visual target. At birth the digits display a closed and flexed posture, but by 1 month they adopt a collected posture in which the hand is relaxed and partially open (72). Nevertheless, newborn infants will close the digits on an object that makes haptic contact with the palm (73) and by at least 4 months of age infants can use haptic cues to shape the hand to match the contours of an object (74). By 2 months of age infants start "hand babbling," producing a variety of spontaneous but complex digit movements that form a variety of Grasp configurations. Movements include extension and flexion of individual digits, sequential digit movements, and pressing individual digit pads together to form vacuous pincer and precision grips [Ref. (75); Video S2 in Supplementary Material]. At 4 months, these movements become self-directed and are used to grasp the infant's own body or clothing. In performing these movements, infants do not look at their hands, suggesting that the movements are shaped by somatosensation rather than by vision.

Not only do the Reach and the Grasp emerge independently in early development, but they require a long developmental period to be integrated under online visual control. When infants first start to Reach to visual targets, they advance an open hand along a jerky trajectory, often missing the target on the first attempt or making multiple contacts between the open hand and target before closing to Grasp it. Thus, they do not preshape the hand to the target and use a Touch-then-Grasp strategy similar to that described above for unsighted adults. As infants age, they become more accurate at using vision to direct an open handed Reach to the target on the first attempt; however, they do not preshape the hand and haptic contact with the target continues to instruct shaping of the Grasp, similar to the first set of optic ataxic patients described in the previous section [**Figure 4**; Ref. (68, 76)]. Thus, the Reach and the Grasp are dissociated in early development and complete integration of the two movements under visual control, such that the hand accurately preshapes prior to target contact, does not appear to be complete until at least 2 years of age (53, 77).

In summary, analyses on the development of prehension provide evidence that the Reach and the Grasp follow independent developmental profiles. Initially, both the Reach and the Grasp emerge under somatosensation and only later come under visual control. Even then, visual guidance of the Reach

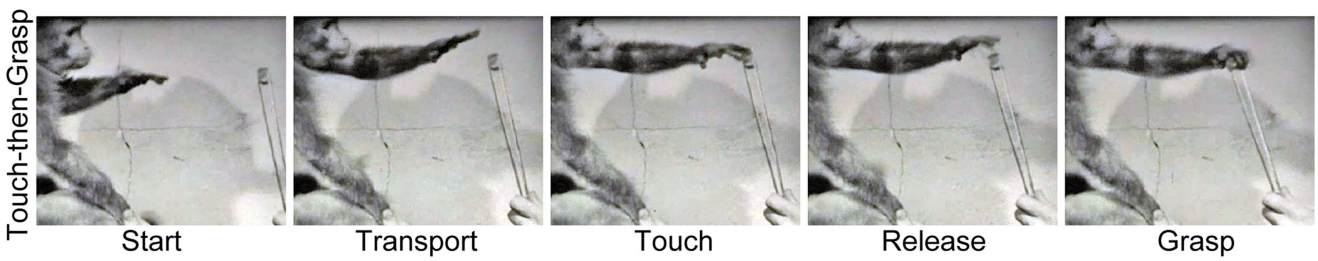

FIGURE 3 | Representative still frames illustrating the Touch-then-Grasp strategy used by a macaque monkey to acquire a visible target 5 months after a bilateral corticospinal tract lesion. Note: even though vision is available the monkey advances an open hand toward the target, and only shapes and closes the hand to grasp the target after it has been touched [videos provided by Lawrence (54)].

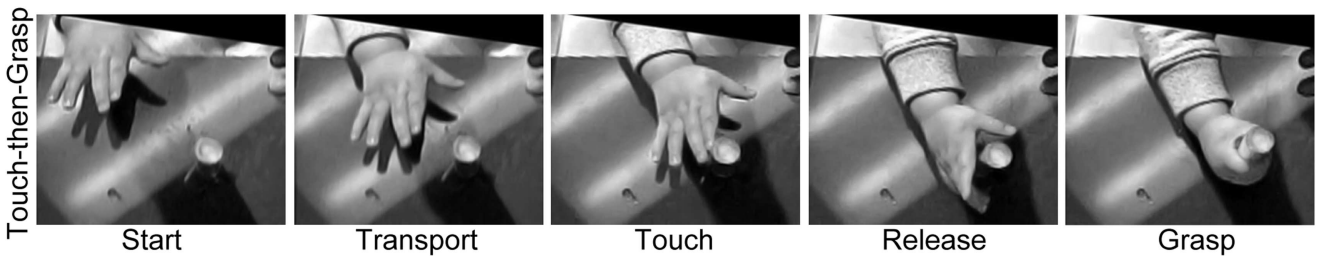

FIGURE 4 | Representative still frames illustrating the Touch-then-Grasp strategy used by a 7-month old human infant in order to acquire a visible target. Note: even though vision is available the infant advances an open hand toward the target, touches the target, and, only after contact, then re-orients, shapes, and closes, the hand to Grasp it.

develops before visual guidance of the Grasp. Finally, integration of the Reach and the Grasp under visual control only appears after a protracted developmental time course lasting into early childhood.

DISTINCT EVOLUTIONARY ORIGINS FOR THE REACH AND THE GRASP

The preceding lines of evidence show that somatosensory and visual information have equal access to the neural pathways that control the Reach and the Grasp. When vision is removed or limited, as occurs with visual occlusion, brain injury, or early in development, the Reach and the Grasp are dissociated by a Touch-then-Grasp strategy that maximizes the use of haptic feedback for guiding each movement independently. Nevertheless, the Reach and the Grasp can be integrated under non-visual control, similar to visually guided prehension, if online haptic feedback concerning the target is available. In the following section we will consider evidence that haptically mediated Reach and Grasp movements are phylogenetically older than those guided by vision.

In phylogenetically early quadrupeds, the neural control of the forelimbs and hindlimbs is tightly coupled to subserve locomotion, but even when stepping a forelimb has independence. Forelimb stepping is achieved by first flexing the forelimb to release contact with the substrate and then extending it to re-establish contact at another location (78). Semi-independent control of a single forelimb likely evolved to allow animals to circumvent obstacles and to navigate over uneven terrain (5, 79–81). Complete independence of a single forelimb allowed the stepping movement to be adapted for a variety of non-locomotor functions such as pushing, swatting, or digging. For instance, a polar bear may flex and extend a single forelimb in order to pin a slippery fish to the ground, a cat may flex and extend a single forelimb to swat at a fly, or a boar may flex and extend a single forelimb to uncover a food item covered by soil. Thus, the wide range of independent forelimb movements produced by various animals, including Reach movements, may be derived from a common origin, stepping.

Our behavioral and kinematic analyses reveal similarities between forelimb stepping and the Reach movement which support the idea of common origin (**Figure 5**). We have examined a variety of movements in rodents and primates, including walking in rats, crawling in humans, and climbing and reaching in both species. In all of these behaviors, the forelimb movement is initiated by flexing the elbow and *lifting* the hand from the substrate. The digits then flex and close in a *collected* posture as the limb is transported forward. The digits then open and *extend* as they approach the target. The hand then *pronates* in the lateral to medial direction and is finally *placed* on the target or substrate (38, 82, 83). Thus, a number of kinematic similarities shape both forelimb stepping and the Reach movement.

For movements of stepping and its derivatives, vision is not essential. Vision is usually directed ahead of the limb's target (84, 85). Thus, the step is performed in the absence of online sensory control until it receives haptic confirmation associated with limb placement. A rat may use vibrissae cues to signal where to step (83) but in its forward movement, this sensory signal precedes the step. Likewise, a rat may use olfactory cues to locate a food item that it will retrieve with a Reach, but the animal must displace its head in order to clear a path for the hand to the target (86). As a result, rats perform both stepping and Reach movements in the absence of online visual control (83, 87). Thus, like a blindfolded human reaching for an unknown target, the rat does not preshape the hand prior to target contact and cannot learn to do so even with extended training (88). Detailed information

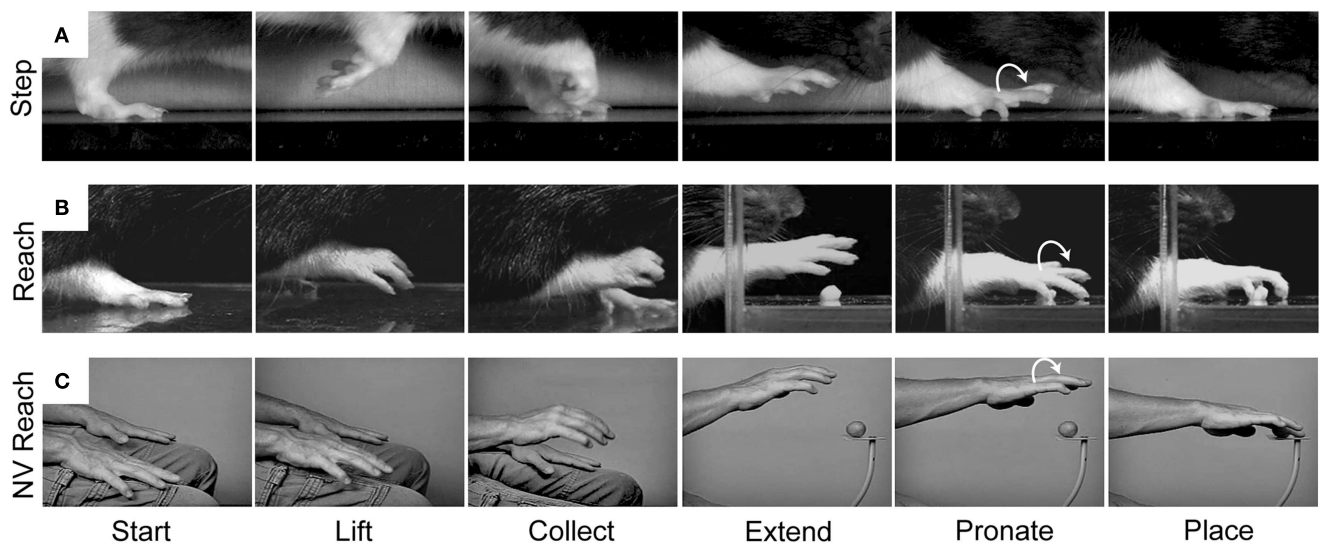

FIGURE 5 | Representative still frames illustrating the kinematic structure of (A) a rat forelimb stepping movement, (B) a rat Reach movement, and (C) a human non-visual Reach movement. Note: all three movements share a common kinematic structure in which the hand is first *lifted* from the underlying substrate, the digits *collect* and *extend* as the arm is advanced forward, and the hand *pronates* before being *placed* on the new substrate. Adapted from Whishaw et al. (83) and Karl et al. (38).

on the sensory control of the forelimb for most actions in most animal species is not available, but available evidence suggests that visual guidance is not prominent in species other than primates. Taken together, comparative evidence for kinematic similarities in the structure of forelimb transport, collection, and lateral to medial pronation, coupled with the distinct absence of hand preshaping, argues that only the Reach movement, not an integrated Reach-to-Grasp movement, is derived from forelimb stepping.

The Grasp action, especially grasping a food item, is a common forelimb movement in many vertebrate orders (16, 17, 89). Grasping not only involves holding a food item and bringing it to the mouth with a hand, but taking an item from the mouth with a hand or taking it from one hand with the other hand, as well as manipulating the item in preparation for consumption. Furthermore, in various non-primate species, specialized hand and digit movements may be used to Grasp and remove the hard shell from a sunflower seed, the spiky legs from a cricket, or the fleshy peel from an orange (90–93). In all of its manifestations these Grasp movements are guided by hapsis. Thus, the demands of a diverse diet have led to the evolution of dexterous and haptically sensitive hands (94–96).

The many manipulations made by the hand in handling food require preshaping by both the hand and the mouth to receive the food item (97). These preshaping movements are the likely origin of hand preshaping for the primate Grasp. Comparisons of rat and human hand preshaping prior to retrieving a food item from the mouth are illustrated in **Figures 6** and **7**. The human is blindfolded and the location of the rodent's eyes prevent it from observing its hands. For both species, online haptic feedback from the food in the mouth guides hand preshaping in order to Grasp the food item. The movement is initiated by *lifting* the hand from a substrate, *preshaping* the hand to the size of the target, and closing the digits on approach to the target in order to *Grasp* it (**Figure 6**). Even though rodents are unable to preshape the hand when reaching to a distal target; they, like primates, readily use oral hapsis to scale hand aperture to the size of a target in the mouth (**Figure 7**), which is also similar to visually guided hand preshaping displayed by primates (40, 97).

After the target is grasped a large and varied vocabulary of specialized grip configurations and independent digit movements may be used to manipulate, explore, or stabilize the food item (**Figure 8**). For further descriptions in non-primates see (17, 91–93, 97). For descriptions in primates see (98–100). That both rodents and primates use haptic information to Grasp a food item in the mouth suggests that haptically guided hand preshaping, as well as manipulatory digit movements, predate visually guided Grasp movements in primates (40, 96).

DISTINCT ANATOMICAL ORIGINS FOR THE REACH AND THE GRASP

In addition to classic work (5), recent electrophysiological and brain imaging studies in non-human primates and humans support the notion that cortical control of the Reach could be derived from a pre-existing locomotion pathway in parietofrontal cortex. Like the Reach, stepping appears to be mediated by a dorsomedial pathway in parietofrontal cortex. Electrical stimulation of this pathway elicits bilateral movements of the forelimbs and hindlimbs that resemble spontaneous running or leaping in monkeys (28–30, 101). In humans, regions

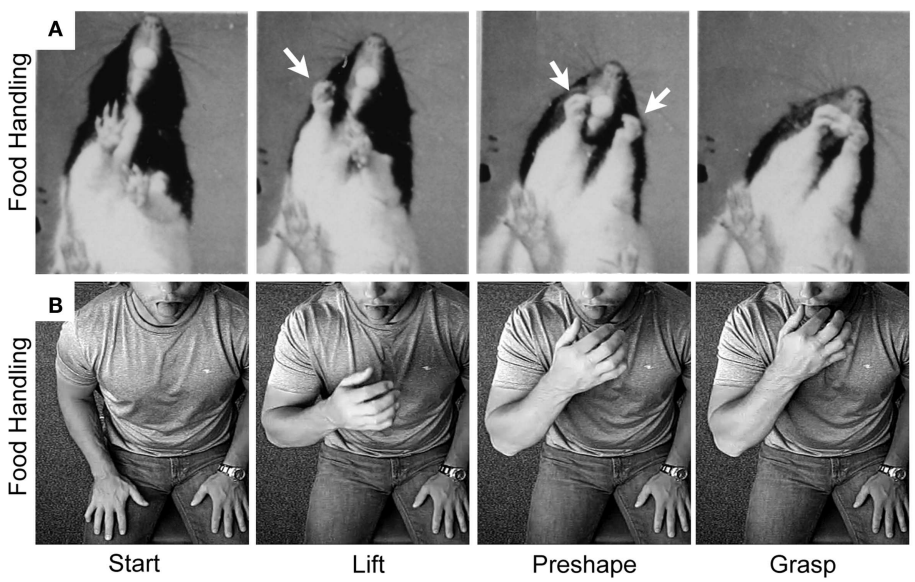

FIGURE 6 | Representative still frames illustrating the kinematic structure of (A) a rat food handling movement and (B) a human food handling movement. Note: both movements share a common kinematic structure in which the hand is first *lifted* from the substrate, the digits then *preshape* to the target, and finally the digits close on approach to the target in order to *grasp* it. White arrows indicate hand preshaping in the rat. Adapted from Whishaw et al. (97) and Karl et al. (38, 40).

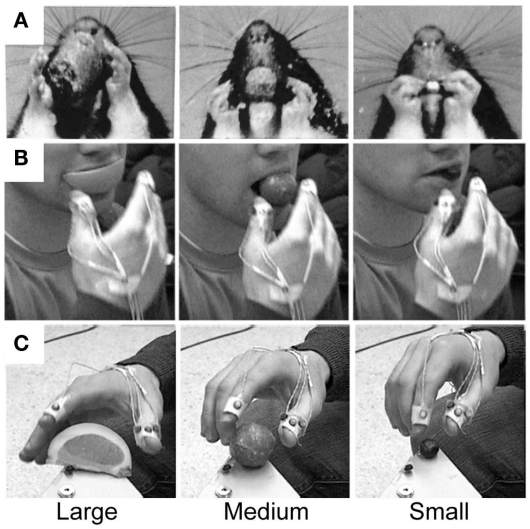

FIGURE 7 | Representative still frames illustrating hand preshaping before touching the target in (A) rat food handling movements, (B) human food handling movements, and (C) human visually guided Grasp movements. Note: in all three situations online haptic (food handling) or visual (Grasp) information is available to guide hand preshaping such that a large peak hand aperture is used to Grasp a large food item, an intermediate peak hand aperture is used to Grasp a medium-sized food item, and a small peak hand aperture is used to Grasp a small food item. Adapted from Whishaw et al. (97) and Karl et al. (40).

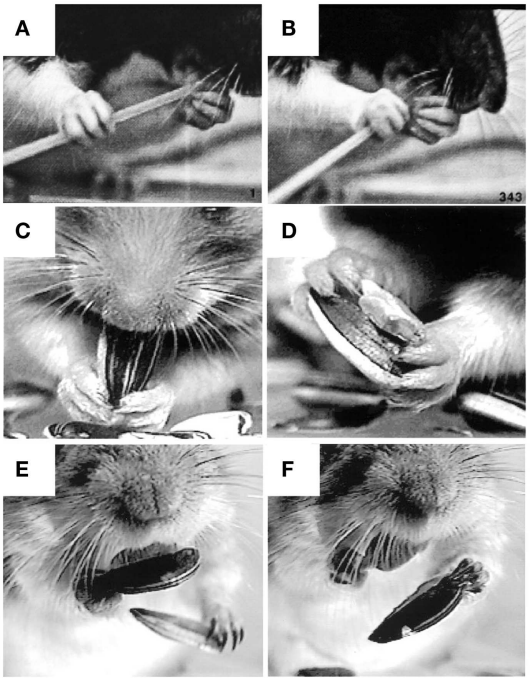

FIGURE 8 | Representative still frames illustrating specialized grip configurations and independent digit movements in rodents during food handling. **(A,B)** A rat eating uncooked spaghetti, the left hand holds the pasta near the mouth with the digit tips (a modified precision grip) while the right hand uses a scissor grip between digits 4 and 5 to push the pasta toward the mouth. **(C,D)** A hamster eating a sunflower seed, both hands hold the seed in a modified precision grip between digit 1 (the thumb) and digits 2 and 3 as the mouth bites into the shell. Two objects can also be held at once, the seed is held in a modified (bilateral) pincer grip between digits 1 and 2, the shell is held in a bilateral power grip between the palm and digits 3 and 4, while digit 5 is positioned on the ventral surface of the seed, likely to stabilize the grip on both objects. **(E,F)** A Mongolian gerbil eating a sunflower seed. A bite from the incisors is used to open the shell (not shown). The bottom half of the shell is held in the digit tips as the left hand uses a precision grip to grasp and discard the top portion of the shell. The left hand then grasps the bottom half of the shell in the digit tips (precision grip) and discards it as the right hand uses a precision grip to hold the seed in the mouth. Adapted from Whishaw et al. (93), Whishaw et al. (92).

of dorsomedial posterior parietal cortex (PPC) mediate reaching to visible targets with the arms and hands, but also subserve pointing and stepping movements to visible targets with the foot (102–106). Although the stepping and Reach pathways overlap in dorsomedial PPC, they appear to diverge in frontal cortex. Thus, regions of overlap (SPOC/V6A, mIPS) may code for a specific behavioral function; i.e., transport of a limb to a different spatial location, whereas regions of divergence (PMd/SMA and M1) might specify the body part used to execute that behavior, i.e., the foot (stepping) or the hand [forelimb stepping/Reach; Ref. (104)].

Food handling, like grasping, may be mediated by a dorsolateral pathway in parietofrontal cortex. Electrical stimulation of this dorsolateral pathway elicits hand-to-mouth movements, in which the hand is lifted toward an open mouth and the digits shape to Grasp (28–30, 101). In humans, a similar region in the inferior parietal lobule is activated when performing grasping movements with either the mouth or hands (107). Furthermore, aIPS, the parietal region of the dorsolateral Grasp pathway, receives strong somatosensory inputs (29, 108–110) and mediates grasping and manipulatory digit movements directed toward both visual and haptic targets (44–46, 111, 112). Thus, it is possible that, like the stepping and Reach pathways, the food handling and Grasp pathways may overlap in parietal cortex (aIPS), which might code for a specific behavioral function, i.e., shaping a body part to grasp/manipulate a target, whereas regions of divergence (M1) could specify the body part used to execute that behavior, i.e., the mouth (bite) or the hand (Grasp).

Interestingly, lesions to V6A, a crucial node in the dorsomedial Reach pathway, disrupt both Reach and Grasp movements (113), although, as demonstrated by Cavina-Pratesi et al. (52), the Grasp impairments could emerge as a secondary consequence of misreaching. Nevertheless, V6A receives inputs from AIP [the macaque homolog of human aIPS; Ref. (108, 114)] and contains orientation- and grip-selective neurons (115–117). Thus, V6A could have originally evolved to serve the Reach, but through its connections with AIP, it may also monitor preshaping of the Grasp as the hand is advanced toward the target. Thus, primate V6A may serve as a visuoproprioceptive "integrator," ensuring that visually guided Reach and Grasp movements unfold in temporal synchrony (116). Indeed, the neural substrate that integrates the Reach and the Grasp must emerge early in the visuomotor pathways in order to integrate the two movements from action

onset. One way to determine whether the grip-selective properties of neurons in V6A are intrinsic to this cortical area, or emerge in response to inputs from AIP, would be to selectively lesion AIP while observing the effect on grip-selective neurons in V6A.

Although non-primate species do not display visually guided hand preshaping during reaching, behavioral evidence suggests that the sensorimotor representations of the Reach and the Grasp should be similar to that of primates with respect to motor control. For instance, the rat has a well-developed forelimb representation in anterior motor cortex consisting of a relatively smaller rostral forelimb area (RFA) and a larger caudal forelimb area [CFA; for a reviews see Ref. (118, 119)]. Microelectrical stimulation of these regions produces brief movements of distal and proximal regions of the contralateral forelimb, respectively. Longer train electrical stimulation in the RFA is more likely to elicit movements involving the hands, including grasping, whereas stimulation in the CFA elicits whole limb movements (120), some of which resemble reaching. Inactivation of these regions disrupts Grasp and Reach movements respectively (Brown and Teskey, unpublished). Additionally, results from brainstem stimulation in freely moving rats suggest separate subcortical regions mediate the Reach (stepping movements) and the Grasp (food handling movements). For example, forced forelimb movements are obtained by electrical stimulation in the region of nucleus gigantocellularis whereas fictive eating (the rats sits on its haunches and engages in food handling and eating without food) from the region of the locus coerulelus (121).

We also suggest that descending projections from cortical motor regions may form the efferent control of the cortical visuomotor Reach and Grasp pathways. The direct projections of the CST are distinctive in primates (122) but have been associated with the production of independent digit movements (54, 123, 124). Yet there are many difficulties with the independent digit theory, including definitional difficulties related to independent digit movements as well as evidence that deficits following cortical injury are related to movement synergies, not independent digit control (125, 126). Independent digit movements are also distinctive in the hand babbling movements of infants as young as 2 months of age (75), well before maturation of the direct connections of the CST is complete (127, 128). In the earliest stages of development and following CST lesions in primates, prehension resembles optic ataxia in that the Reach and the Grasp do not appear to integrate under visual control, but are characterized instead by a distinct absence of hand preshaping as well as the use of modified Touch-then-Grasp strategies. The prolonged developmental period required to integrate the Reach and the Grasp also seems to parallel the long maturational period characteristic of the direct projections of the CST. Taken together, this evidence seems to suggest that, in primates, visual integration of the Reach and the Grasp co-evolved with direct corticospinal projections from motor cortex.

Collectively, anatomical studies confirm predictions from behavioral work that separate pathways should subserve the Reach and the Grasp in non-primate species and that these species could be further examined to identify the neural origins of primate Reach and Grasp movements. Specifically, it is proposed that the neural circuits for stepping are the evolutionary antecedent for the Reach whereas the neural circuits for food handling are the evolutionary antecedent for the Grasp. Early in their evolution these movements were importantly dependent on non-visual guidance, including somatosensation and olfaction, whereas visual control of the Reach and the Grasp appears to have emerged later as a primate specialization. The proposition that visually guided Reach and Grasp movements might be derived from pre-existing non-visual stepping and food handling circuits fits well with recent evidence that movement representations in primate parietofrontal cortex are both effector- and modality-independent (42).

CONCLUSION

Healthy adults use vision to integrate the Reach and the Grasp into a unified prehensile act by preshaping the hand and digits to the size and shape of a visible target as the hand is advanced toward it. This behavior is critically dependent on foveal vision. Nevertheless, when visual inputs are limited or disrupted as occurs during early development, under visual occlusion, or following brain injury, prehension decomposes into its constituent movements: a Reach that advances an open hand in order to haptically locate the target and a haptically guided Grasp that shapes the hand and digits for target purchase.

The independence of the Reach and the Grasp under non-visual control supports the proposition of the Dual Visuomotor Channel Theory that the neural substrates of the Reach and the Grasp are distinct and derived from different evolutionary origins. Collective evidence suggests that the primate Reach is one of a number of species-specific adaptations derived from forelimb stepping, whereas the primate Grasp is one of a number of species-specific adaptations derived from food handling. Thus, distinct motor circuits for the "Reach" and the "Grasp" may have emerged relatively early in evolution and were likely influenced more by non-visual than visual inputs. Expansion of the primate visual system would have given rise to a number of new connections between occipital and parietofrontal cortex, allowing vision to harness these pre-existing "Reach" and "Grasp" circuits resulting in multiple visuomotor pathways from occipital to parietofrontal cortex (**Figure 9**). No longer constrained by the necessity of haptic control, the Reach and the Grasp could be executed simultaneously, rather than sequentially, giving primates the unique ability to preshape the hand to the intrinsic properties of a visual target before touching it.

Finally, distinct neural and evolutionary origins for the Reach and the Grasp would allow for a multiplicity of Grasp movements including various single handed pincer, precision, or power grasps, as well as any combination of two handed grasps to be combined with a multiplicity of Reach movements including single handed reaches, two handed reaches, pushes, throws, or swats, all of which can executed under various forms of sensory or cognitive control. Thus, the proposition that distinct motor circuits for the Reach and the Grasp evolved separately and only came under visual control late in the evolutionary process supports the idea that the Reach and the Grasp pathways in parietofrontal cortex are accessed not only by vision, but also by a variety of non-visual and cognitive inputs in order to produce a diverse repertoire of adaptive behaviors upon which natural selection may act.

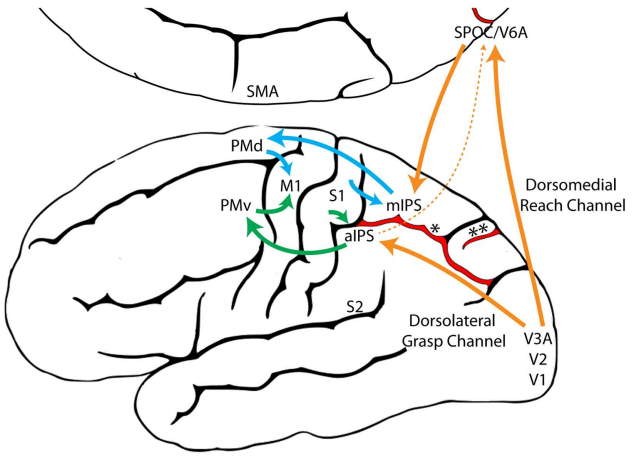

FIGURE 9 | A model illustrating the proposed evolutionary origins for dual visuomotor Reach and Grasp channels in primate parietofrontal cortex. The original dorsomedial stepping/Reach circuit (blue) and the dorsolateral food handling/Grasp circuit (Green) evolved first and were subsequently harnessed by the primate visual system (Orange) through neural re-use (129). (aIPS, anterior intraparietal sulcus; M1, primary motor cortex; mIPS, medial intraparietal sulcus; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; SMA, supplementary motor area; SPOC, Superior parieto-occipital cortex; V1, primary visual cortex; V2, secondary visual cortex; V3A, visual area 3A; V6A, visual area 6A, *, intraparietal sulcus, **, parieto-occipital sulcus).

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fneur.2013.00208/abstract

Video S1 | PreReach movements made with the mouth in a 2-month old human infant. Adapted from Foroud and Whishaw (66).

Video S2 | Hand babbling in a 2-month old human infant. Note the production of independent digits movements and vacuous pincer and precision grips. Adapted from Wallace and Whishaw (75).

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Hand functioning in children with cerebral palsy

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Brain lesions may disturb hand functioning in children with cerebral palsy (CP), making it difficult or even impossible for them to perform several manual activities. Most conventional treatments for hand dysfunction in CP assume that reducing the hand dysfunctions will improve the capacity to manage activities (i.e., manual ability, MA). The aim of this study was to investigate the directional relationships (direct and indirect pathways) through which hand skills influence MA in children with CP. A total of 136 children with CP (mean age: 10 years; range: 6–16 years; 35 quadriplegics, 24 diplegics, 77 hemiplegics) were assessed. Six hand skills were measured on both hands: touch-pressure detection (Semmes–Weinstein esthesiometer), stereognosis (Manual Form Perception Test), proprioception (passive mobilization of the metacarpophalangeal joints), grip strength (GS) (Jamar dynamometer), gross manual dexterity (GMD) (Box and Block Test), and fine finger dexterity (Purdue Pegboard Test). MA was measured with the ABILHAND-Kids questionnaire. Correlation coefficients were used to determine the linear associations between observed variables. A path analysis of structural equation modeling was applied to test different models of causal relationships among the observed variables. Purely sensory impairments did seem not to play a significant role in the capacity to perform manual activities. According to path analysis, GMD in both hands and stereognosis in the dominant hand were directly related to MA, whereas GS was indirectly related to MA through its relationship with GMD. However, one-third of the variance in MA measures could not be explained by hand skills. It can be concluded that MA is not simply the integration of hand skills in daily activities and should be treated *per se*, supporting activity-based interventions.

Keywords: cerebral palsy, hand, manual ability, activities of daily living, body functions, dexterity, path analysis, relationships

INTRODUCTION

Hand functioning, the ability of the hands to perform properly in various contexts, requires the integrity of the central nervous system and, therefore, may be disturbed by different brain disorders. Cerebral palsy (CP) is the most prevalent form of physical disability in children (1), occurring in 1 out of 303 live births (http://www.cdc.gov/ncbddd/cp/index.html). Almost 50% of children with CP present an arm–hand dysfunction (2, 3). Children with unilateral spastic CP seldom use their paretic hand spontaneously in daily activities (2, 4). For these reasons, increasing attention in the last decade has focused on hand functioning in children with CP.

Abbreviations: AIC, Akaike information criterion; AROM, active range of motion; β, standardized path coefficient; BIC, Bayes information criterion; χ^2, chi-squared statistic; CAIC, consistent Akaike information criterion; CIMT, constraint-induced movement therapy; CP, cerebral palsy; DH, dominant hand; FFD, fine finger dexterity; GMD, gross manual dexterity; GMFCS, gross motor function classification system; GS, grip strength; HABIT, hand–arm bimanual intensive training; ICF, international classification of functioning, disability, and health; IQ, intellectual quotient; MA, manual ability; MBRs, mental body representations; *n*, number; NDH, non-dominant hand; P, proprioception; Q1, first quartile; Q3, third quartile; RMSEA, root mean square error of approximation; S, stereognosis; SD, standard deviation; SE, standard error; TD, touch-pressure detection.

The impact of CP on a child's hand functioning may be formalized through the theoretical framework of the International Classification of Functioning, Disability, and Health (ICF) (5). According to the ICF, CP may affect three separate but related domains of functioning: body functions and structures (body domain), activities (individual domain), and participation (social domain). In the present work, only the body and individual domains were considered, as the social dimension cannot be reduced to the sole functioning of the hands. Body functions include the physiological or psychological functions of the different body systems. Body structures refer to the anatomic parts of the body (e.g., organs, limbs, and their components). By definition, CP is a consequence of early brain lesions that may affect the corticospinal tract. CP may impact the hand and its components (e.g., muscles, joints, and bones), as well as several body functions (e.g., muscle strength, control of rapid coordinated movements, touch-pressure detection, and recognition of common objects and shapes). CP may also limit the ICF domain of activities, which refers to the ability to execute an essential task or action of daily living (e.g., eating, drinking, grooming, or dressing). In this paper, the term "hand skills" will be used to refer to hand functions (ICF body domain) and hand mobility (ICF activity domain, mobility subdomain). The term "manual ability" (MA) will be used to refer to the children's

capacity to manage daily activities requiring the use of hands and upper limbs (ICF activity domain, self-care subdomain) (6).

One fundamental rehabilitation goal is to improve the child's ability to manage daily activities necessary for autonomous living (7). Most conventional treatments endeavor to reduce hand impairments by normalizing movement patterns, stretching spastic muscles, strengthening weakened muscles, etc., assuming that body impairments are largely responsible for the difficulties experienced in daily activities (2). However, the ICF stresses the importance of addressing the impact of CP on the child's hand functioning beyond the body level. The ICF has contributed to a recent shift away from body functions and toward the activities and participation perspectives (8). Recent neurorehabilitation concepts have emphasized what children do in their actual environment, rather than what they can do in a standardized environment (9). Newly developed activity-based interventions, including constraint-induced movement therapy (CIMT) (10) and hand–arm bimanual intensive therapy (HABIT) (11), provide evidence for the improvement of hand functioning (12–14).

Understanding the interrelationships between hand skills and how these are related to MA in children with CP is crucial for planning and implementing the most appropriate rehabilitation interventions. According to previous studies in children and adolescents with CP, MA was not related to passive range of motion (15), but was moderately to highly related to other hand motor skills (e.g., active range of motion, muscle tone/strength/coordination, dexterity, and quality of movement) (3, 15–20). Touch-pressure detection and proprioception were weakly or not associated with MA, whereas two-point discrimination and stereognosis were moderately to highly related to MA (3, 15). However, these studies used correlation coefficients or multiple regression analyses to study the relationships between hand skills and MA. Although informative, these statistical techniques do not account for the potentially complex interrelationships among hand skills, such as causal chains in which some hand skills may influence other mediating variables, which, in turn, may predict the outcome variable (i.e., MA).

Path analysis is a more powerful tool for interpreting the relationships among a set of variables. By including "mediators," path analysis can identify directional relationships (both direct and indirect pathways) through which hand skills influence MA. To our knowledge, only one study has applied path analysis in children with CP to study the directional relationship among spasticity, weakness, gross motor function, and activities (21). Spasticity and strength had significant indirect effects on activities, through their effects on gross motor function. According to us, gross motor function mediates between body functions and activities as it reflects a combination of both ICF domains. In the same way, dexterity which is one of the hand skills that best predicts MA (3) and the independence in daily activities (22–25) involves both the ICF domains of body functions and activities (i.e., mobility subdomain including lifting/carrying objects, fine hand use, and hand and arm use). Therefore, we hypothesized that dexterity might link hand functions to MA. Our purpose in the present study was to investigate the directional relationships through which hand skills influence MA in children with CP, and to explore whether dexterity mediates the relationships between hand functions and MA.

MATERIALS AND METHODS

PARTICIPANTS

A cross-sectional analysis was conducted with data derived from two existing studies (26, 27) ($n = 124$) and pre-treatment data from an unpublished study investigating the efficacy of intensive bimanual training ($n = 12$). These studies were previously approved by the ethics committee of the Université catholique de Louvain. All children in this study were over 6 years old, to ensure that they had mature manipulative skills in activities of daily living. Children in the study presented no major intellectual deficit (IQ $\geq$ 60 or normal school level) and were recruited through several centers dedicated to CP. All 12 children from the second (27) and the unpublished studies presented unilateral spastic CP. Consistent with previous hand–arm bimanual intensive trials, children from the unpublished study had to be able to grasp light objects and lift the more affected arm 15 cm above a table surface and were excluded if they presented: (1) uncontrolled seizures, (2) botulinum toxin injections or orthopedic surgery in the upper or lower extremities within the previous 12 months or planned within the study period, and (3) visual problems likely to interfere with treatment/testing. The entire sample included mainly but not exclusively children with spastic CP (84% spastic syndrome, 4% dyskinetic syndrome, 1% ataxic syndrome, and 11% mixed syndrome). The participant characteristics are shown in **Table 1**.

OUTCOME MEASURES

Six hand skills were assessed on both hands, starting with the dominant hand (DH): stereognosis (S), proprioception (P),

Table 1 | Participants' characteristics ($n = 136$).

Characteristics	*n*
Age (years)	10.0 ± 2.6 (6–16)
SEX	
Girls	56
Boys	80
LIMB DISTRIBUTION	
Quadriplegia	35
Diplegia	24
Hemiplegia	77
Right	38
Left	39
SYMPTOMATIC CLASSIFICATION	
Spastic syndrome	124
Dyskinetic syndrome[a]	5
Ataxic syndrome	2
Mixed syndrome	15
GMFCS	
Level I: most independent motor function	61
Level II	38
Level III	12
Level IV	21
Level V: least independent motor function	4

GMFCS, gross motor function classification system.

[a]*Athetosic, dystonic, and choreic movements.*

touch-pressure detection (TD), grip strength (GS), gross manual dexterity (GMD), and fine finger dexterity (FFD). Using the modified Manual Form Perception Test, S was determined as the number of objects out of 10 that a child could correctly identify by touch (28). P was measured by passively moving the metacarpophalangeal joints of the thumb and index finger, and counting the number of joint movement directions that a blindfolded child correctly identified out of 10 trials (5 each for the thumb and index finger) (28). TD was measured by applying the filaments of the Semmes–Weinstein esthesiometer (Lafayette Instrument Company, Loughborough, UK) to the tip of a blindfolded child's index finger, and recording the force required to bend the thinnest filament that the child could detect (29). GS was determined as the average maximal force exerted on a Jamar hydraulic hand dynamometer (Therapeutic Equipment Corporation, Clifton, NJ, USA) across three trials (30). Using the Box and Block Test (31), GMD was determined as the maximum number of blocks transported individually from one compartment of a box to another in 1 min (32). FFD was measured from three trials of the Purdue Pegboard Test (33) (Lafayette Instrument Company, Sagamore Parkway North, USA) as the average number of pegs picked up from a cup and placed into the holes of a board within 30 s (34).

Manual ability was measured with the ABILHAND-Kids questionnaire (26). For each child, the child's parents rated 21 mostly bimanual activities on a 3-level response scale (0: impossible, 1: difficult, or 2: easy), according to their child's perceived difficulty in performing the activity. Each activity had to be completed without technical or human assistance, regardless of the limb(s) or adaptive strategies used. Activities not attempted in the last 3 months were not scored and were encoded as missing responses. As reported in a previous study (26), ordinal total scores obtained on the ABILHAND-Kids questionnaire were transformed into interval-level measures according to the Rasch model (35). Interval-level measures were expressed in logits (i.e., the natural logarithm of the odds of success of a child for an activity). These measures were subsequently recalculated into the percentage of the range of logit measures of the scale (0–100), to facilitate their clinical interpretation.

DATA ANALYSIS

Descriptive statistics were performed for each variable, to examine the children's clinical characteristics. Pearson's correlation coefficients were used to explore the magnitude of bivariate linear associations among hand skills and between hand skills and MA, according to Guilfords' guidelines (36).

All hand skills that significantly related to MA were subsequently included in a path analysis of structural equation modeling to test a set of multiple regression equations simultaneously, and to assess the directional relationships (both direct and indirect) through which the predictors influence the outcome variable (37).

Path analysis requires the development of one hypothesized initial model (e.g., of the directional relationships among the set of variables) that is tested against the observed data and progressively refined through successive analyses to fit the data. The theoretical initial model was based on evidence from ICF theoretical considerations (5), relevant literature, and bivariate results. The maximum likelihood method was used to estimate the strength and significance of hypothesized connections among the variables included in the path model. Both unstandardized and standardized path coefficients were estimated.

Unstandardized path coefficients indicate the expected amount of change in MA per unit change in one predictor, while all other predictors are controlled. Unstandardized path coefficients cannot give the relative contribution of each predictor to each dependent variable because they reflect the different metrics used to assess the variables. However, they are useful for testing the path model with a different sample, or with the same sample at different time points. Standardized path coefficients indicate the expected amount of change in MA per standard deviation (SD) change in one predictor, while all other predictors are controlled. Standardized path coefficients estimate the magnitude of relationships among different variables. They can be understood as correlation measures showing the direct effect of an independent or mediating variable on a dependent variable when other predictors are controlled. Non-significant path coefficients imply that the parameters do not differ from zero and could be deleted from the model.

Various fit indices were used to assess the adequacy of the hypothesized path model and to determine how well it explains the data (37). A good fit of the model to the data is indicated by a non-significant chi-squared (χ^2) statistic ($p > 0.05$), a root mean square error of approximation (RMSEA) below 0.06 (with a lower bound of the 90% confidence interval <0.05 and an upper bound <0.10), an adjusted goodness-of-fit index above 0.90, and goodness-of-fit, normed fit, comparative fit, and Tucker–Lewis indices above 0.95 (37, 38).

Path analysis also provides modification indices, which suggest causal pathways that may be added to improve the goodness-of-fit indices. Additional pathways were only included in the model if they made sense clinically. The path model was modified several times by systematically removing non-significant path coefficients and adding the causal pathways suggested by the modification indices, until the goodness-of-fit indices indicated that the path model fit the data well. Predictive fit indices favoring simpler models, including the Akaike information criterion (AIC), consistent Akaike information criterion (CAIC), and Bayes information criterion (BIC), were considered to choose the more parsimonious model (37). The path model with the lowest AIC, CAIC, and BIC values was chosen as the final model.

The Statistical Package for Social Sciences (SPSS) version 20.0 was used for all statistical analyses. AMOS version 21.0 was used for the path analysis. All assumptions underlying the path analysis were verified; namely, the linearity, normality, and constant variance of the residuals, the absence of influential outliers, and the absence of multicollinearity. To prevent problems with collinearity, when independent variables were intercorrelated by more than 0.80, only one variable was selected. Selection was made on the basis of the clinical sense and the magnitude of the relationship with the dependent variable. The alpha level of significance was fixed at 0.05 for all statistical tests.

RESULTS

DESCRIPTIVE ANALYSIS OF HAND SKILLS AND MANUAL ABILITY

Table 2 summarizes the measures of hand skills and MA. Raw scores for hand motor skills were converted into standardized

Table 2 | Descriptive statistics of manual ability and hand skills.

Variables	Mean	SD	Median	Q1	Q3	Range	*Z*-score (mean ± SD)
DEPENDENT VARIABLE							
Manual ability (% logits)	62.69	22.00	–	–	–	0–100	–
INDEPENDENT VARIABLES: HAND SKILLS							
S_DH (n/10)	–	–	10.00	9.00	10.00	1–10	–
S_NDH (n/10)	–	–	9.00	6.00	10.00	0–10	–
P_DH (n/10)	–	–	10.00	10.00	10.00	0–10	–
P_NDH (n/10)	–	–	10.00	8.25	10.00	0–10	–
TD_DH [$\log_{10}$ (10 × mg)]	–	–	2.80	2.40	3.20	2–7	–
TD_NDH [$\log_{10}$ (10 × mg)]	–	–	2.80	2.40	3.60	2–7	–
GS_DH (kg)	13.77	7.77	–	–	–	0–42	−1.93 ± 1.67
GS_NDH (kg)	7.37	6.17	–	–	–	0–27	−3.06 ± 1.64
GMD_DH (n/1 min)	39.61	18.28	–	–	–	0–86	−2.40 ± 2.72
GMD_NDH (n/1 min)	24.33	16.82	–	–	–	0–67	−4.88 ± 2.80
FFD_DH (n/30 s)	8.69	4.70	–	–	–	0–18	−5.38 ± 4.16
FFD_NDH (n/30 s)	3.14	4.01	–	–	–	0–14	−7.78 ± 3.24

SD, standard deviation; Q1, first quartile; Q3, third quartile; DH, dominant hand; NDH, non-dominant hand; n, number; S, stereognosis; P, proprioception; TD, touch-pressure detection; GS, grip strength; GMD, gross manual dexterity; FFD, fine finger dexterity.

scores (*Z*-scores), according to normative data (30, 34, 39). For our sample, the mean MA measure was 63 ± 22 on a logit scale from 0 to 100. All hand skills were more impaired in the non-dominant hand (NDH) compared to the DH. Gross motor and FFD deficits were observed in both hands for all CP types. This finding indicates that in hemiplegics, the dexterity of the "non-paretic" hand may also be affected, especially in the achievement of fine finger movements. Children with CP were more severely affected in their dexterity compared to other hand skills.

BIVARIATE ASSOCIATIONS WITHIN HAND SKILLS AND WITH MANUAL ABILITY

Table 3 reports the correlation coefficients among hand skills and between hand skills and MA. In both hands, MA was significantly but moderately related to hand motor skills and S, but weakly related to P. Although MA was not significantly related to TD in the DH, it was weakly related to TD in the NDH. GMD and FFD presented the highest correlations with MA for both hands, followed by S in the DH and GS in the NDH. In both hands, GMD and FFD were very highly intercorrelated (≥0.87).

To prevent problems with collinearity, only GMD was selected for path analyses. A high association was observed between GMD and GS in the NDH. In both hands, S was moderately related to all other motor and sensory hand skills, except TD in the DH, for which a weak relationship with S was observed. Overall, moderate relationships were found among sensory hand skills. Weak relationships appeared between sensory (TD, P) and motor hand skills, except for GS in the DH, which was only related to S. Moderate to high correlations were observed among hand motor skills. Finally, weak (S and FFD) to moderate (P, TD, GS, and GMD) associations were found between hands for each hand skill.

PATH MODEL OF HAND FUNCTIONING IN CHILDREN WITH CEREBRAL PALSY

Figure 1 illustrates the final path model of hand functioning in children with CP. The entire model accounted for 66% of the variance in MA. The chi-squared value ($\chi^2 = 8.12$, 7 df, $p = 0.32$) representing the overall goodness-of-fit was not significant, supporting the fit of the path model. The path model showed an adequate fit to the data according to all of the other fit indices, except for the upper bound of the 90% confidence interval of the RMSEA (i.e., 0.12), which was slightly higher than the optimal fit criterion (i.e., <0.10).

Table 4 reports the unstandardized path coefficients, their associated standard error (SE), and their significance. All causal pathways were significant, demonstrating that all parameter estimates differed significantly from zero. **Table 4** also shows the standardized path coefficients (β; see **Figure 1**), which reflect the relative importance of each causal pathway. The GMD in both hands ($\beta_{GMD\_DH \rightarrow MA} = 0.27$; $\beta_{GMD\_NDH \rightarrow MA} = 0.43$; $p < 0.001$) and S in the DH ($\beta_{S\_DH \rightarrow MA} = 0.29$; $p < 0.001$) were the only hand skills to contribute directly to MA. The GS and purely sensory skills (P, TD) did not have a significant direct relationship with MA. However, in both hands, GS indirectly contributed to MA through its impact on GMD ($\beta_{GS\_DH \rightarrow GMD\_DH} = 0.71$; $\beta_{GS\_NDH \rightarrow GMD\_NDH} = 0.75$; $p < 0.001$). The GS in the DH and NDH explained 50 and 57% of the variance in GMD, respectively. The GMD in the DH was indirectly related to MA through its influence on S ($\beta_{GMD\_DH \rightarrow S\_DH} = 0.56$; $p < 0.001$) and accounted for 31% of the variance in S.

Table 5 shows the standardized direct, indirect, and total contributions of hand skills on MA. The indirect effect was calculated as the product of the direct effects that comprised it. For instance, in the DH, GS indirectly affected MA through two pathways: its direct influence on GMD ($0.71 \times 0.27 = 0.19$) and its indirect

Table 3 | Pearson correlation coefficient matrix of hand skills and manual ability.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Manual ability (% logits)	1.00												
2. S_DH (n/10)	0.61***	1.00											
3. S_NDH (n/10)	0.42***	0.37***	1.00										
4. P_DH (n/10)	0.31***	0.58***	0.35***	1.00									
5. P_NDH (n/10)	0.25**	0.25**	0.53***	0.57***	1.00								
6. TD_DH_log [$\log_{10}$ (10 × mg)]	−0.12	−0.30***	−0.09	−0.41***	−0.22*	1.00							
7. TD_NDH_log [$\log_{10}$ (10 × mg)]	−0.21*	−0.27**	−0.59***	−0.41***	−0.55***	0.57***	1.00						
8. GS_DH (kg)	0.48***	0.45***	0.11	0.14	0.02	−0.15	−0.09	1.00					
9. GS_NDH (kg)	0.57***	0.32***	0.54***	0.17*	0.35***	−0.05	−0.30***	0.54***	1.00				
10. GMD_DH (n/1 min)	0.69***	0.55***	0.17*	0.24**	0.09	−0.29***	−0.20*	0.69***	0.43***	1.00			
11. GMD_NDH (n/1 min)	0.72***	0.43***	0.59***	0.23**	0.36***	−0.12	−0.37***	0.36***	0.76***	0.61***	1.00		
12. FFD_DH (n/30 s)	0.68***	0.57***	0.09	0.24**	−0.01	−0.28***	−0.14	0.63***	0.29***	0.90***	0.53***	1.00	
13. FFD_NDH (n/30 s)	0.57***	0.28**	0.52***	0.15	0.34***	−0.09	−0.32***	0.15	0.68***	0.43***	0.87***	0.39***	1.00

DH, dominant hand; NDH, non-dominant hand; S, stereognosis; P, proprioception; TD, touch-pressure detection; GS, grip strength; GMD, gross manual dexterity; FFD, fine finger dexterity.
**$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.*

influence on S ($0.71 \times 0.56 \times 0.29 = 0.12$). Thus, the global indirect effect of GS in the DH on MA was equal to 0.31. A similar indirect contribution of GS on MA was observed in the NDH ($0.75 \times 0.43 = 0.32$). Among all of the hand skills investigated in the study, GMD were the strongest contributors to MA in both hands. Although GMD in the NDH had a higher direct impact on MA ($\beta_{GMD\_NDH \rightarrow MA} = 0.43$) than GMD in the DH ($\beta_{GMD\_DH \rightarrow MA} = 0.27$), similar total contributions were found for both hands due to the indirect effects of GMD on MA through S in the DH ($\beta_{GMD\_DH \rightarrow S\_DH \rightarrow MA} = 0.16$).

DISCUSSION

This study is the first attempt to establish a model for understanding hand functioning in children with CP. According to the path analysis, GMD in both hands and S in the DH were directly related to MA, whereas GS was indirectly related to MA through its relationship with GMD. However, one-third of the variance in MA was not explained by the hand skills investigated in this study.

The path analysis provided a comprehensive picture of hand functioning in children with CP by identifying several mediators through which hand skills influence MA. Among the hand skills investigated, GMD measures in both hands were the strongest contributors to MA. Although related, dexterity is a separate concept from MA. Dexterity refers to the physiological functions of the hand and central nervous system that enable the execution of rapid and coordinated hand movements and mobility, without purposeful functioning. Dexterity tasks are generally performed in a short period of time. Such tasks are not representative of daily activities performed continuously throughout the day, in which fatigue may play a role (39, 40). Moreover, dexterity tasks are too artificial in nature and require too limited of movement patterns to reproduce the meaningful situations encountered in daily life (41, 42). By contrast, MA refers to the use of combined hand functions aimed at executing activities generally considered to be essential for an individual's daily living. Several factors (e.g., learned non-use phenomenon, motivation, cognitive skills, familial and social environments, etc.) may explain why people with similar dexterity skills might present varying MA levels (3, 40). To prevent problems with collinearity, in this study, only GMD was selected in the path model. GMD was preferred to FFD, as GMD measures in both hands presented the highest correlations with MA. Moreover, in our experience, the Box and Block Test is friendlier and more sensitive than the Purdue Pegboard Test to differentiate more affected CP children. However, a similar path model fitting the data was found when FFD was included instead of GMD.

Apart from GMD in both hands, S in the DH was the only hand skill investigated in the study that contributed directly to MA. A high relationship between S and MA was also previously reported in children with unilateral congenital CP (15). The influence of GMD on S confirms that the recognition of an object by tactile sensation requires that the object be moved in the hand to perceive its shape. Active in-hand manipulation is considered to be more efficient in object identification than passive manipulation (43). Thus, failure to identify some objects by touch might result from manipulative deficits, rather than from real sensory impairments (3). Carlson and Brooks (44) showed that healthy individuals presented reduced S when placed in a simulated hemiplegic hand

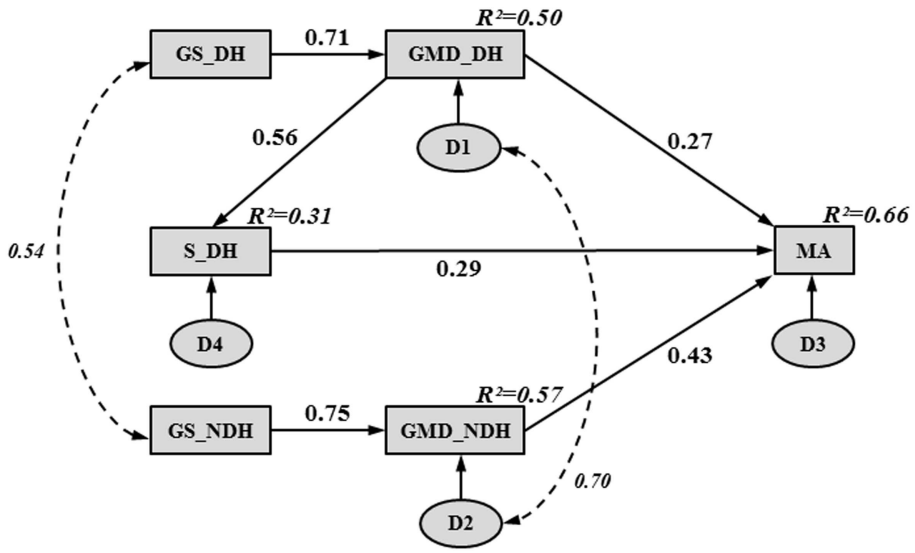

FIGURE 1 | Final path model, illustrating hand functioning in children with CP. Rectangles and ovals represent observed and unobserved variables, respectively. A single-headed arrow indicates a direct effect between two variables, pointing from the "cause" (arrow tail) to the "effect" (arrow head). A curved, double dashed arrow indicates a correlation between two variables without any causal assumption. Numbers beside the single- and double-headed arrows correspond to standardized path coefficients. Numbers in the upper right-hand corner of each rectangle represent squared multiple correlations (R^2) (i.e., proportion of the variance in the dependent variable accounted for by the set of independent variables). The letter "D" inside an oval represents the unobservable disturbance (i.e., measurement error and the variance amount of a dependent variable unexplained by the predictors) associated with each dependent and mediating variable.

Table 4 | Maximum likelihood parameter estimates for the final path model of hand functioning in children with CP.

Parameter	Unstandardized	SE	*p*-Value	Standardized
DIRECT EFFECTS				
GS_DH → GMD_DH	1.71	0.11	<0.001	0.71
GS_NDH → GMD_NDH	2.01	0.12	<0.001	0.75
GMD_DH → S_DH	0.04	0.01	<0.001	0.56
GMD_DH → MA	0.32	0.08	<0.001	0.27
GMD_NDH → MA	0.56	0.08	<0.001	0.43
S_DH → MA	4.30	0.91	<0.001	0.29
COVARIANCES				
GS_DH ↔ GS_NDH	25.61	4.65	<0.001	0.54
D1 ↔ D2	99.20	14.94	<0.001	0.70
VARIANCES				
GS_DH	59.88	7.29	<0.001	
GS_NDH	37.85	4.61	<0.001	
D1 (GMD_DH)	172.63	21.04	<0.001	
D2 (GMD_NDH)	117.31	14.29	<0.001	
D3 (MA)	160.69	19.56	<0.001	
D4 (S_DH)	1.47	0.18	<0.001	

SE, standard error; DH, dominant hand; NDH, non-dominant hand; GS, grip strength; GMD, gross manual dexterity; S, stereognosis; MA, manual ability; D, disturbance (i.e., unexplained variance).

Table 5 | Direct, indirect, and total effects of hand skills on manual ability.

Hand skills	Effects[a]		
	Direct	**Indirect**	**Total**
S_DH	0.29	–	0.29
GS_DH	–	0.31	0.31
GS_NDH	–	0.32	0.32
GMD_DH	0.27	0.16	0.43
GMD_NDH	0.43	–	0.43

[a] *Standardized path coefficients.*

DH, dominant hand; NDH, non-dominant hand; S, stereognosis; GS, grip strength; GMD, gross manual dexterity; MA, manual ability.

position compared to a normal hand position. Other studies have confirmed the importance of hand mobility in object recognition, through the moderate associations between S and dexterity (45–49). Our path analysis revealed that GMD could account for 31% of the variance in S.

In both hands, GS indirectly contributed to MA through its impact on GMD, confirming the relationships observed in the literature between hand strength and dexterity (3, 46, 47, 50). Although deficient GS may influence a child's ability to hold and maintain the grip of objects, objects can be efficiently stabilized in other ways (e.g., against a table surface or body) to perform manual activities. According to Sakzewski et al. (47), a GS >1 kg may be adequate for the NDH to be an effective assisting hand in bimanual tasks. In our sample, only 13% of the children presented a GS below 1 kg in their NDH, and no more than two children were severely affected in both hands. Although children with CP can

develop functional compensatory strategies using the less affected hand solely, the path model emphasizes that the success of manual activities, in terms of strength and dexterity, requires cooperation of both hands.

Sensory inputs are important in anticipatory control and grip-lift tasks (43, 51). However, in this study, TD and P were weakly or not related to MA, consistent with other findings in the literature (3, 14, 45). It can be hypothesized that TD and P were not sufficiently impaired in our sample to affect the achievement of manual activities in a significant way (52, 53). Krumlinde-Sundholm and Eliasson (45) and Gordon and Duff (46) found that TD was less impaired than two-point discrimination in children with unilateral spastic CP. They suggested that the children might have had deficient lateral inhibition or tactile spatial resolution, which are required for two-point discrimination (45, 46). The children's peripheral nerve fibers may have been relatively intact, as reflected by TD (54). TD and P involve low-level sensory processing of somatic stimuli (55). However, higher-level mental body representations (MBRs) that are generated from multisensory inputs are crucial for our daily interaction with the outside environment and may play a role in controlling motor behavior (56). MBRs refer to abstract representations of one's body derived from sensory inputs, like TD or P, but capable to reciprocally influence primary tactile processing and to modulate the perception of external objects that may be body-referenced, thereby playing a role in perception and/or action (57). MBRs develop slowly during ontogenesis in healthy children (58) and do present abnormalities in cortical activation in children with CP (59, 60). This suggests that children with CP may be unable to fully integrate external stimuli into high-level sensorimotor processes (such as MBRs), which may disturb motor output. Taking MBRs into account in future work [see Gandevia et al. (61) and Longo et al. (62) for MBRs measurement] may reveal whether this aspect contributes to MA.

Disturbances representing the unexplained variances of MA and other mediating variables (GMD in both hands and S in the DH) had to be added in the path model (see **Figure 1**) since the model is supposed to show all variables that affect the dependent variables. Without disturbances, the path model would make the implausible claim that a dependent variable is measured without any measurement error and is an exact linear combination of the predictors. The significant disturbance covariance between GMD in the DH and NDH indicates that these mediating variables shared at least one common omitted cause (e.g., severity of the disorder). This illustrates the complexity of understanding the relationships among hand skills and between them and MA. Moreover, good fit of a path model does not guarantee that all relevant predictors have been included in the model. Hand skills other than those measured in this work may also impact the achievement of manual activities in CP children. It would be interesting to test the potential contribution on the model of body structures, such as the corticospinal tract dysgenesis measured by the diffusion tensor imaging symmetry index, as a moderate association of this structure with the ABILHAND-Kids questionnaire has been observed (27). Tactile spatial resolution as measured by the grating orientation task should be investigated in the future; unlike the two-point discrimination test, the stimulus-induced neural image is issued only from spatial cues (63). Spasticity is another hand skill that would be interesting to explore in CP children. Reduction of spasticity remains a primary focus in the clinical management of CP, with the assumption that it will lead to an improvement in MA. The reduction of muscle tone (e.g., by botulinum toxin) improves the active range of motion (AROM) of the antagonist muscles, which could create new potentials to learn and improve manual skills (64, 65). However, this was not confirmed by the study of Rameckers et al. (65) showing that though reduced tone leads to an increase in AROM, this gain was not translated into more upper limb function and thus children were not able to benefit from the changes induced by botulinum toxin. Only one study (15) has demonstrated moderate relationships between ABILHAND-Kids measures and spasticity as tested by the Modified Ashworth Scale, a scale that does not comply with the concept of spasticity (i.e., a velocity-dependent increase in muscle tone) (66). Apart from dexterity, other potential mediators between hand functions and MA could be tested in a path model. For instance, the quality of movement, as measured by the Quality of Upper Extremity Skills Test (67) or the Melbourne Assessment of Unilateral Upper Limb Function (68), and the actual use of the affected hand in bimanual activities, as measured by the Assisting Hand Assessment (69), could link hand impairments to MA because they include items related to both the ICF domains of the body functions and activities (i.e., subdomain of mobility) (9).

By exploring the process by which hand skills are related to MA, this study highlights potential treatment priorities to improve hand functioning in children with CP. First, the strengthening of hand muscles may indirectly contribute to improve MA, through its impact on dexterity. In the past, muscle strengthening was not recommended for children with CP because it was believed that muscle strengthening would increase spasticity (21). However, in their study of nine hemiplegic children, Vaz et al. (70) observed significant strength gains due to wrist muscle strengthening by electrostimulation, but no change in passive stiffness. A recent study of 10 children with CP found good short-term efficacy of repetitive intensive strengthening training of the hand, in terms of muscle strength, muscle size, kinematics, and motor function (71). Although additional studies are required to confirm the efficacy of strength training on MA, we believe that weakness of the hand muscles, including spastic muscles, should be treated. Second, dexterity training of children with CP can be helpful to improve MA. The GMD in both hands were the strongest contributors to MA. The GMD mediated the relationship between hand functions and MA, possibly because GMD reflects a combination of both the ICF domains of body functions and activities. Third, our finding that hand skills only partially predicted MA in children with CP has several clinical implications. A therapist cannot assume that an improvement in hand skills will result in a correspondingly higher MA. Interventions focused solely on reducing hand impairments may be questionable, especially as it is more important for CP children to manage daily activities autonomously than it is for them to have "normal" hand functions (72). This conclusion does not mean that interventions based on body functions are useless; indeed, they may be important, especially for preventing secondary impairments (e.g., contractures or deformities) (73). However, as MA is not simply the integration of hand skills in daily activities, MA should be treated *per se*,

supporting the usefulness of activity-based interventions such as CIMT or HABIT.

Some limitations should be noted in the interpretations of our findings. The current cross-sectional dataset limits our ability to make causal inferences. A longitudinal study design with multiple end-point measurements is required to ascertain the temporal sequence and to confirm the causal relationships between variables. An additional limitation is the sample size. Although we used a moderate sample size for SEM (37), the necessary number of subjects depends on the model complexity; more parsimonious models (i.e., with less parameter estimates) require smaller samples than more complex models (37). A common sample size guideline for path analysis suggests that 10–20 subjects per parameter are sufficient for reliable model precision (37). Our sample size was adequate, as the ratio of subjects to parameters was 10:1 (i.e., 136/14). As 52% of our sample was constituted by spastic hemiplegic children, the identified path model might reflect the hand functioning of spastic hemiplegics more than that of all CP types. However, children were recruited from different settings (e.g., a CP reference center, university hospitals, special education schools, and rehabilitation centers); thus, the original sampling likely provided a fairly representative CP sample.

The proposed path model is only one possible model of hand functioning in children with CP. A good fit indicates that the model is consistent with the relationships observed in the data. However, there may be other models that also fit the data well. Whenever possible, the relative fit of alternative theoretically plausible models should be considered (74). Several alternative models that included small modifications were tested, all of which presented worse goodness-of-fit and predictive fit indices than the proposed model. Although this result strengthens our confidence in the proposed model, future studies are required to validate our model by confirming models that are based on other independent samples with larger sample sizes and longitudinal study designs (74). The robustness of the model could be tested by selecting other measures of the involved variables. As more evidence is accumulated across studies, we can be more confident in the accuracy of the proposed model.

Although the present study should be regarded as preliminary in light of its limitations, it offers potentially helpful clinical guidelines about the relevant hand skills that should be accounted for when designing hand-care interventions, as well as treatment priorities that should be set up to improve hand functioning in children with CP. Hand muscle strengthening and dexterity training may be useful to improve MA in children with CP. However, MA is not simply the integration of hand skills in daily activities and should be treated *per se*, supporting the usefulness of activity-based interventions.

AUTHOR CONTRIBUTIONS

Carlyne Arnould performed the statistical analyses, conducted the literature search, and drafted the manuscript. Carlyne Arnould and Yannick Bleyenheuft participated in the data collection. All authors contributed to the study design, participated in the data interpretation, critically revised the draft of the manuscript for important intellectual content, and contributed to the writing. All authors have read and approved the final manuscript. Data sharing statement: dataset is available from Carlyne Arnould at carlyne.arnould@gmail.com.

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The two visual systems hypothesis: new challenges and insights from visual form agnosic patient DF

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Patient DF, who developed visual form agnosia following carbon monoxide poisoning, is still able to use vision to adjust the configuration of her grasping hand to the geometry of a goal object. This striking dissociation between perception and action in DF provided a key piece of evidence for the formulation of Goodale and Milner's Two Visual Systems Hypothesis (TVSH). According to the TVSH, the ventral stream plays a critical role in constructing our visual percepts, whereas the dorsal stream mediates the visual control of action, such as visually guided grasping. In this review, we discuss recent studies of DF that provide new insights into the functional organization of the dorsal and ventral streams. We confirm recent evidence that DF has dorsal as well as ventral brain damage – and that her dorsal-stream lesions and surrounding atrophy have increased in size since her first published brain scan. We argue that the damage to DF's dorsal stream explains her deficits in directing actions at targets in the periphery. We then focus on DF's ability to accurately adjust her in-flight hand aperture to changes in the width of goal objects (grip scaling) whose dimensions she cannot explicitly report. An examination of several studies of DF's grip scaling under natural conditions reveals a modest though significant deficit. Importantly, however, she continues to show a robust dissociation between form vision for perception and form vision-for-action. We also review recent studies that explore the role of online visual feedback and terminal haptic feedback in the programming and control of her grasping. These studies make it clear that DF is no more reliant on visual or haptic feedback than are neurologically intact individuals. In short, we argue that her ability to grasp objects depends on visual feedforward processing carried out by visuomotor networks in her dorsal stream that function in the much the same way as they do in neurologically intact individuals.

Keywords: patient DF, two visual systems hypothesis, grasping, perception and action, dorsal and ventral streams

Just a few days after her 34th birthday in 1988, a young woman was taking a shower in her newly renovated cottage and was nearly asphyxiated by carbon monoxide from a poorly vented water heater. Although she had passed out from hypoxia, her partner found her before she died and rushed her to hospital. When she emerged from her coma, it was clear that her brain had been badly damaged from lack of oxygen. Her vision was particularly affected. She could no longer recognize common objects by sight or even her husband and friends. In the days and weeks that followed her accident, she showed some improvement, but in the end she was left with a profound visual form agnosia; in other words, she could no longer identify objects on the basis of their shape. Indeed, in later testing, it became apparent that DF (as she is now known in the literature) could not identify even the simplest of geometric figures, although her ability to see colors and visual textures remained relatively intact.

DF's ability to perceive the form of objects is so compromised that she cannot distinguish a rectangular block of wood from a square one with the same surface area (**Figure 1A**). Such blocks are often referred to as "Efron" blocks, after the psychologist, Robert Efron, who first devised shapes such as these to test for visual form agnosia (1). DF cannot even manually estimate the widths of the blocks by opening her finger and thumb a matching amount (2, 3). Nevertheless, one aspect of DF's visually guided behavior with respect to object form has remained remarkably preserved. When she reaches out to pick up one of the Efron blocks, the aperture between her thumb and finger scales in flight to the object's width (2–7). Similarly, even though DF cannot distinguish perceptually amongst objects on the basis of their orientation and shape, she orients her wrist correctly when posting her hand or a wooden card through a slot (2, 8, 9) and places her fingers on stable grasp points when picking up smooth-spline, pebble-like shapes [**Figure 1B**; see Ref. (10)]. In other words, despite a profound deficit in form perception, DF seems able to use information about object form to guide her grasping movements.

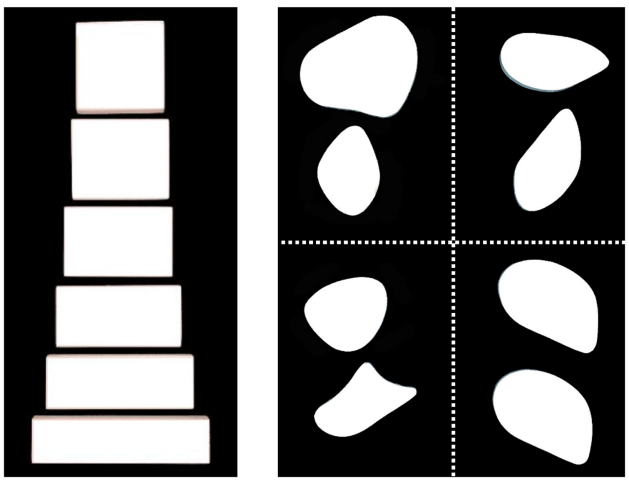

FIGURE 1 | (A) Examples from a set of Efron blocks that, by definition, are matched for surface area, texture, mass, and color, but vary in width and length (1). In the grasping task, DF reached out to pick these objects up across their width. In a typical perceptual task, she is asked to indicate manually the width of the block by adjusting her thumb and index-finger a matching amount or to provide same/different judgments about pairs of these objects. **(B)** Examples of the pebble-like shapes used in Goodale et al. (10). DF was asked to either (i) reach out to pick up the shapes presented at one of two possible positions one at a time or (ii) give explicit same/different judgments about pairs of shapes when they had different shapes and different orientations (top left), the same shape but different orientations (top right), different shapes but same orientations (bottom left), and same shape and orientation (bottom right).

DF's dissociation was one of the key pieces of evidence for the original formulation of the Two Visual Systems Hypothesis (TVSH) put forward by Goodale and Milner in 1992 (11). According to the TVSH, the ventral stream of projections from early visual areas to the inferotemporal cortex mediates vision for perception, whereas the dorsal stream of projections to the posterior parietal cortex mediates the visual control of actions. DF was later shown to have bilateral damage in her ventral stream, particularly in a region of the lateral occipital cortex (area LOC; see **Figure 1**) implicated in object recognition [for review, see Ref. (12)]. Other patients, who have damage to the dorsal but not the ventral stream, show clear deficits in visuomotor control but relatively spared visual perception (10, 13, 14). Although this double dissociation is by itself compelling, the TVSH is also supported by a broad range of additional evidence extending from monkey neurophysiology to neuroimaging studies of both patients and neurologically intact individuals [for review, see Ref. (15–18)].

Nevertheless, it is important to acknowledge that DF's lesions are not restricted to her ventral stream. Her brain shows the typical pattern of diffuse atrophy that is seen in patients who have experienced hypoxia from carbon monoxide poisoning, but in her case the cortical thinning is most evident in the posterior regions of the cerebral cortex (see **Figure 2**). Moreover, in addition to the bilateral damage to LOC in her ventral stream, the original clinical scans also showed evidence of localized damage in the parieto-occipital cortex (POC) of her left hemisphere (2). Subsequent high-resolution MRI scans confirmed the presence of a POC lesion in the left hemisphere while noting extensive bilateral atrophy in the posterior regions of the intraparietal sulcus and in POC of the right hemisphere (19), and the most recent scans indicate that the lesion to POC is now evidently bilateral (20), suggesting that the atrophy has increased in size in these and other areas (see **Figure 2**). Nevertheless, functional magnetic resonance imaging (fMRI) makes it clear that, despite the lesions to the POC and atrophy in the surrounding tissue, there is robust activation in the anterior intraparietal sulcus of DF's brain during visually guided grasping [Ref. (19); see **Figure 3**]. This dorsal-stream area has long been associated with the planning and execution of prehensile movements in both monkeys (21–24) and neurologically intact humans (19, 25–32). Importantly, the activation in DF's anterior intraparietal cortex occurs despite the fact that she has functionally complete bilateral damage of LOC, suggesting that the computations that mediate her spared visual control of grasping are not dependent on form processing in the ventral stream.

The bilateral damage to area POC in DF's brain warrants some discussion of the role of this brain area, particularly since it forms part of the dorsal stream. After all, the TVSH would predict that damage to this area would affect visually guided action. In fact, a mounting body of evidence implicates POC in the control of visually guided reaching, particularly to targets presented in the periphery [for review see Ref. (34–38)]. In an important study, Karnath and Perenin (38) carried out an analysis of lesion sites in 16 optic ataxic patients with unilateral damage to either the left or the right posterior parietal cortex. The authors contrasted these patients with control patients who had sustained damage to their parietal cortex but who did not exhibit optic ataxia. Their analysis showed that the greatest degree of lesion overlap that was unique to the optic ataxic patients occurred in POC and in the precuneus. Critically, all of the patients with optic ataxia showed misreaching errors when reaching out to touch targets presented in the periphery of their contralesional field. Although there is clear evidence that optic ataxia can include visuomotor deficits in central vision [e.g., Ref. (13, 14, 39, 40)], it is well-known that optic ataxia more frequently manifests itself as misreaching to targets presented in the periphery (41, 42). In fact, peripheral and centrally guided reaches might well rely on separate networks in the posterior parietal cortex (43, 44). Clavagnier et al. (43) have argued that the POC forms part of a fronto-parietal network of areas that is critical for visually guided reaches to peripherally presented targets.

Given the damage to DF's POC, it is perhaps not surprising that this region shows unusually little, if any, fMRI activation in this region when she reaches out to touch targets (19) and that she exhibits a gross deficit when reaching out to point to targets in the periphery, but not when pointing to targets presented centrally (33, 45). Thus, DF's deficit in peripheral reaching is likely due to the damage in her POC. There is also some indication that the POC in monkey and in man plays a role in the control of grasps that are directed at peripheral targets (46–48). For example, patient MH, who developed optic ataxia following a unilateral POC lesion, not only shows a deficit in pointing to targets presented in the periphery of his contralesional field, but he also shows a deficit in grip scaling when grasping these same objects. Critically, however, if the

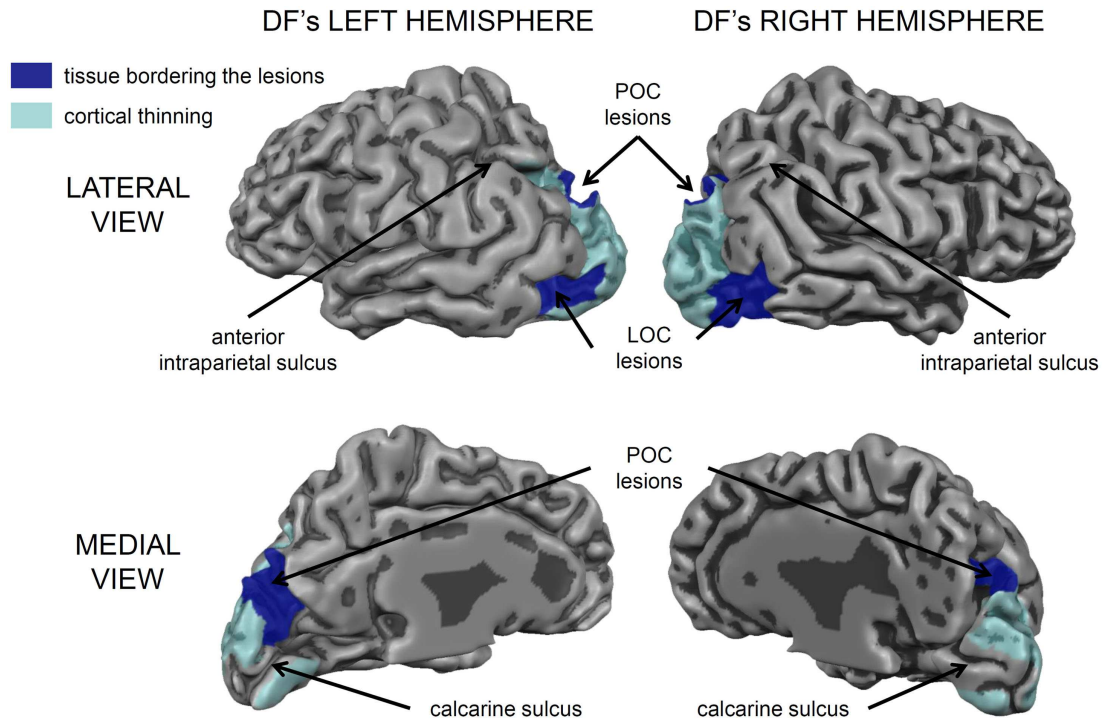

FIGURE 2 | A 3D rendering of the cortical gray matter boundary of DF's brain. The peripheral surface of her gyri is depicted as lighter and more reflective, whereas, the sulci are depicted a darker gray. The areas of cortical thinning are painted in translucent light blue and encompass much of peri- and extrastriate cortex, especially in the left hemisphere [see Ref. (20) for a detailed analysis]. There are also prominent bilateral lesions in the lateral occipital cortex (LOC) and additional lesions in the parieto-occipital cortex (POC) marked in dark blue. Importantly, the cortical tissue surrounding most of the calcarine sulcus, corresponding to primary visual cortex (V1) is intact, as are most of the frontal, temporal, and parietal cortices. The small lesion in the anterior part of the upper bank of the calcarine sulcus in her left hemisphere accounts for the partial quadrantanopia in her lower visual field [see Ref. (10, 33)].

objects are closer and he does not have to reach out toward them before picking them up, MH's grip scaling is normal. This suggests that his grasping deficit is secondary to his deficit in reaching (49). Interestingly, DF also shows a deficit in grip scaling when reaching out to pick up targets located in her peripheral visual field (33). But again, this deficit in grasping targets in the periphery might be secondary to her demonstrated deficit in reaching into the periphery, as it is in patient MH.

Nevertheless, DF's visuomotor performance, even centrally, is not completely normal in all situations. Himmelbach and colleagues (50) revisited DF's grasping with the aim of testing for a dissociation using the independent sample *t*-tests recommended by Crawford et al. (51). Himmelbach et al. compared her performance [as reported in Ref. (2, 10)] with that of 20 new age-matched control participants on three different visuomotor tasks: posting a hand-held card through a slot, picking up Efron blocks of varying width, and picking up smooth-spline pebble-like shapes (2, 10). Although DF's grip scaling (as measured by correlations) with rectangular objects fell within the range of the new control participants, the grasp points she selected when picking up the pebble-like shapes were not as optimal as those of the new control participants tested by Himmelbach et al. Her performance on the card-posting task was also slightly, but significantly, poorer than that of the controls. Nevertheless, as the authors themselves admit, the tests also revealed that DF's data set satisfied Crawford et al.'s (52) criterion for a "strong/differential" dissociation. Unlike the criterion for a "classic" dissociation in which the patient shows a deficit in one task but not the other, the criterion for a "strong/differential" dissociation allows for a deficit in both tasks, but, critically, requires a dramatically greater deficit in one task than in the other. In other words, despite the presence of slight impairments, DF's performance on the action tasks were consistently better than her performance on the corresponding perceptual tasks – and this difference was much larger for her than it was for the controls.

Although DF's spared visuomotor abilities have been examined in a number of different settings, it is her ability to scale her grip aperture to the relevant dimension of a goal object when picking it up that has been tested most often. No matter how the computations underlying the programming and control of grasping are conceptualized [e.g., Ref. (53–59)], there is general agreement that the accurate grasping of a goal object normally requires a visual analysis of the object's shape so that the final positions of the thumb and fingers can be computed correctly with respect to the relevant dimension of the object, such as its width. Any error in this computation could lead to the object being knocked away or fumbled. When assessing DF's grasping ability, investigators have typically relied on the known positive linear relationship between

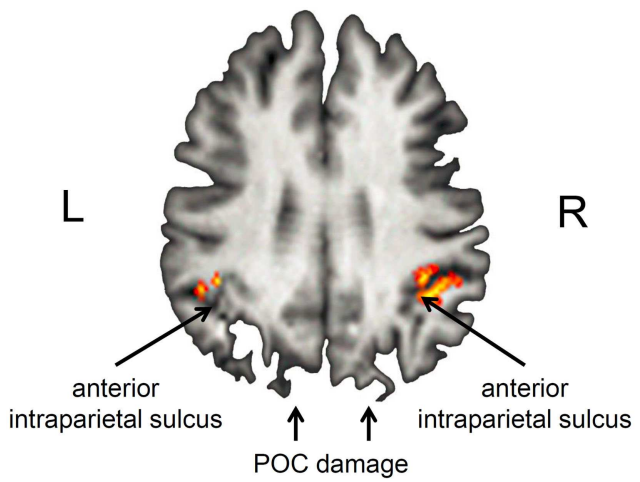

FIGURE 3 | Horizontal section through DF's brain illustrating grasp- and reach-related activation in the anterior intraparietal sulcus (aIPS). Grasp-specific activation is largely restricted to the right hemisphere. Note that these regions are activated despite the presence of bilateral damage to the parieto-occipital cortex (POC). Unlike healthy controls, there was little or no activation associated with reaching in the POC (19).

the maximum opening of the hand mid-flight and object's targeted dimension (see **Figure 4**). Given the survey of DF's dorsal-stream damage discussed above and in light of Himmelbach's findings, we examined DF's grip scaling (as measured by regression slopes) across a range of studies in which she grasped centrally located targets under naturalistic viewing conditions which included online visual feedback (2–7). Critically, the targets in all these studies were drawn from a set of blocks that varied in width and length but were matched for surface area, texture, mass, and color, so that she could not discriminate one from another in perceptual tests. DF clearly scales her grip aperture to the widths of these targets when reaching out to pick them up (see **Figure 4**). Nevertheless, she does show a modest, though significant, deficit when compared to the controls. Critically, from study to study, DF's estimations of the widths of these targets remain at chance, whereas, not surprisingly, the estimations made by the controls are essentially perfect. Moreover, a formal test of the difference in performance across the two conditions indicates a significant strong/differential dissociation (52). In short, over the course of two decades of testing, DF's dissociation between object vision-for-action and object vision for perception remains as strong as ever.

As remarkable as DF's visually guided grasping is, however, it is clearly not without limitations. In fact, there are a number of seemingly simple task modifications that have a remarkably detrimental effect on her grip scaling. For example, if a target object is shown to DF and then taken away, she is unable to scale her grasp appropriately when she is asked to show how she would pick the target up should it have remained there. In healthy participants, of course, grip aperture still correlates well with the object's width, even for delays as long as 30 s. In DF, however, all evidence of grip scaling disappears after a delay of only 2 s (3). DF's poor performance cannot be due to a general impairment in memory: she has no difficulty showing how she would pick up an imaginary orange or a strawberry, objects that she would have encountered before her accident or would have handled in the past. In other words, when she pretends to pick up an imaginary orange, her hand opens wider than it does for an imaginary strawberry (3). Moreover, she is as accurate as normally sighted controls when asked to open her finger and thumb a particular amount (e.g., "show me how wide 5 cm is") with her eyes closed. Indeed, her manual estimations in this task are much better than they are when she is asked to indicate the width of an Efron block placed directly in front of her. It is important to note that even though the grasping movements made by normal participants in the delay condition are scaled to the width of the remembered objects, they look very different from those directed at objects that are physically present. This is because the participants are "pantomiming" their grasps in the delay conditions, and are thus relying on a stored perceptual representation of the object they have just seen. Presumably, DF's failure to scale her grasp after a delay arises from the fact that she cannot use a stored percept of the object to drive a pantomimed grasping movement because she never "perceived" the target object in the first place.

DF's inability to pantomime grasps becomes relevant in the context of a more recent series of experiments on DF's grasping abilities, which prompted the suggestion that her ability to grasp objects accurately relies critically on haptic feedback rather than on visual feedforward processing as is the case in normal individuals. Using an ingenious mirror apparatus, Schenk (60) demonstrated that DF's grip scaling is completely abolished in a task in which the target remains visible (as a virtual image in the mirror) yet is physically absent (behind the mirror) so that when her hand closes down on the apparent edges of the virtual target, it closes down on "thin air." Schenk argued that DF's failure to show grip scaling in this situation is due to the absence of haptic feedback, which would compensate for her poor visual abilities. According to Schenk, DF's grip scaling relies on the integration of visual and haptic feedback about location of the finger and thumb endpoints that are, presumably, applied in a predictive manner on subsequent trials [for a discussion of Schenk's interpretation and related issues, see Ref. (61, 62)]. When such haptic feedback is absent, Schenk argues, DF's ability to grasp objects falls apart because her degraded form vision cannot, by itself, support visually guided grasping.

We have offered an alternative, more straightforward explanation. We contend that grasping tasks in which the target is visible but not available to touch are actually pantomime tasks in which the participant has to pretend to contact the object. For the visuomotor systems in the dorsal stream to remain engaged, we would argue, there must be some sort of tactile confirmation that the visible target has been contacted at the end of the movement. In the absence of such feedback, participants revert to pantomiming and pretend to grasp the object they see in the mirror. This conclusion is supported by the fact that the slopes of the function relating grip aperture to object width in the normal participants in the absent-object task are much steeper than those typically observed in normal grasping in which the target object is physically present (63). In fact, the slopes resemble those seen in manual

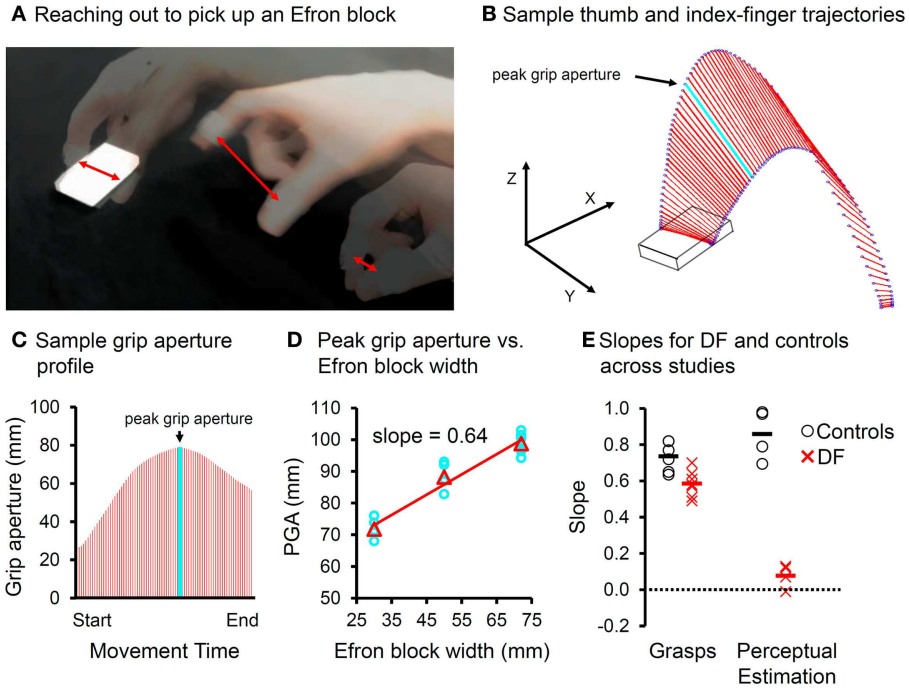

FIGURE 4 | (A) Superimposed snapshots of a reach-to-grasp action directed at an Efron block. Red double-headed arrows indicate "grip aperture," the Euclidean distance between the tracked markers placed on the tips of the thumb and index-finger **(B)** sample trajectories of the thumb and index-finger (blue circles) during a precision pincer grasp as the hand reaches out toward the object. The grip aperture is indicated in red. The light blue line reflects the peak grip aperture, which is achieved well-before the fingers contact the object. **(C)** Grip aperture plotted as a function of time (e.g., percent movement time). The peak grip aperture is again indicated in light blue. **(D)** Peak grip aperture shows a positive linear relationship to the target size of the object, and so it is thought to reflect the visuomotor system's anticipatory estimate of the target's width. The slopes can be used as indicators of "grip scaling." **(E)** The slopes for grasping and manual estimation for both the controls (open circles) and DF (X's) across studies in which Efron blocks were used, the visual conditions were "ecological" (i.e., online visual feedback was available), and the controls were gender-matched and age-appropriate for DF. Although DF scales her grasp to the width of the Efron blocks, her slopes are significantly shallower than those of the controls, using either independent or paired-samples variants of the *t*-test ($p_{max} < 0.04$). The slopes of DF's manual estimations are essentially zero and clearly different from those of the controls ($p_{max} < 6 \times 10^{-3}$). Critically, the difference in slopes between the grasping and manual estimation tasks falls well-outside of the range of the controls ($p_{max} < 5 \times 10^{-3}$). In other words, across a number of comparable studies of DF's grasping and perceptual estimation ability, her performance when grasping Efron blocks is sharply dissociated from her performance when perceptually estimating their width.

estimations of object width, suggesting that participants are relying on a perceptual representation of the target to drive their behavior rather than engaging more "encapsulated" visuomotor networks in the dorsal stream that normally mediate visually guided grasping. In short, in the absence of any tactile feedback, the participants default to a pantomime grasp. DF, of course, is at an enormous disadvantage in this situation because she does not perceive the form of the virtual image in the mirror and thus cannot generate a pantomimed response. As a consequence, her grip aperture bears no relationship to the width of the target in this situation.

To test this idea, we recently examined DF's performance using the same mirror set-up used by Schenk (60). In our experiment, however, there was always an object behind the mirror for her to grasp. Importantly, the width of that object never changed, even though the width of the object viewed in the mirror varied from trial to trial (5, 6). With this arrangement, DF always experienced tactile feedback at the end of the movement, but the feedback was completely uninformative about whether or not her grasp was properly tuned to the width of the object in the mirror. Contrary to what Schenk's visuohaptic calibration hypothesis would predict, we found that DF continued to show excellent grip scaling in this task. In other words, DF was able to use visual information in a feedforward manner to scale her grasp in the complete absence of reliable haptic feedback. Tactile contact by itself was evidently enough to keep the visuomotor systems in her dorsal stream engaged.

It is worth mentioning another prediction that follows from the visuohaptic calibration hypothesis (60, 64). According to Schenk, the reason DF is unable to manually estimate the width of an object is that, unlike in the grasping task, she experiences no haptic feedback about the object's width after she makes each estimate. We tested this prediction directly by allowing DF to pick up the object immediately after she had made her estimate (6, 7). Again, contrary to the visuohaptic calibration hypothesis, we found that DF continued to be unable to indicate the width of the object despite having accurate haptic information about the width of the target after every estimate. It would appear that an explicit estimate of size, reflecting what she perceived (or perhaps more correctly, did not perceive) of the object's width, could not take advantage of the haptic feedback.

As we pointed out earlier, the TVSH does not rest entirely on the evidence from DF. Support for the central ideas of the hypothesis comes from a broad range of studies, from monkey neurophysiology to human neuroimaging. Moreover, there is also converging evidence from other patients with visual form agnosia. Patient JS, for example, has bilateral lesions in the ventral stream that were more medial than DF's, but showed a similar dissociation between visual form perception and the visual control of grasping (65). In fact, there are a number of anecdotal reports in the long literature on visual form agnosia that such patients are able to reach out and grasp objects with surprising accuracy [e.g., Ref. (66)].

Patient DF's ability to use object form to guide the configuration of her grasping hand in the absence of conscious awareness of that form is reminiscent of what Weiskrantz and his colleagues called "blindsight" in an influential article published in *The Lancet* in 1977 (67). Patients with blindsight are able to respond to visual stimuli presented in their blind field despite a complete absence of visual phenomenology in that field. In fact, subsequent investigations of patients with "action" blind sight [for review, see Ref. (68)] have revealed a dissociation between prehension and perceptual size-estimation (69–73). These patients typically have lesions to the earliest visual cortical areas, including primary visual cortex or even the pathways from the lateral geniculate nucleus that innervate these areas. In a recent paper, Whitwell, Striemer, and Goodale (73) found that a young woman with a unilateral lesion of V1 was nevertheless able to scale her hand to the width of objects that she could not perceive. This observation coupled with many others demonstrating spared visuomotor control in patients with V1 lesions suggests that the posterior parietal cortex enjoys privileged access to visual inputs that bypass the retino-geniculo-striate route. One possible route for such transmission is the well-known set of projections from the superior colliculus in the midbrain to the pulvinar – and from there to the middle temporal area (MT) and the posterior parietal cortex. There are other candidate pathways as well [for review see Ref. (15)]. It seems unlikely that these extra-geniculo-striate projections evolved to be a "back up" should V1 happen to be damaged, but rather play a more integral role in the mediation of visually guided movements in neurologically intact individuals. It seems likely that these pathways normally supply the dorsal stream with essential information for the visual control of movements such as reaching and grasping – and that in DF's brain such pathways would also be at work.

In summary, the demonstration that DF has a remarkable ability to use information about object form and orientation to control skilled actions despite having a massive deficit in form vision has stood the test of time. Although a number of critics have tried to argue otherwise, it appears that she is able to use feedforward visual information about the shape of objects to guide her hand and fingers as she reaches out to grasp them – and her spared ability to do this does not depend on some sort of abnormal recruitment of haptic information to augment her compromised visual processing. Instead, it appears that vision-for-action in DF, at least as it applies to the control of grasping, depends on the recruitment of relatively intact visuomotor networks in her dorsal stream, and that these networks are engaged in much the same manner as they are in the normal healthy brain.

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Targeting the full length of the motor end plate regions in the mouse forelimb increases the uptake of Fluoro-Gold into corresponding spinal cord motor neurons

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Lower motor neuron dysfunction is one of the most debilitating motor conditions. In this regard, transgenic mouse models of various lower motor neuron dysfunctions provide insight into the mechanisms underlying these pathologies and can also aid the development of new therapies. Viral-mediated gene therapy can take advantage of the muscle-motor neuron topographical relationship to shuttle therapeutic genes into specific populations of motor neurons in these mouse models. In this context, motor end plates (MEPs) are highly specialized regions on the skeletal musculature that offer direct access to the pre-synaptic nerve terminals, henceforth to the spinal cord motor neurons. The aim of this study was two-folded. First, it was to characterize the exact position of the MEP regions for several muscles of the mouse forelimb using acetylcholinesterase histochemistry. This MEP-muscle map was then used to guide a series of intramuscular injections of Fluoro-Gold (FG) in order to characterize the distribution of the innervating motor neurons. This analysis revealed that the MEPs are typically organized in an orthogonal fashion across the muscle fibers and extends throughout the full width of each muscle. Furthermore, targeting the full length of the MEP regions gave rise labeled motor neurons that are organized into columns spanning through more spinal cord segments than previously reported. The present analysis suggests that targeting the full width of the muscles' MEP regions with FG increases the somatic availability of the tracer. This process ensures a greater uptake of the tracer by the pre-synaptic nerve terminals, hence maximizing the labeling in spinal cord motor neurons. This investigation should have positive implications for future studies involving the somatic delivery of therapeutic genes into motor neurons for the treatment of various motor dysfunctions.

Keywords: motor end plates, motor neurons, Fluoro-Gold, mouse forelimb, motor neuron columns, retrograde tracing

INTRODUCTION

Knowledge regarding the organization of the lower motor neuron system has significantly developed through the work of Sherrington (1892), Romanes (1941, 1946, 1951), and Rexed (1954). Collectively, their work has established that motor neurons in the ventral horn of the spinal cord that innervate skeletal muscles are arranged into longitudinal columns. More recently, retrograde tracers, either applied to the peripheral nerve stump or injected intramuscularly, have been instrumental in defining the connectivity between individual skeletal muscles and the innervating motor neuron columns in various mammalian species (Kristensson and Olsson, 1971a,b; McHanwell and Biscoe, 1981; Jenny and Inukai, 1983; Nicolopoulos-Stournaras and Iles, 1983; Brichta et al., 1987; Callister et al., 1987; Hörner and Kümmel, 1993; Novikova et al., 1997; Vanderhorst and Holstege, 1997; McKenna et al., 2000; Choi et al., 2002; Tosolini and Morris, 2012; Bácskai et al., 2013a,b). Together, these studies further characterize the organization of motor neuron columns throughout the spinal cord.

Dysfunctions or diseases of the lower motor neurons are amongst the most debilitating motor disorders. In this regard, the emergence of numerous transgenic mouse models of lower motor neuron conditions provide insight into the mechanisms underlying these pathologies (Gurney et al., 1994; Wong et al., 1995, 2002; Hsieh-Li et al., 2000; Kaspar et al., 2003; Ishiyama et al., 2004; Turner et al., 2009; Wegorzewska et al., 2009; Kimura et al., 2010; Towne et al., 2010; Xu et al., 2010; Guo et al., 2011; Riboldi et al., 2011; Pratt et al., 2013). For example, the Cu/Zn superoxide dismutase type-1 (SOD-1) mouse model was developed in order to further understand the etiology and pathogenesis of a subtype of amyotrophic lateral sclerosis (Gurney et al., 1994; Wong et al., 1995; Raoul et al., 2005; Zhong et al., 2009; Towne et al., 2010; Riboldi et al., 2011). This is also the case for the survival motor neuron 1 (SMN) knockout mouse model of spinal muscular atrophy (SMA) (Hsieh-Li et al., 2000). With these mouse models, viral-mediated gene therapy can take advantage of the muscle-motor neuron topographical relationship to retrogradely shuttle therapeutic genes into specific populations of motor neurons (for

recent reviews, see Bo et al., 2011; Wang et al., 2011; Federici and Boulis, 2012; Franz et al., 2012; Lentz et al., 2012). This approach has been explored using intramuscular bolus injections (Baumgartner and Shine, 1997, 1998; Kaspar et al., 2003; Nakajima et al., 2008, 2010; Uchida et al., 2012; Benkhelifa-Ziyyat et al., 2013). With this gene delivery method, however, the levels of transgene expression in motor neurons and, therefore, the outcomes of the therapy often remain suboptimal.

Motor end plates (MEPs) are highly specialized regions on the skeletal musculature that offer direct access to the pre-synaptic nerve terminals, henceforth to the spinal cord motor neurons. We have recently described the location and span of the MEP regions for several muscles of the rat forelimb (Tosolini and Morris, 2012). Targeting the entire MEP region with retrograde tracers has revealed that the motor neuron columns supplying the rat forelimb span more cervical segments and exhibit greater overlap with neighbor columns than previously reported (Tosolini and Morris, 2012). The aim of the present investigation was to extend this knowledge to the mouse, the species of choice for gene targeting in animal models of various motor conditions (Hsieh-Li et al., 2000; Kaspar et al., 2003; Ishiyama et al., 2004; Turner et al., 2009; Wegorzewska et al., 2009; Kimura et al., 2010; Towne et al., 2010; Xu et al., 2010; Guo et al., 2011; Riboldi et al., 2011; Pratt et al., 2013).

MATERIALS AND METHODS

ANIMALS

All experimental procedures complied with the Animal Care and Ethics Committee of the University of New South Wales and were performed in accordance with the National Health and Medical Research Council of Australia regulations for animal experimentation. A total of 38 adult male C57BL/6 mice (ARC, Western Australia) weighing between 20 and 30 g at the time of surgery were used in this study. The mice were housed in groups of five in an animal holding room under 12-h light–dark cycle. Water and chow were available *ad libitum* throughout the course of the experiment.

ACETYLCHOLINESTERASE HISTOCHEMISTRY

Acetylcholinesterase histochemistry (AChE) was performed on mice carcasses as per Tosolini and Morris (2012). Six lightly perfused mice were obtained through tissue sharing. The skin was removed from the carcasses and the entire bodies were immersed for 4 h at 4˚C in a solution containing 200 ml of phosphate buffer (PB), 290 mg acetylthiocholine iodide, 600 mg glycine, and 420 mg copper sulfate (all reagents from Sigma-Aldrich, St. Louis, MO, USA). The carcasses were subsequently washed for 2 min in distilled water and developed by rapid immersion (i.e., 5–10 s) in a 10% ammonium sulfide solution.

SURGERY

Anesthesia was induced with isoflurane (Provet, Sydney, NSW, Australia; 1–2% in O_2). The fur covering the targeted areas was shaved and cleaned with 70% ethanol. For each muscle under investigation, a small incision was made directly in the skin to expose the muscle of interest. Fluoro-Gold (FG) (Fluorochrome, Denver, CO, USA) injections were manually performed through graded glass micropipettes (DKSH, Zurich, Switzerland) along the entire MEP region. Great care was taken to preserve the fasciae covering both the targeted muscles and those in the surrounding. Special care was also taken to ensure that the blood vessels surrounding the muscles were left intact. After the injections, the muscles were wiped with gauze to remove any tracer that may have inadvertently seeped from the injected muscle. A total of 47 series of intramuscular injections along the full extent of the MEP region were performed into the following muscles: acromiotrapezius ($n = 6$), acromiodeltiodeus ($n = 6$), spinodeltoideus ($n = 5$), biceps brachii ($n = 6$), triceps brachii ($n = 6$), extensor carpi ulnaris ($n = 4$), extensor carpi radialis ($n = 6$), flexor carpi ulnaris ($n = 4$), and flexor digitorum profundus ($n = 4$). For these injections, the volume of FG varied between 2 and 6 µl depending on the size of the muscle (i.e., triceps brachii received 6 µl whereas extensor carpi ulnaris received 2 µl). Triceps brachii was also targeted with either a 3-µl bolus injection of FG into the thickest part of the muscle ($n = 6$) or with 3 µl injections restricted to the anterior or the posterior portion of its MEP ($n = 4$). In additional animals, 3 µl of FG was applied directly onto the intact fasciae covering triceps brachii ($n = 4$). The skin was subsequently closed with surgical clips (Texas Scientific Instruments LLC, Boerne, TX, USA).

HISTOLOGICAL PROCESSING AND DISSECTION

After the intramuscular injections of FG, the mice were kept for 7 days to allow for optimal retrograde transport of the neuronal tracer. After this period of time, the mice received a lethal dose of Lethabarb (Virbac, Sydney, NSW, Australia) and were intracardially perfused with 0.1 M PB followed by 4% paraformaldehyde in 0.1 M PB. Dissections of the spinal cord were made from the dorsal aspect whereby the paravertebral muscles were reflected/removed and the cervical vertebral column was exposed. The bony spinous process of C2 was identified and then removed, exposing the C2 dorsal roots, which were then colored with a permanent marker. Vertebrae C3–T1 were subsequently removed one by one and the dorsal roots were colored in alternating colors (i.e., C2, C4, C6, and C8 were colored with a green marker and C3, C5, C7, T1 were colored with a blue marker). After this process, the cervical spinal cord was cut transversely into two-segment blocks (i.e., C2–C3, C4–C5, C6–C7, and C8–T1 blocks). For each block, a fiducial mark was made *in situ* in the white matter, half way between two adjacent roots to indicate the boundary between the segments. The blocks were then removed from the body, post-fixed overnight in a solution containing 4% paraformaldehyde in 0.1 M PB and then cryoprotected in a 30% sucrose solution (Sigma-Aldrich, St. Louis, MO, USA) in distilled water for 2 days at 4˚C. Each block of spinal cord tissue was cut longitudinally in 50 µm-thick sections and mounted onto microscope slides. The slides were air-dried and then coverslipped with an anti-fade medium containing DAPI (Invitrogen, Carlsbad, CA, USA).

DATA ANALYSIS AND PRESENTATION

After the AChE procedure, the bodies were photographed, and Adobe Photoshop CS6 was used to transpose the average locations

of the MEPs onto a diagrammatic representation of the mouse forelimb adapted from Komárek (2004) and DeLaurier et al. (2008).

The spinal cord tissue sections were photographed and analyzed under epifluorescence to detect FG-labeled motor neurons. Motor neurons were considered positively labeled when FG granulations were present within both the soma and at least one axon/dendrite (Vanderhorst and Holstege, 1997; Tosolini and Morris, 2012; Bácskai et al., 2013a,b). Adjacent tissue was also scrutinized to eliminate double counting of motor neurons. For each tissue section, FG labeled motor neurons were plotted as single black dots on a separate layer of a diagrammatic representation of the spinal cord using Adobe Photoshop CS6. Root exit points, the position of the central canal and the fiducial marks created during dissection were used as spatial references. The Adobe Photoshop layers were subsequently stacked together to create a single two-dimensional representation of the position of the motor neurons innervating each forelimb muscle. For each muscle, individual data plots were then presented side by side on a schematic diagram of a spinal cord (**Figure 3**). The data plots derived from intramuscular injections performed on the left forelimb were transposed onto the right spinal cord to maintain consistency with the representation. For all muscles, data plots were then combined to form a representative motor neuron column and were represented concurrently in rostro-caudal, dorso-ventral, and medio-lateral axes in the same figure (**Figure 5**).

RESULTS

MOTOR END PLATE DELINEATION

Overall, for each muscle the location of the MEP region was similar between animals. The location and span of the MEPs for each muscle investigated is shown in **Figure 1**. **Figure 1A** is a photograph showing the lateral view of a mouse forelimb after an AChE reaction. The MEPs can be seen as black speckles traversing the muscle fibers. On this photograph, the boundaries of each muscle as well as the direction of their muscle fibers can also be observed, allowing for *in situ* muscle orientation. **Figures 1B,C** are schematic representations of the lateral and medial forelimb on which the location of the MEPs were transposed. The MEPs are typically organized in an orthogonal fashion across the muscle fibers. The MEP regions can be seen as extending across the full width of the muscles, passing through, but not limited to the region commonly referred to as the muscle "belly" (**Figures 1A–C**). It is worthwhile to note that some muscles do not have a "belly" region but still have a clearly observable MEP region. This is the case for acromiotrapezius, spinodeltoideus, and acromiodeltoideus. As each muscle has its own shape, so too does its MEP region. In most instances, the MEP regions are sinusoidal-like or V-shaped, however they never appeared to form a straight line. For muscles with multiple heads such as triceps and biceps brachii, the MEP region is located in the common part of the muscle, not in the heads. The thin and narrow muscles, such as those acting on the wrist joint (extensor carpi radialis, extensor carpi ulnaris, flexor digitorum profundus, and flexor carpi ulnaris) have their MEP

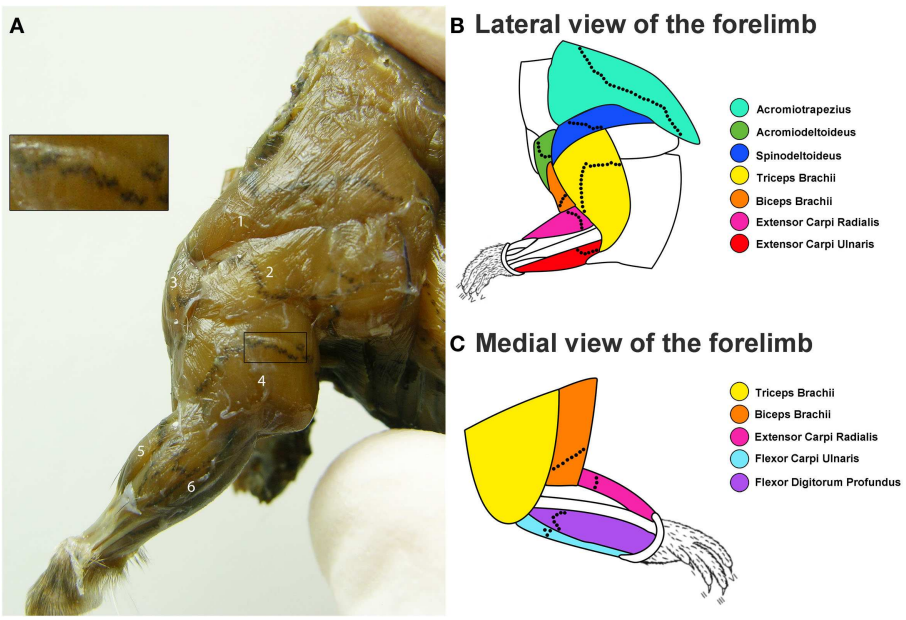

FIGURE 1 | Distribution of the motor end plate (MEP) regions for the mouse forelimb. **(A)** Lateral view of the mouse forelimb after an aceytlcholinesterase (AChE) histochemical reaction to reveal the location of the MEPs. In this figure, the MEPs appear as black speckles traversing the brown muscle fibers. From this lateral view, the following muscles can be seen: (1) acromiotrapezius, (2) spinodeltoideus, (3) acromiodeltoideus, (4) triceps brachii, (5) extensor carpi radialis, and (6) extensor carpi ulnari. The insert shows a close up view of the MEPs from a portion of the triceps brachii muscle. **(B,C)** Composite diagrams representing the location of the MEPs from the lateral **(B)** and medial **(C)** view of the forelimb. The color-coded forelimb muscles targeted are: acromiotrapezius (turquoise), acromiodeltoideus (green), spinodeltoideus (dark blue), biceps brachii (orange), triceps brachii (yellow), extensor carpi ulnaris (red), extensor carpi radialis (magenta), flexor carpi ulnaris (light blue), and flexor digitorum profundus (purple). The black dotted lines on each muscle are representative locations of the MEP region.

region located closer to the elbow joint rather than in the middle of the muscle (**Figures 1A–C**).

FLUORO-GOLD LABELED MOTOR NEURONS

The intramuscular injections of FG gave rise to intense labeling of motor neurons in proximity to the border between the gray and white matter. **Figure 2** is a photomicrograph of a right cervical cord to illustrate a typical column of labeled motor neurons. On this figure, FG granulations are present within multiple motor neuron somas and their processes.

DISTRIBUTION OF MOTOR NEURON COLUMNS SUPPLYING INDIVIDUALLY TARGETED MUSCLES

A total of nine forelimb muscles were targeted with intramuscular injections of FG: three muscles acting on the shoulder joint (acromiotrapezius, acromiodeltoideus, and spinodeltoideus), two muscles acting on the elbow joint (biceps brachii and triceps brachii), and four muscles acting on the wrist joint (extensor carpi radialis, extensor carpi ulnaris, flexor carpi ulnaris, and flexor digitorum profundus).

Acromiotrapezius

Acromiotrapezius, one of the muscles forming the trapezius muscle group (Komárek, 2004; DeLaurier et al., 2008), can be seen on the lateral aspect of the mouse forelimb (**Figures 1A,B**). Acromiotrapezius is a thin but large muscle connecting the spinous processes of vertebrae to the acromion process of the scapula. It is involved in retraction, elevation, and depression of the scapula. Six series of injections were performed along the MEP region of acromiotrapezius, four of which gave rise to intense labeling in the ventral horn of the cervical spinal cord. Data from these successful series injections ($n = 4$) were included in the present analysis. Such injections resulted in labeled motor neurons forming a column spanning segments C2–C6 of the spinal cord (**Figure 3A**).

Acromiodeltoideus

Acromiodeltoideus, one of the two muscles comprising the deltoid muscle group (Komárek, 2004; DeLaurier et al., 2008), is located on the lateral aspect of the mouse forelimb (**Figures 1A,B**). Acromiodeltoideus also acts on the glenohumeral joint. It is a small triangular muscle located at the anterior point of the shoulder at the junction of spinodeltoideus and biceps brachii (**Figures 1A,B**). Six series of injections of FG were performed in acromiodeltoideus and all six gave rise to intense labeling spanning segments C3–C7 of the cervical spinal cord (**Figure 3B**). Data from these successful series of injections ($n = 6$) were included in the present analysis.

Spinodeltoideus

Together with acromiodeltoideus, spinodeltoideus belongs to the deltoid muscle group (Komárek, 2004; DeLaurier et al., 2008). Spinodeltoideus is a trapezoidal-shaped muscle present on the lateral surface of the mouse forelimb (**Figures 1A,B**), immediately adjacent to acromiotrapezius. As is the case with acromiotrapezius and acromiodeltoideus, spinodeltoideus acts on the glenohumeral joint. Five series of intramuscular injections were performed on spinodeltoideus. Of these five series of injections, four series gave rise to bright labeling of motor neurons spanning segments C3–C6 of the cervical spinal cord (**Figure 3C**). Data from these four successful injections ($n = 4$) were included in the present analysis. It is worthwhile to mention that, in one case, two labeled motor neurons were present in C7. These two motor neurons were not taken into account in further analysis.

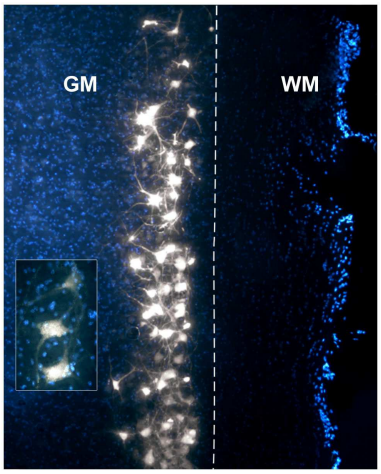

FIGURE 2 | Photomicrograph of a DAPI-stained longitudinal section through a right cervical spinal cord demonstrating a typical column of Fluoro-Gold (FG)-labeled motor neurons. The insert displays a higher magnification of motor neurons with clear FG granulations in the somas and axons/dendrites. GM, gray matter; WM, white matter. The dashed line represents the border between GM and WM.

Biceps brachii

Biceps brachii is located on the ventral aspect of the upper forelimb (**Figures 1A–C**) (Komárek, 2004; DeLaurier et al., 2008). The two proximal heads of biceps brachii unite to form one distal muscle mass that flexes the elbow joint. A total of six series of intramuscular injections of FG were performed into biceps brachii and all of the series gave rise to intense labeling of motor neurons between segments C3 and C7 of the spinal cord (**Figure 3D**). Data from these six successful series of injections ($n = 6$) were included in the present analysis.

Triceps brachii

Triceps brachii is located on the dorsal aspect of the upper forelimb (**Figures 1A–C**) (Komárek, 2004; DeLaurier et al., 2008). Similarly to biceps brachii, the three proximal heads of triceps brachii join to form one belly that extends the elbow joint. A total of six series of intramuscular injections of FG were performed into triceps brachii, four series of which resulted in intense labeling of a motor neuron column that spans segments C4–T1 of the spinal cord (**Figure 3E**). Data from these four series of injections ($n = 4$) were included in the present analysis. In one series case, however, three labeled motor neurons were present in rostral T1. These three motor neurons were not taken into account in further analysis.

Extensor carpi ulnaris

Extensor carpi ulnaris is one of the two muscles targeted in the present investigation that extends the wrist joint (Komárek,

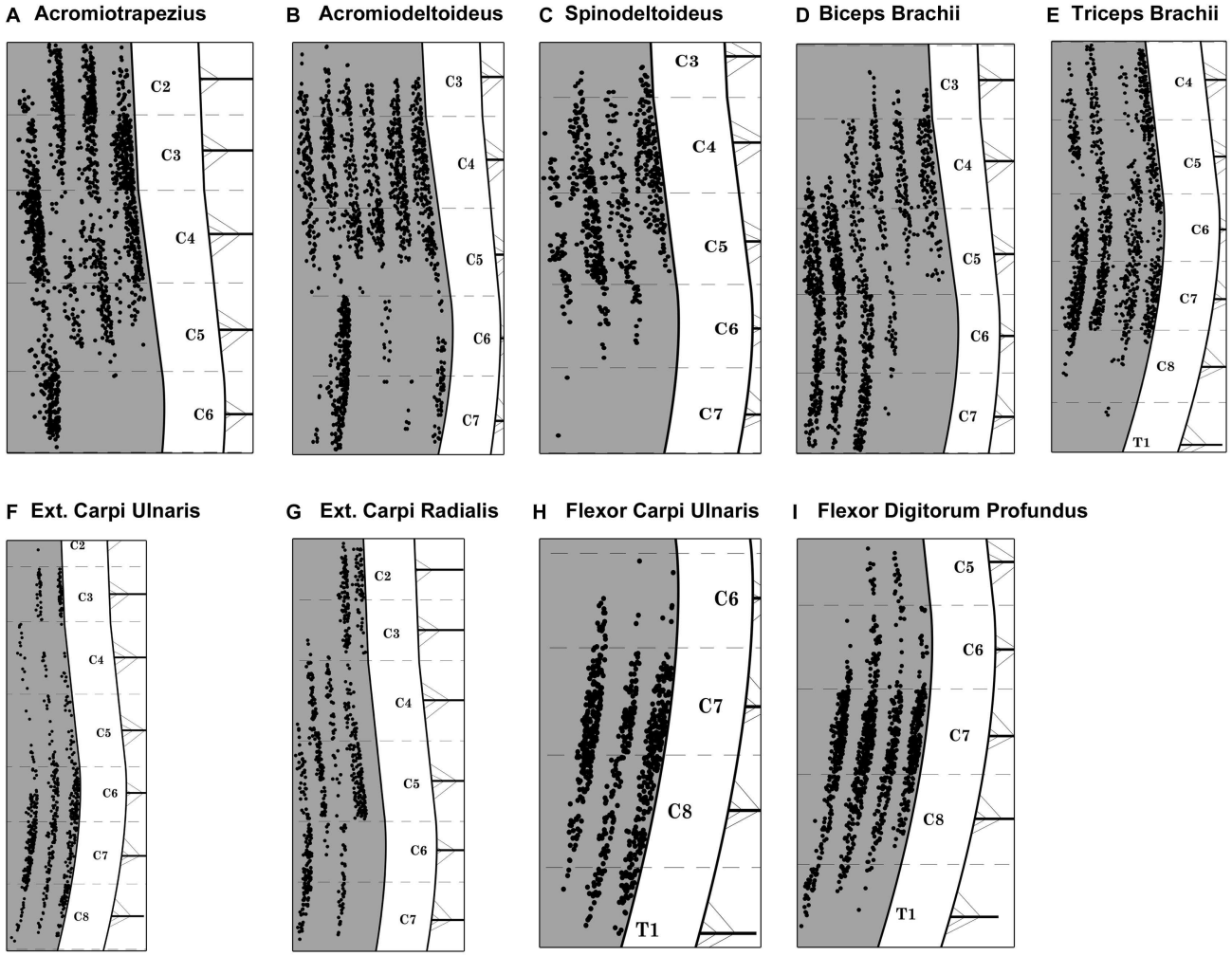

FIGURE 3 | Composite diagram illustrating the distribution of labeled motor neurons from each targeted muscle. Each black dot represents one labeled motor neuron and each columnar-shaped data set represents the FG-labeling observed after intramuscular injections in one muscle. **(A)** Acromiotrapezius, **(B)** acromiodeltoideus, **(C)** spinodeltoideus, **(D)** biceps brachii, **(E)** triceps brachii, **(F)** extensor carpi ulnaris, **(G)** extensor carpi radialis, **(H)** flexor carpi ulnaris, and **(I)** flexor digitorum profundus. Spinal cord levels are indicated in the white matter on the right hand side of each diagram. Each cervical/thoracic spinal cord segment is demarcated by dashed lines. These lines correspond to the halfway point between two nerve roots.

2004; DeLaurier et al., 2008). Extensor carpi ulnaris is a thin and shallow muscle located on the dorsal aspect of the distal forelimb (**Figures 1A,B**). Overall, there were four series of intramuscular injections performed in extensor carpi ulnaris, with three series giving rise to intense motor neuron labeling located between segments C2 and C8 of the cervical spinal cord (**Figure 3F**). Data from these three successful series of injections ($n = 3$) were included in the present analysis. In one case, however, one labeled motor neuron was present in caudal C2. This motor neuron was not taken into account in further analysis.

Extensor carpi radialis

Together with extensor carpi ulnaris, extensor carpi radialis, which is located on the dorsal part of the mouse distal forelimb, extends the wrist joint (**Figures 1A–C**) (Komárek, 2004; DeLaurier et al., 2008). Extensor carpi radialis is comprised of a smaller brevis and a larger longus compartments; however, both parts were targeted together. A total of six series of intramuscular injections of FG were performed into extensor carpi radialis. Of these six series, five series of injections gave rise to consistent labeling of motor neurons spanning cervical segments C2–C7 (**Figure 3G**). Data from these five injections ($n = 5$) were included in the present analysis.

Flexor carpi ulnaris

Flexor carpi ulnaris, a flexor muscle that acts on the wrist joint, is located on the ventral aspect of the distal part of the mouse forelimb (**Figure 1C**) (Komárek, 2004; DeLaurier et al., 2008). A total of four series of intramuscular injections of FG were performed in flexor carpi ulnaris. Of these injections, three gave rise to intense labeling of motor neurons forming a column between segments C6 and T1 of the spinal cord (**Figure 3H**). Data from these three injections ($n = 3$) were included in the present analysis.

Flexor digitorum profundus

As is the case for flexor carpi ulnaris, flexor digitorum profundus is a wrist flexor located on the ventral aspect of the mouse distal forelimb (**Figure 1C**) (Komárek, 2004; DeLaurier et al., 2008). A total of four series of intramuscular injections were performed into flexor digitorum profundus and all four series gave rise to intense motor neuron labeling between segments C5 and T1 of the spinal cord (see **Figure 3I**). Data from these four injections ($n = 4$) were included in the present analysis.

PARTIAL TARGETING OF THE MOTOR END PLATE REGION IN TRICEPS BRACHII

Figure 4A is a schematic representation of the portions of the MEP region of triceps brachii that were selectively targeted with FG. These regions are the anterior and posterior halves and the center of the MEP region. **Figure 4B** shows the distribution of labeled motor neurons resulting from these partial injections. Injections throughout the full length of the MEP region gave rise to columns of labeled motor neurons that extends from segment C4 through the rostral part of C8. In comparison, the partial targeting of the MEP region gave rise to correspondingly partial labeling of triceps brachii's motor neuron column. More specifically, the targeting of the anterior portion of the MEP region resulted in the labeling of motor neurons forming a column mainly spanning segments C4–C5. Conversely, FG injections limited to the posterior part of the MEP region for triceps brachii gave rise to a column of positively labeled motor neurons, the bulk of which was confined within segments C7 and the rostralmost aspect of C8. Moreover, single injections of FG in the center of the MEP region produced labeling in fewer motor neurons mainly confined to segment C7. Furthermore, the application of FG onto the external surface of triceps brachii's fascia resulted in a negligible number of labeled motor neurons.

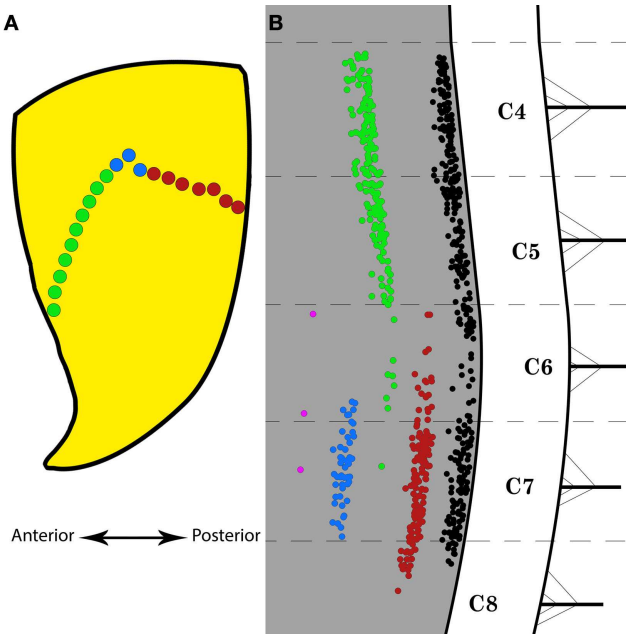

FIGURE 4 | Selective Fluoro-Gold (FG) targeting of the motor end plate region in triceps brachii and the resulting labeling in the spinal motor neurons. **(A)** Schematic representation of the motor end plates (MEPs) selectively targeted on the triceps brachii muscle. The green and red dots represent the anterior and posterior halves of the entire MEP region, respectively. The blue dots indicate the location of a bolus injection of FG in the belly of the muscle. The double-headed arrow indicates the antero-posterior direction. **(B)** Distribution of labeled motor neurons resulting from selective MEP injections of FG as indicated in **(A)**. The black motor neuron column is taken from **Figure 3E** and represents the typical labeling observed after full-length MEP injections in triceps brachii. The red motor neuron column was obtained after FG injections along the posterior half of the MEP region. The green motor neuron column was obtained after FG injections along the anterior half of the MEP region. The blue motor neuron column was obtained after FG bolus injections in the belly of triceps brachii. The magenta "column" was obtained after application of FG onto the external surface of the fascia over triceps brachii. Each cervical/thoracic spinal cord segment is demarcated by dashed lines. These lines correspond to the halfway point between two nerve roots.

OVERALL ORGANIZATION OF THE MOTOR NEURON COLUMNS SUPPLYING THE MOUSE FORELIMB

Figure 5 is a diagrammatic representation of the motor neuron columns for all muscles targeted in the present investigation. On the rostro-caudal axis, these columns of motor neurons encompass segments C2–T1 of the mouse spinal cord (**Figure 5A**). As shown in this figure, a great amount of overlap can be observed between these motor neuron columns. Motor neuron columns innervating the muscles acting on the glenohumoral joint (i.e., acromiotrapezius, acromiodeltoideus, and spinodeltoideus) extend from segments C2 to C7 whereas the motor neuron columns supplying the muscles acting on the elbow joint (i.e., biceps brachii and triceps brachii) span segments C3–C8. Moreover, the extensor (i.e., extensor carpi radialis, extensor carpi ulnaris) and flexor (i.e., flexor carpi ulnaris and flexor digitorum profundus) muscles acting on the wrist joint are innervated by motor neuron columns spanning segments C2–C8 and C5–T1, respectively. Overall, there is a topographical relationship, on the rostro-caudal axes, between the different muscles targeted in the present analysis and the motor neuron columns that innervate them. This relationship is such that the proximal-most muscles (e.g., acromiotrapezius) are innervated by motor neuron columns located in the rostral segments of the cervical spinal cord whereas the distal-most muscles (e.g., flexor carpi ulnaris) are supplied by columns located more caudally. Exceptions to this organizational scheme are the motor neuron columns for the two wrist extensors, namely extensor carpi radialis and extensor carpi ulnaris. Although these muscles are located in the distal part of the forelimb, they are supplied by motor neuron columns located in rostral segments of the spinal cord. **Figure 5B** shows the motor neuron columns in the transverse plane at spinal cord levels C3, C5, and C7. On this plane, the columns of motor neurons also exhibit a high level of overlap with each other. Overall, there is a dorso-ventral topographical relationship between the muscles targeted in the present investigation and the motor neurons that supply them. This relationship is such that the proximal (e.g., acromiotrapezius) and distal muscles (e.g., flexor carpi ulnaris) are innervated by motor neuron

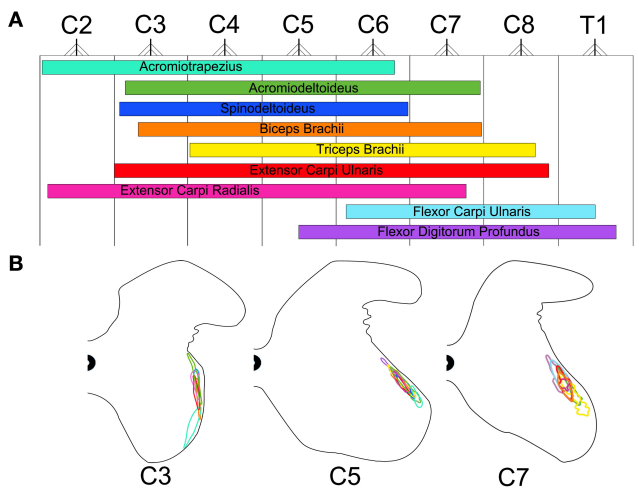

FIGURE 5 | Color-coded schematic map of the motor neuron columns innervating the Fluoro-Gold targeted forelimb muscles. The color schemes remain consistent with that of **Figures 1B,C**. **(A)** Rostro-caudal map of the motor neuron columns innervating the targeted forelimb muscles. These columns were obtained by combining plots from **Figure 3**. The nerve root exit points represent the halfway point between spinal cord segments throughout C2–T1. **(B)** Dorso-ventral and medio-lateral map of the motor neuron columns innervating the targeted forelimb muscles for spinal cord segments C3, C5, and C7. The gray matter contours were adapted from Watson et al. (2009).

columns located ventrally and dorsally, respectively, within the ventral horn spinal cord.

DISCUSSION

We have recently targeted the entire MEP region of several muscles of the rat forelimb with retrograde tracers (Tosolini and Morris, 2012). The results of this analysis showed that the motor neuron columns that supply these muscles extend over more cervical spinal cord segments and display greater overlap with one another than formerly reported. The aim of the present investigation was to transfer this knowledge to the species of choice for the design of genetically engineered models of motor dysfunction, namely the mouse. The main outcomes of this study are first, the production of a detailed map of the motor end plate region for nine muscles of the mouse forelimb. This map was subsequently used as a guide to target the entire motor end plate region of these muscles with FG. The second main outcome is the characterization of the topographical organization that exists between the mouse forelimb muscles and the motor neuron columns that innervate them.

MOTOR END PLATE ANALYSIS

To our knowledge, the present study is the first description of the MEP regions in the mouse forelimb. This analysis revealed that the MEP region for the different muscles targeted is located orthogonally to the direction of the muscle fibers. It is noteworthy that the MEP region is not consistently located within the fleshy part of a muscle (i.e., the muscle "belly"). Moreover, some muscles such as acromiotrapezius, spinodeltoideus, and acromiodeltoideus are flat and therefore do not have such a fleshy region. In these muscles as well as in triceps and biceps brachii, the MEP region traverses the entire width of the muscle (see **Figure 1**). The thin and narrow muscles, such as extensor carpi radialis, extensor carpi ulnaris, flexor digitorum profundus, and flexor carpi ulnaris have a fleshy part where the MEP regions are located. However, in these muscles, the so-called "belly" is not located in the center of the muscle but closer to the elbow joint. In most cases, the MEP region does not form a straight band. Rather, MEPs often exhibit a sinusoidal-like curve or are V-shaped. These findings are consistent with previous MEP characterization in the rat forelimb (Tosolini and Morris, 2012).

Fluoro-Gold was also injected along partial aspects of the MEP region in triceps brachii (see **Figure 4A**). The rostro-caudal extent of the labeling obtained from these partial MEP injections was then compared with that resulting from injections along the full length of the MEP region (see **Figure 4B**). Injections of FG restricted to the anterior half of the MEP region gave rise to a column of labeled motor neurons spanning only the rostral part of the columns of neurons obtained after complete MEP region injections. Conversely, FG injections restricted to the posterior half of the MEP region in triceps brachii produced a column of labeled motor neurons spanning only the caudal half of the column of motor neurons obtained after complete MEP region injections. Interestingly, combined labeling from the anterior and posterior MEP injections resulted in a column of similar span to that produced from the injections of the complete MEP region. These data suggest the existence of a MEP/motor neuron topographical relationship, although more data points need to be generated to confirm this *a priori* interesting finding. Should such organization be confirmed for triceps brachii, the MEP/motor neuron relationship will have to be established for the other muscles of the mouse forelimb. Injections selectively targeting the belly of triceps brachii were also performed (see **Figure 4**). As compared with the complete MEP data, these injections gave rise to shorter columns with substantially less FG-positive motor neurons. Together, these data can explain, at least partly, why targeting the entire MEP region with FG gave rise to labeled motor neurons spanning more spinal cord segments than recently observed (e.g., Bácskai et al., 2013a).

METHODOLOGICAL CONSIDERATIONS

In the present analysis, functionally diverse muscle groups that act on the three major joints of the forelimb were targeted with FG, namely the shoulder, elbow, and wrist. Superficial muscles of the mouse forelimb were selected for this tract-tracing experiment because these muscles are easily accessible. Indeed, the deeper muscles of the forelimb require a significant amount of dissection before they can be exposed and subjected to neuronal tracer injections, hence creating a risk for contamination of the tracers to surrounding muscles. It is our opinion that the superficial muscles of the forelimb have greater translational relevance than the deeper ones, as they are more likely to be the target in clinical trials involving somatic gene therapy.

In our hands, FG has proven to be a reliable and robust retrograde tracer that produces intense labeling of the neuronal somas

and processes, therefore allowing for easy identification and direct count of motor neurons (see **Figure 2**). Importantly, unlike some other neuronal tracers, FG does not have the tendency to leak out of the cells (Schmued and Fallon, 1986). Yet, leakage has been recently reported after intramuscular injections of FG in the mouse forelimb (Bácskai et al., 2013a). It is important to note that muscle fasciae consist of tough layers of fibrous connective tissue that act as a natural barrier to tracer leakage (Haase and Hrycyshyn, 1986). In the present study, great care was taken to leave the muscle fasciae intact, aside from the penetrations of the ultra-thin micropipettes. It is also worth mentioning that the outermost aspect of the fasciae (i.e., the fasciae that can be readily visualized once the skin over the muscle of interest is cut open) was difficult to puncture even with the sharpest glass micropipettes. As the innermost fasciae (i.e., the fasciae that cover the innermost part of the muscles) would offer the same resistance against puncture, we are confident that the minute amount of tracer injected did not contaminate the deep muscles underlying those of interest. Additionally, the exposed region was routinely wiped off immediately after each injection in order to remove any tracer that may have seeped out. Special care was also taken to ensure that the blood vessels surrounding the muscles were not perforated. Taken together, these precautions ensured that insignificant spurious labeling was generated in the present investigation. However, leakage of the tracer to adjacent muscles cannot be entirely ruled out. To investigate the possibility that, despite these extensive precautions, some tracer might have been taken up by non-targeted surrounding muscles, FG was directly applied onto the external fascia covering triceps brachii (see **Figure 4B**). The result of such application of FG resulted in negligible labeling, therefore confirming that, at least in our hands, spurious labeling was not significant.

Overall, all injections of FG in the same muscles resulted in similar labeling for each animal. However, some variability was observed, in the extent, on the rostro-caudal axes, of the motor neuron column (see **Figure 3**). The same observation has been reported in previous tract-tracing investigations of the organization of motor neurons supplying the skeletal muscles (Hollyday, 1980; McHanwell and Biscoe, 1981; Nicolopoulos-Stournaras and Iles, 1983; Vanderhorst and Holstege, 1997; McKenna et al., 2000; Coonan et al., 2003; Tosolini and Morris, 2012; Bácskai et al., 2013a,b). This variability could be due to intraspecies differences with regard to the overall number of motor neurons innervating a muscle, a phenomenon that, in turn, influences the length and/or spatial distribution of the motor neuron columns. Interestingly, we also found slight differences in spatial distribution of motor neuron columns within the same animal, i.e., between homologous muscles in the left and right forelimbs. It is possible that this finding reflects forelimb use preference (i.e., handedness). Forelimb preference when reaching is well documented in the rat (Gharbawie et al., 2007; Alaverdashvili et al., 2008; Alaverdashvili and Whishaw, 2010; Morris et al., 2011); however, it is still a matter of debate in the mouse (Neveu et al., 1988; Takeda and Endo, 1993; Waters and Denenberg, 1994; Biddle and Eales, 1996; Bulman-Fleming et al., 1997). In the present study, forelimb use preference was not determined prior to the delivery of neuronal tracer. Thus, whether forelimb use preference affects the distribution of the motor neuron columns remains to be investigated. On the other hand, one cannot rule out that there could have been differences in the uptake of FG across different injections targeting the same muscle. Indeed, although great care was taken to minimize inter-injection variability, the uptake of FG could have been suboptimal in some cases. In these instances, the number of labeled motor neurons can actually be considered as an underestimation of the actual population (Nicolopoulos-Stournaras and Iles, 1983; Tosolini and Morris, 2012).

TRANSLATIONAL RELEVANCE

Neurological conditions that affect lower motor neurons are among the most debilitating motor disorders. Genetically based mouse models are currently available for conditions that directly affect the output of spinal cord motor neurons on the skeletal musculature. In particular, these include models of amyotrophic lateral sclerosis, i.e., the SOD-1-G93A and A315T-TDP-43 strains (Jackson Laboratory, Bar Harbor, ME, USA) (Kaspar et al., 2003; Ishiyama et al., 2004; Turner et al., 2009; Wegorzewska et al., 2009; Towne et al., 2010; Xu et al., 2010; Guo et al., 2011; Riboldi et al., 2011). Likewise, models of Duchenne's muscular dystrophy, i.e., the various mdx strains (Jackson Laboratory, Bar Harbor, ME, USA) (Kimura et al., 2010; Pratt et al., 2013) and of SMA, i.e., the $SMN^{-/-}$ strain (Hsieh-Li et al., 2000) are also available. Mice with these above-mentioned mutations display a typical motor phenotype with upper and lower limb deficits.

Several treatment strategies have been designed in an attempt to reverse the motor phenotype in these mutant mice. For instance, both pharmaceutical and cell-based therapies have been performed through different routes of administration (Raoul et al., 2005; Henriques et al., 2011; Teng et al., 2012). These therapeutic approaches have been shown to slow the progression of the motor phenotype, to increase lifespan of the affected animals but are yet to eradicate these conditions. Targeted delivery of therapeutic agents to motor neurons for the treatment of ALS-like phenotypes has also been achieved via intramuscular injections and the ensuing retrograde transport along the peripheral nerve (e.g., Kaspar et al., 2003; Wu et al., 2009; Calvo et al., 2011). Intramuscular injection and retrograde delivery of therapeutic molecules is a minimally invasive surgical procedure and, in combination with viral vectors, offers promising potential for translational gene therapy aiming at the restoration of motor function (Baumgartner and Shine, 1998; Giménez y Ribotta et al., 1998; Kaspar et al., 2003; Nakajima et al., 2008; Towne et al., 2010; Uchida et al., 2012; Benkhelifa-Ziyyat et al., 2013). The present anatomical investigation has clearly shown that targeting the full width of the muscles' MEP region can maximize the success of somatic delivery of therapeutic molecules to spinal cord motor neurons. Thus, knowledge regarding the precise anatomical relationship between the different muscles of the mouse forelimb and the location of: (1) their MEP region and (2) the spinal cord motor neuron columns that supply them will prove to be valuable tools to further investigate new treatment for ALS and other related motor disorders.

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Nerve transfers to restore upper extremity function: a paradigm shift

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Brachial plexus and peripheral nerve injuries lead to significant upper extremity dysfunction and disability. Traditionally, both have been treated with nerve grafting when a tensionless, end-to-end repair is not feasible. Despite our best efforts, functional outcomes of this procedure are less than ideal due to the long distances that the axons must regenerate to reach their end organs. Over the past 20 years our understanding of nerve anatomy, topography, and regeneration has improved and the surgical technique of nerve transfers has been developed. Due to improved functional outcomes, decreased morbidity, and surgical time, we are now experiencing a paradigm shift in the treatment of brachial plexus and peripheral nerve injuries from nerve grafting to nerve transfers (1, 2).

Motor function after nerve injury is dependent on both time to reinnervation and the number of motor axons reinnervating the target muscle (3). Nerve transfers capitalize on these two factors and are the reason for their clinical success. Nerve transfers, by definition, involve coapting a healthy, expendable donor nerve or fascicle to a denervated recipient nerve to restore function to the recipient end-organ (skin for sensation or muscle for motor function). They can be performed closer to the recipient target allowing for earlier reinnervation of the muscle and quicker return of function. Further advantages include that nerve transfers are performed outside the zone of injury and scarred field, can be performed on patients with delayed presentation, and can avoid interpositional nerve grafting, which leads to increased numbers of regenerating nerve fibers making it to the target organ (3).

The ideal timing of nerve transfers has not yet been established, but reinnervation of the muscle by 12–18 months after injury is a common goal. Indications are evolving and currently include patients with proximal nerve root avulsions, high level peripheral nerve injuries, large neuromas-in-continuity, and/or multi-level nerve injuries. In our group, we use nerve transfers to treat most brachial plexus injuries (avulsions or not) and peripheral nerve injuries in upper arm or proximal forearm. We usually reserve nerve grafting for nerve injuries in the distal forearm or hand because the regenerative distances and time to reinnervation of the muscle are short. At these distal injuries, functional outcomes with grafting are similar to those seen with nerve transfers and donor site morbidity from a nerve transfer is avoided.

In brachial plexus injuries, a hierarchy of return of function exists with efforts directed to restoring elbow flexion first, followed by shoulder function, then hand function. For upper trunk injuries, multiple combinations of nerve transfers have been described. The double fascicular nerve transfer is the most common nerve transfer performed to return elbow flexion. This transfer involves coapting redundant nerve fascicles from the median and ulnar nerves to the biceps brachii and brachialis branches of the musculocutaneous nerve. Many have reported their experience with this transfer and patients have achieved at least Medical Research Council (MRC) strength of 3 with most achieving grade 4 or greater without evidence of donor site morbidity (4–6). For restoration of shoulder function transfers of the spinal accessory nerve to the suprascapular nerve and a branch of the triceps to the axillary nerve are most commonly performed. Thoracodorsal nerve and intercostal nerves transferred to the long thoracic nerve are also common to restore scapular stability provided by the serratus anterior muscle. Restoration of shoulder abduction and external rotation has been successfully reported with these nerve transfers (7, 8). In lower plexus injuries, the brachialis branch of the musculocutaneous nerve can be transferred with encouraging results to the anterior interosseous nerve to restore prehension. Previously, these lower plexus injuries were treated with free functional muscle transfers given the great regenerative distance from the brachial plexus to the forearm musculature. However, free functional muscle transfers are associated with increased morbidity, operative time, and lengthy hospital stays. The brachialis to anterior interosseous nerve transfer avoids these drawbacks and establishes a platform for restoring function to the hand.

In addition to their use for brachial plexus injuries, nerve transfers to restore hand function following peripheral nerve injuries are also gaining momentum. New transfers continue to be developed as our understanding of nerve topography grows. Ulnar nerve injuries result in loss of power grip, pinch strength, and hand dexterity. The pronator quadratus branch of the anterior interosseous nerve can be transferred to the motor component of the ulnar nerve distally in the forearm to reinnervate the intrinsic muscles of the hand (9). It was originally described as an end-to-end coaptation if no regeneration of the ulnar nerve is expected, but recently Mackinnon and colleagues have shown efficacy of an end-to-side "supercharge" coaptation enabling proximal regeneration of the ulnar nerve to proceed as well (10). Upper extremity trauma frequently results in radial nerve injuries impairing both

wrist and finger extension. Although tendon transfers are functional for patients with radial nerve palsies, nerve transfers from the median to radial nerves allow for independent thumb and finger extension (11). To restore median nerve function, transfer of branches of the radial nerve, the brachialis branch, and branches of the ulnar nerve have been described with good outcomes (12). Focusing on synergism and redundancy of function has led to the success of these transfers.

An exciting application of nerve transfers is in the field of spinal cord injury (SCI). Drs. Susan Mackinnon and Ida Fox at Washington University in St. Louis, MO, USA are leading developers in the use of nerve transfers to restore upper extremity function in patients with cervical SCI. These transfers are being developed to increase volitional control and improve independence. Unlike brachial plexus or peripheral nerve injuries, SCI patients have intact lower motoneurons below the level of injury and thus, the motoneuron – peripheral nerve – muscle end-organ connection remains intact. For this reason, the muscle is "preserved" and nerve transfers in SCI patients can be performed without the time sensitivity found with a peripheral nerve injury. Specific transfers for SCI include transfer of the brachialis branch of the musculocutaneous nerve to the anterior interosseous nerve to improve prehension and transfer of the deltoid nerve branches to the triceps branches to improve elbow extension. Evaluation and collaboration among the physiatrists, therapists, and surgeon are critical to identifying ideal candidates, developing operative plans, and ultimately achieving success with nerve transfers in this patient population.

In conclusion, nerve transfers are an essential tool for the peripheral nerve surgeon to improve upper extremity function after nerve injury. I would argue that nerve transfers are the preferred treatment for high peripheral nerve injuries and for most patterns of brachial plexus injury. In addition, they will likely play an increasing role in managing SCI patients. Return of earlier, more effective upper extremity function supports the importance of this surgical technique. As we critically analyze and report our outcomes with nerve transfers, further indications and expectations of return of function will be elucidated. The paradigm shift; however, is happening now. Nerve transfers viewed as "standard of care" may not be far away. Currently, they certainly hold great promise and should be considered in restoring upper extremity function in patients with devastating nerve injuries.

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Skilled reaching and grasping in the rat: lacking effect of corticospinal lesion

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The corticospinal system is a major motor pathway in the control of skilled voluntary movements such as reaching and grasping. It has developed considerably phylogenetically to reach a peak in humans. Because rodents possess advanced forelimb movements that can be used for reaching and grasping food, it is commonly considered that the corticospinal tract (CST) is of major importance for this control also in rodents. A close homology to primate reaching and grasping has been described but with obvious limitations as to independent digit movements, which are lacking in rodents. Nevertheless, it was believed that there are, as in the primate, direct cortico-motoneuronal connections. Later, it was shown that there are no such connections. The fastest excitatory pathway is disynaptic, mediated via cortico-reticulospinal neurons and in the spinal cord the excitation is mainly polysynaptically mediated via segmental interneurons. Earlier behavioral studies have aimed at investigating the role of the CST by using pyramidotomy in the brainstem. However, in addition to interrupting the CST, a pyramidal transection abolishes the input to reticulospinal neurons. It is therefore not possible to conclude if the deficits after pyramidotomy result from interruption of the CST or the input to reticulospinal neurons or both. We have re-investigated the role of the CST by examining the effect of a CST lesion in the C1–C2 spinal segments on the success rate of reaching and grasping. This lesion spares the cortico-reticulospinal pathway. In contrast to investigations using pyramidal transections, the present study did not demonstrate marked deficits in reaching and grasping. We propose that the difference in results can be explained by the intact cortical input to reticulospinal neurons in our study and thus implicate an important role of this pathway in the control of reaching and grasping in the rat.

Keywords: skilled forelimb movements, reaching, grasping, corticospinal tract lesion, reticulospinal, interneuron, motorneuron

INTRODUCTION

Skilled reaching and grasping are among the most complex voluntary movements that many different species of animals perform for their daily living and survival. The corticospinal tract (CST) plays a major role in the control of skilled voluntary movements such as reaching and grasping in higher species (1–5). This function has developed during phylogeny and has reached its peak in man (3, 6, 7). Rodents also perform skilled forelimb movements and it is generally accepted that the CST has an important role for reaching and digit grasping (8, 9). At first, microcircuit analysis using anatomical tracing and electrophysiology suggested, as in the primate, the existence of direct cortico-motoneuronal connections (10). These results further emphasized the idea that independent digit movements can only be performed if there are direct cortico-motoneuronal connections. Later, it was shown that there are no such connections in rodents (11, 12). It has been shown that the fastest excitatory pathways from motor cortex in the rat are mediated disynaptically via a cortico-reticulospinal–motoneuronal pathway (11). In the earlier behavioral studies, the lesion was made in the pyramid (8) at the brainstem level or in the motor cortex (9) and therefore interrupted not only the CST, but also cortico-reticular projections. We have now investigated the contribution of the CST in the rat by comparison of the success rate of reaching and grasping of a small morsel of food with the forepaw before and after transection of the axons in the dorsal column in the C1/C2 segmental border. This lesion eliminates the corticospinal input to the segmental interneurons (sINs), but spares the input to reticulospinal neurons as shown in **Figure 3D** (11).

MATERIALS AND METHODS

ETHICS

The study was conducted in accordance with national laws (The Swedish Animal Protection Act and Animal Protection Ordinance) including approval by a regional ethical committee (Swedish Board of Agriculture).

SUBJECTS AND HOUSING

The experiments were made on 13 female Wistar and 5 Sprague-Dawley rats (age 2–3 months, weight 300–400 g). They were housed in groups of six to eight in a cage (1.5 m wide, 0.5 m deep, and 0.5 m high) with grid walls and enriched with bedding,

tubes, hammocks, and shelters. A test box (25 cm wide, 30 cm deep, 35 cm high; c.f. **Figure 1**) for behavioral experiments was directly attached at the end of the home cage.

BEHAVIORAL TEST

The animals were trained in a behavioral paradigm testing the ability to remove a morsel of food placed 10–15 mm behind a vertical slit. The rats were first familiarized with a simplified test by allowing the entire group, for three nights (separated by 48 h), *ad libitum* retrieval of crushed morsels of their regular food pellets (Harlan Teklad) through a slit in the front wall of the test box. The morsels were contained in a vertical cylinder with a hole placed directly behind the slit. Thereafter, the animals were trained at daytime in a setup mounted in the test box only during experiments. It resembled that of Whishaw et al. (8), but with the difference that the slit was not positioned in the front wall of the test box but was located between glass walls (1 mm thick) protruding 55 mm from the front wall (c.f. **Figure 1**). The bottom wall was positioned 23 mm above the floor. The farther side wall (background) was painted black to improve the contrast of the paw in the video images. The height of the slit was 20 mm and the width 13 mm. The intention of this arrangement was to allow for comparison with a test in the cat, developed by Górska and Sybirska (13), with retrieval of food from a horizontal tube at shoulder level. The morsels consisted of cut pieces (about 2 mm × 3 mm) of Rotastak Milk Drops (Armitage Pet Care). During the experiments, the home cage was separated into two parts with a slideable hatch so that only animals kept in the part to which the test box was attached could enter it and perform the test. In the first experiment, the entire group of rats had access to the test box but this number was successively reduced in the next two to five experiments so that the animals were normally tested one at a time. Occasionally, two animals had access to the test box and were tested in the same experiment. The rats were moderately fasted during the preceding night (about 10 pellets of their regular food and 2 dl of glucose solution were left in the cage). The preoperative training period consisted of 10–20 sessions with a minimum inter-session interval of 2 days. Each experiment continued until the animal was satiated (20–100 trials).

Of the four animals, the behavior of which are described in this study, three (corresponding to lesions in **Figures 2A,C,D**) performed the movement with the left forelimb and the fourth animal (**Figure 2B**) with the right forelimb. In two of them (**Figures 2A,B**), the movements were recorded with a Motionscope PCI camera system (Redlake Imaging, San Diego, USA) with parallel sampling (two cameras, 250 Hz sampling rate, shutter 1/1250; sampling period 2 s) of views from above and from the lateral side. The entire experiments were also recorded on digital video cameras (40 Hz, Panasonic, Japan). In the two others (**Figures 2C,D**), the movements were recorded with digital video cameras (100 Hz, Sony, Japan).

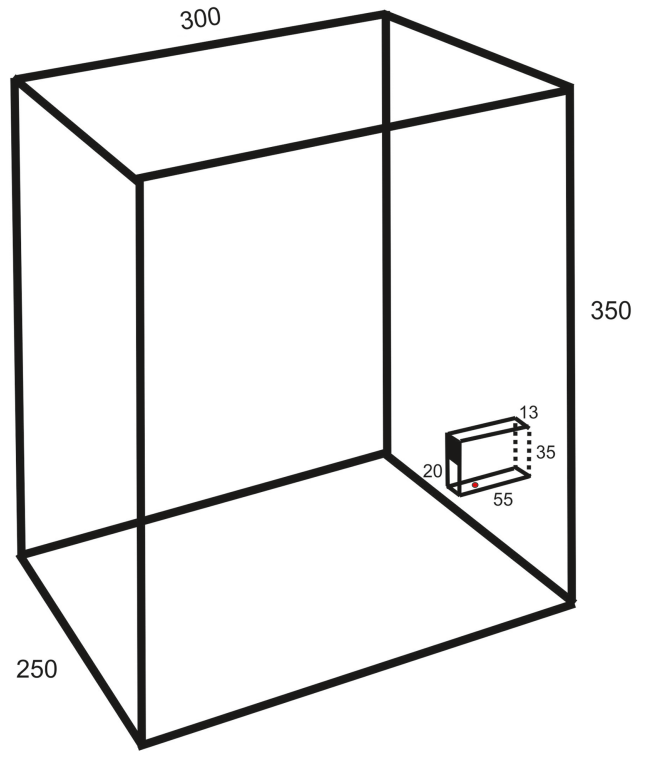

FIGURE 1 | Schematic drawing of the test box (oblique lateral view; measurements in millimeters) showing the behavioral paradigm with a vertical slit formed by glass walls. The upper part of the space between the vertical walls is covered so that the height of the slit is 20 mm.

MOVEMENT ANALYSIS

The movements were evaluated qualitatively from the video images. In addition, the success rate, defined as the percentage of trials in which the morsel was grasped and brought to the mouth [c.f. (9)] was calculated from all trials performed in an experiment. This parameter was 90% at the end of the pre-operative training. Postoperatively, the success rate was measured on the first day each individual animal participated in the test and which corresponded to day 8, 16, 11, and 7, respectively in the animals the

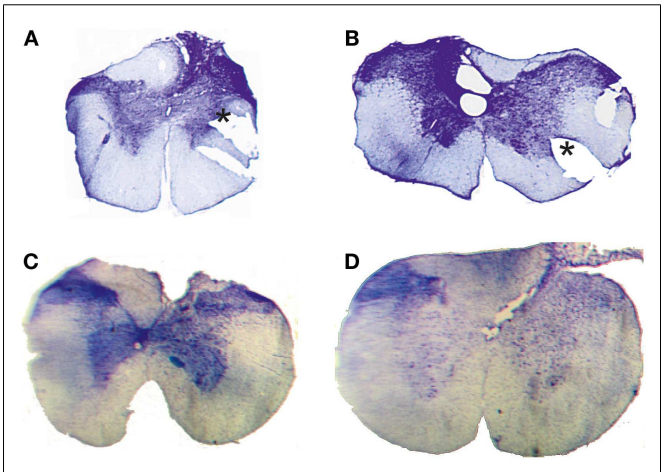

FIGURE 2 | Histological control. Transverse extent of spinal selective lesions in four rats **(A–D)** giving complete interruption of the CST but with remaining fibers in unlesioned areas of the DC. The asterisks in **(A,B)** show the position of a notch cut, after fixation, in the lateral funicle on one side to identify that side of each section when mounting them.

lesions of which are shown in **Figures 2A–D**. In the one animal, which performed the arpeggio movement and which was filmed with high-speed video, the movement was rated according to Whishaw et al. (14) using a three-point scale (2 = arpeggio present, 1 = arpeggio slightly abbreviated but recognizable, 0 = arpeggio absent). Since the number of animals with adequate lesion was limited ($n = 4$; c.f. results) and high-speed video was used only in two of them, the material was considered too small for statistical analysis.

SURGERY AND ANIMAL CARE

Anesthesia was induced by isoflurane after which Dormicum (i.p.), Hypnorm (i.p.), Atropine (s.c.), Carprofen (s.c.), and Ringer's Acetate solution (s.c.) were administered. The animal was positioned on a heating pad, intubated, and artificially ventilated. The anesthesia was maintained with isoflurane using a Univentor unit (Agnthos, Lidingö, Sweden). Rectal temperature, sPO2, ECG, and end-tidal CO2 was continuously monitored. The border between the C1 and C2 vertebras was exposed by a midline incision and retraction of the dorsal neck muscles. In some cases, a small laminectomy was made of either the caudal part of C1 or the rostral part of C2. The dura was opened transversally and the dorsal column partially transected with watch-makers forceps. The forceps were inserted into the dorsal column contralateral to the intended side of lesion. Starting from the dorsal root entry zone, an oblique lesion was made in the contralateral dorsal column along the pia layer overlying the dorsal horn. When reaching the ventral part of the dorsal column the lesion was enlarged to a bilateral transection of that area. The neck muscles were sutured with Vicryl (4–0) and the skin with Monocryl (4–0) sutures. Buprenorphine (s.c.) and Ringer's acetate solution (s.c.) were given immediately postoperatively. Vital values were monitored until sPO2 could be maintained above 95% with spontaneous breathing in room air. The animal was then put on a heating pad in a separate part of the home cage. During the first postoperative week, the animals were kept at least two together in an area of the home cage with a height reduced to 15 cm to restrict them from climbing on the grid walls.

CONTROL OF LESIONS

Electrophysiology

The completeness of the CST lesion was assessed in acute electrophysiological experiments. The preparation has previously been described in detail Alstermark et al. (11). In brief, the animals were anesthetized with a mixture of fentanyl and midazolam (2.8 ml/kg i.p.) and then by α-chloralose (60 mg/kg i.v.). Atropin (0.5 mg), dexamethasone (2 mg) was administered just after the induction of anesthesia. During recordings, the animals were immobilized with pancuronium bromide and artificially ventilated. The rostral part of the C1 and the C4-Th1 spinal segments were exposed by laminectomy and a posterior craniotomy was performed over the cerebellum to allow for insertion of stimulating electrodes in the pyramid. Corticofugal fibers were stimulated in the contralateral pyramid at 0.5 mm lateral to the midline, 2 mm rostral to the Obex level with a rostral angle of 30° using tungsten electrodes. Recording of the descending volley was made from the surface of the DC, cord dorsum potential (CDP), rostral and caudal to the lesion using a silver ball electrode. Using glass micro-electrodes, intracellular records were obtained from forelimb motoneurons (MNs) in C6–C8, with a minimal membrane potential of 40 mV. All signals were sampled using a Digidata 1200 recording system and analyzed off-line with Clampfit (Axon Instruments, Foster City, CA, USA).

Histology

At the end of the acute experiments the animals were sacrificed with pentobarbital (i.v.) and perfused with 3% formaldehyde solution after which the spinal cord was removed. The C1–C2 segments were freeze-sectioned (50 μm), stained with cresyl violet and the transverse extent of the lesion evaluated histologically.

RESULTS

C1–C2 CST/DC LESIONS

Complete transection of the CST with minimal damage to more dorsally located fibers was achieved only in 4 animals out of 18. In the other cases, which were excluded, the CST lesion was incomplete or the entire dorsal column was transected. **Figure 2** shows the histological extent of the four successful C1/C2 DC lesions. In all cases, the lesion covered the most ventral part of the DC bilaterally were the CST is located. In addition, the lesions interrupted, to different extents, also more dorsally located fibers in the DC. On the side ipsilateral to the performing limb (right side in **Figure 2B**, left side in **Figures 2A,C,D**), the largest dorsal extent was found in lesion **Figure 2B**. In the other animals, the lesions were more confined to the ventral part of the DC.

ELECTROPHYSIOLOGY

In all animals, it was verified that the lesion had completely abolished the corticospinal volley recorded below the lesion. The experimental setup is outlined schematically in **Figure 3D**. The volley was recorded from the surface of the DC in C1 and C4. It can be seen that the lesion completely abolished the corticospinal volley in C4. The negative component (upward) was eliminated and only the stimulus artifact remained. In two animals (lesion in **Figures 2C,D**), intracellular recordings were made from forelimb MNs innervating wrist and digit extensor or flexor muscles. In **Figures 3A,B** is illustrated intracellular recordings from a flexor MN (upper traces).

A single electrical stimulus given in the contralateral pyramid (Pyr) evoked no effect, whereas a double pulse elicited excitatory postsynaptic potentials (EPSPs) as shown in **Figure 3B**. Latency measurements from the second electrical stimulus to the onset of the EPSPs are shown in **Figure 3C**. The latencies ranged between 2.5 and 4.6 ms ($n = 15$, 3.48 ± 0.17 ms). In the lower traces of the cord dorsum recordings, a small synaptic volley was observed (arrow head) after the second pyramidal stimulus. This synaptic volley had a latency of 1.9 ms from the stimulus as indicated by the arrow head in C. Thus, the shortest EPSP latencies shown in the histogram (2.5–3.0 ms) were in a monosynaptic range measured from the synaptic volley (below 1 ms), whereas longer latencies (3.0–4.0) were in a disynaptic range. The longest latencies (4.2–4.6) could be compatible with a trisynaptic range. The shortest latency EPSPs recorded after the C1–C2 CST lesion are compatible with transmission by reticulospinal neurons projecting directly to forelimb MNs (11), as shown in **Figure 3D** in green color.

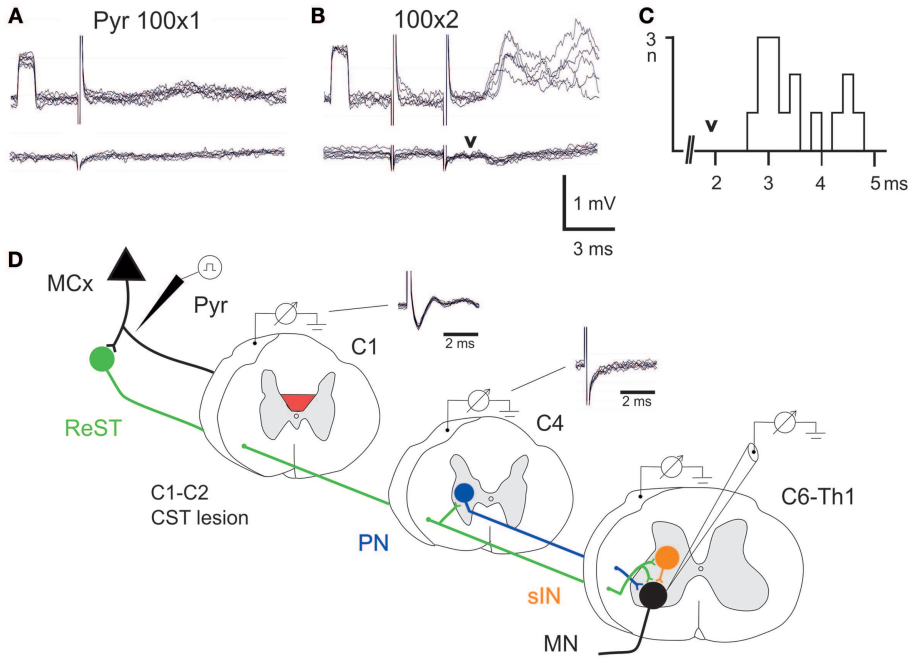

FIGURE 3 | Electrophysiological control. (A,B), upper traces are intracellular recordings from a MN antidromically identified by stimulation of the ulnar/median nerves. Lower traces were recorded from the cord dorsum in the same segment (C7) as the intracellular recordings. The contralateral pyramid was stimulated electrically with a single pulse at 100 μA in **(A)** and with two pulses in **(B,C)**, histogram of EPSP latencies measured from the second pyramidal stimulus to the onset. Arrow head indicates the arrival of the synaptic volley in C7. **(D)** Schematic circuit diagram of demonstrated cortico-motoneuronal pathways. ReST in green, PN pathway in blue and sINs in orange. Note that the ReST has both monosynaptic projection to MNs and disynaptic projection via PNs and sINs. The red area indicates the lesion of CST in C1–C2. Cord dorsum recordings from C1 and C4 evoked by a single stimulation in the contralateral pyramid at 100 μA.

A disynaptic excitatory pathway could include both C3–C4 propriospinal neurons (PNs; blue) and sINs; orange that are activated by reticulospinal neurons (15). Thus, these findings are in agreement with those obtained after acute transection of the CST in the rat (11) and suggest that these pathways can operate also after a chronic CST lesion.

BEHAVIOR

Figure 4 shows two examples of reaching and grasping movements obtained preoperatively (**Figure 4A**) and on the 8th postoperative day (**Figure 4B**) in the same animal (lesion shown in **Figure 2A**). Video sequences from the same rat are illustrated in Movies S1 and S2 in Supplementary Material (pre- and post-operative). It is evident that, in both cases, the animal was able to reach for the morsel of food without signs of dysmetria, to perform a digit grasping movement resulting in successful retrieval and to bring the morsel to the mouth. Both pre- and post-operatively, digit grasping involved preparatory extension and abduction of the digits (frames 120 ms pre-operatively and 100 ms post-operatively) and an arpeggio movement (16) i.e., a combination of pronation and digit flexion, during which the digits were successively put down on the surface, starting with digit 5 (frames 136, 144, and 152 ms pre-operatively and frames 108, 116, and 124 ms post-operatively. In both movements, the morsel was first touched by digit 3 and then grasped by combined flexion and adduction of the digits. Then the paw was supinated before bringing the food to the mouth. The pre- and post-operative movements are markedly comparable and note the remaining supination, which was lacking following pyramidotomy (8). In the present study, supination was evident in the post-operative movements invariably and the paw was never put down pronated (flat) onto the floor after retrieval. The post-operative arpeggio movements in this rat resembled those observed pre-operatively and the mean rating on a three point scale (c.f. methods) was 1.3 ($n = 22$) pre-operatively and 1.4 ($n = 12$) in the first post-operative experiment. Similar findings were made in the other rats with the exception that one of them (corresponding to the lesion in **Figure 2B**) did not perform arpeggio movements in the test, neither pre- nor post-operatively. The success rate in each individual animal on the first post-operative day in which the animal participated in the test was 94% (**Figure 2A**, day 8), 96% (**Figure 2B**, day 16), 96% (**Figure 2C**, day 11), and 88% (**Figure 2D**, day 7).

DISCUSSION

The present results suggest that the control of reaching and grasping with the forelimb in the rat is not critically dependent on spinal circuits controlled by the CST. Following the CST lesion at C1–C2 levels, all the rats showed similar success rate as before the lesion in reaching and grasping the piece of food with the forepaw. Our results differ from previous findings suggesting a role of the CST in the control of the forelimb (8, 9). Earlier, much emphasis was given to a direct excitatory cortico-motoneuronal

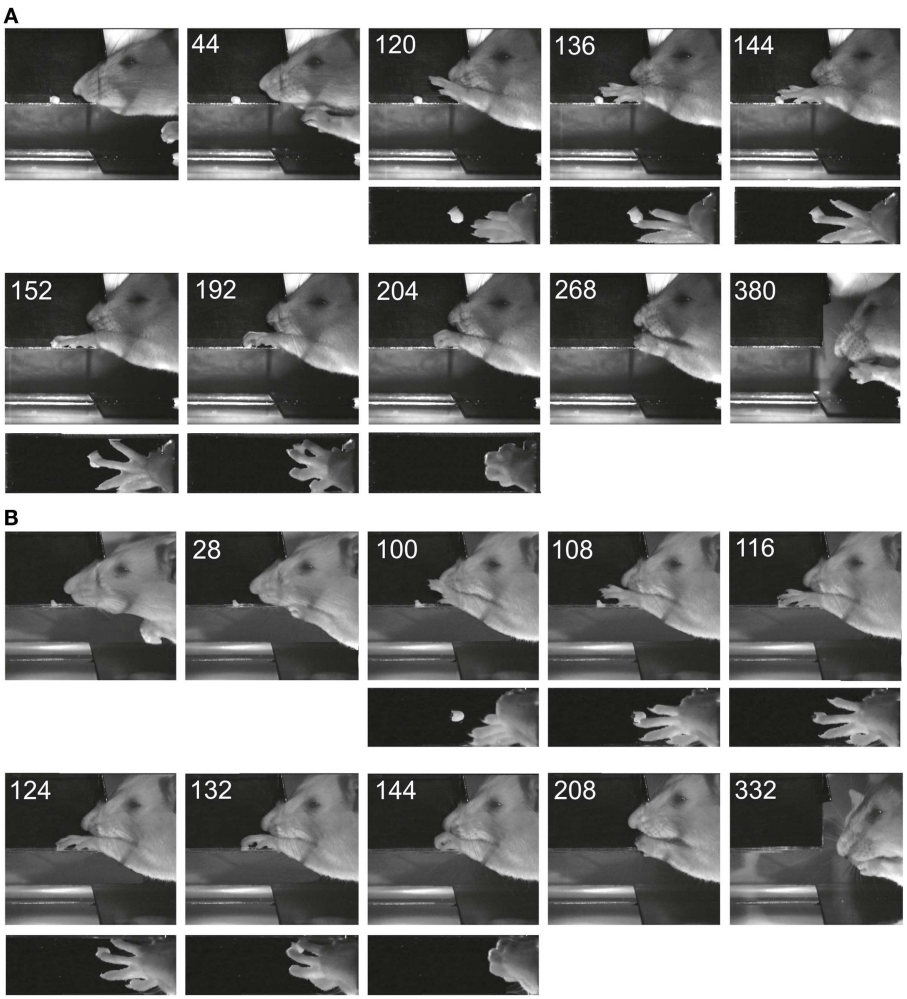

FIGURE 4 | Images of reaching and digit grasping movements obtained with high-speed video (250 Hz) preoperatively (A) and 8 days postoperatively (B) viewed from the lateral side and from above. Times are given in milliseconds relative to the first image. The background has been digitally retouched.

that was considered to provide a high degree of dexterity in the rat (10), but later it was proposed that also indirect cortico-motoneuronal CST pathways were important (9, 11). In fact, it was shown that there is no such direct pathway. Instead, the fastest excitatory pathway from the motor cortex is mediated disynaptically via a cortico-reticulospinal–motoneuronal pathway, whereas long latency excitation is mediated via sINs (11, 12). Our present electrophysiological control experiments confirm that disynaptic excitation could still be evoked in forelimb MNs after the chronic CST lesion. One explanation for the different results in the present study compared to those of Whishaw et al. (8) and Alaverdashvili and Whishaw (9), may be that in the latter two studies the pyramidal lesion and motor cortex lesion eliminated the cortical input to reticulospinal neurons as well as to the spinal cord.

Since our lesions did not interrupt rubral pathways, we do not exclude that they may also contribute to this forelimb motor control. It was shown that lesions of the nucleus ruber resulted in defective control of reaching and paw movements (14), especially the searching (arpeggio) component during pronation (17) and the importance of the rubrospinal tract in the control of grasping was demonstrated in the cat (4). Interestingly, Whishaw et al. (14) found that even following combined lesions of the pyramid and of nucleus ruber, the rats could still reach and grasp despite their deficits. Whishaw et al. (14) in fact emphasized that "*some components of skilled limb use are supported by descending neural pathways or spinal cord circuits other than the crossed rubrospinal or corticospinal projections.*" Our results suggest that one candidate could be the cortico-reticulospinal pathway.

From a phylogenetic perspective, it is interesting that there is a striking similarity in the kinematics of reaching and grasping in the rat and mouse (18). In the mouse, it was recently shown that reticulospinal neurons in the lower brain stem are important in the control of these movements (19). This finding is supported by electrophysiological experiments using intracellular recordings from adult mouse forelimb MNs that demonstrated a disynaptic cortico-reticulospinal excitatory pathway (20). In contrast to the rat, the CST evoked excitation in mouse forelimb MNs was much weaker and less frequent (20). These authors proposed that

the CST may be less involved in the control of MNs, but may be more so in the control of segmental reflex systems in the mouse. The present results suggest that the same be true in the rat. It appears that there is a gradual expansion in the control of spinal circuits by the CST during phylogenesis, with a weak control in the mouse, stronger in the rat of sINs, even stronger in the cat with additional projection to C3–C4 PNs and strongest in primates with the additional direct CM projection (21). In contrast, the cortico-reticulospinal input still remains throughout these species although it is becoming weaker. Even so, the cortico-reticulospinal pathway to forelimb MNs was shown to be highly plastic and could be strengthened after partial spinal cord lesion involving both the CST and rubrospinal tract in the cat (22). Our finding is of interest from a phylogenetic perspective since it shows that similar skilled movements like reaching and grasping can be controlled by different motor pathways in different species of animals.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fneur.2014.00103/abstract

Movie S1 | Examples of pre-operative movements in the rat illustrated in Figure 4.

Movie S2 | Examples of post-operative movements in the rat illustrated in Figure 4.

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Kinematics of the reach-to-grasp movement in vascular parkinsonism: a comparison with idiopathic Parkinson's disease patients

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The performance of patients with vascular parkinsonism (VPD) on a reach-to-grasp task was compared with that of patients affected by idiopathic Parkinson's disease (IPD) and age-matched control subjects. The aim of the study was to determine how patients with VPD and IPD compare at the level of the kinematic organization of prehensile actions. We examined how subjects concurrently executed the transport and grasp components of reach-to-grasp movements when grasping differently sized objects. When comparing both VPD and IPD groups to control subjects, all patients showed longer movement duration and smaller hand opening, reflecting bradykinesia and hypometria, respectively. Furthermore, for all patients, the onset of the manipulation component was delayed with respect to the onset of the transport component. However, for patients with VPD this delay was significantly smaller than that found for the IPD group. It is proposed that this reflects a deficit – which is moderate for VPD as compared to IPD patients – in the simultaneous (or sequential) implementation of different segments of a complex movement. Altogether these findings suggest that kinematic analysis of reach-to-grasp movement has the ability to provide potential instruments to characterize different forms of parkinsonism.

Keywords: bradykinesia, hypometria, idiopathic Parkinson's disease, kinematics, reach-to-grasp, vascular parkinsonism

INTRODUCTION

In both behavioral and neural terms, human reach-to-grasp behavior can be dissociated into separate transport and grip components (1–6). In the first instance, kinematic analysis of the reaching phase shows that during the transport of the hand toward the object, the fingers begin to pre-shape, by progressively opening the grip with straighten fingers and subsequently by closing the grip until it matches the object size. The analysis of the grasping phase confirms that key landmarks, such as the point in time in which grip size is the largest (maximum grip aperture) occurs well before the fingers come into contact with the object, indicating that the motor configuration that is formed by the hand in contact with the object represents the end result of a motor sequence that begins well ahead of the action of grasping itself (7–11). In the second instance, neural computations regarding the reach component occur within the medial intraparietal and the superior parieto-occipital cortex (2, 5) whereas the neural underpinnings of the grasp component occur within a lateral parieto-frontal circuit involving the anterior intraparietal area and both the dorsal and the ventral premotor areas (12).

While there is an extensive literature demonstrating the key roles of fronto-parietal networks in reaching for and grasping objects (6, 13–15), there are less studies examining the role played by subcortical structures – such as the basal ganglia – during the performance of similar tasks in humans (16). An important perspective on the role of cortico-basal ganglia circuits in the unfolding of the reach-to-grasp movement have so far come from the study of patients with idiopathic Parkinson's disease (IPD), wherein reduced tonic levels of dopamine in midbrain neurons results in a disrupted functionality of the thalamocortical–basal ganglia circuit, which is responsible for the motor irregularities (17, 18). It has been suggested that upper-limb motor deficits in IPD can be decomposed into at least two major aspects, namely intensive (amplitude, speed) and coordinative [integration and/or coordination of multiple movement components; (19–23)]. As for the intensive performance, the evidence indicates an absolute slower implementation of actions with respect to healthy controls (HC), but no shortfalls in the ability to modify the spatiotemporal characteristics of the prehension pattern in response to experimentally imposed changes (19). Individuals diagnosed with IPD are thus able to correctly regulate movement parameters and the overall form of the motor program appears to be maintained (24). Rather, it was the coordinated activation of the two components that revealed abnormalities in patients diagnosed with IPD. For instance, the onset of the grasping component was delayed with respect to the onset of the reaching component (19, 20). These results suggest that the grasping deficit shown by patients diagnosed with IPD in the activation of concurrent motor programs

apply not only to the motor programs that are completely independent, but also to those only largely independent, which do show functional coordination.

The evidence so far reviewed refers to studies comparing the performance of patients diagnosed with IPD with neurologically healthy participants. To date, still little is known on how other forms of Parkinsonism impact on the kinematic organization of reach-to-grasp movements, especially the forms linked to corticobasal degeneration. Among these syndromes there is vascular parkinsonism [VPD; (25)], a clinically heterogeneous syndrome that can be separated from IPD on the basis of the presence of additional focal signs, the absence of three typical signs, namely resting tremor in the upper limbs, true akinesia, and definite benefit from levodopa assumption (26). The lesions responsible for VPD are mostly basal ganglia lacunes and/or subcortical white matter vasculopathy of the Binswanger's type (27, 28). In rare cases, a single striatal infarct, striatal cribriform cavities, or ischemic changes in the substantia nigra have induced this type of parkinsonism (29). All in all, the pathophysiology of VPD is still poorly understood and we are not able to fully explain the reason why, despite same apparent lesion loads, some patients do develop parkinsonism while others do not. Therefore, it appears crucial to explore alternative markers with the goal of facilitating the characterization of this disorder.

With respect to motor assessment, gait disorders have primarily been considered and characterized in the VPD population, mostly because reminiscent of – nevertheless distinct from – the gait issues found in patients with IPD (30, 31). Typically, the gait is wide-based, marked by start and turn hesitation as well as by slow and short shuffling steps (32). To refer to such motor problems, terms like "lower body," "lower half" parkinsonism, or "frontal-type" gait disorders have been forged (30). Conversely, in terms of upper-limb movements, minimal or no dysfunctions have been reported. To date, available literature is suggestive of no true upper-limb akinesia or resting tremor, and preserved arm swing (26, 33). A point worth noting, however, is that such conclusions have been drawn on the basis of observational studies and no thorough kinematical investigations of upper-limb movements in VPD patients has been conducted.

Indeed, a close inspection of the causes underlying gait deficits in VPD might provide the ground for investigating more exhaustively upper-limb movements in this population. Gait problems in VPD are largely caused by ischemic damage to the "motor cortex–basal ganglia" and "frontal cortex–basal ganglia" connections (33). An aspect limiting the ability of central motor control systems to generate appropriately modulated descending commands. Because the above-mentioned connections are also relevant for the coordination of upper-limb movements, pathological descending signals might also affect the unfolding of this kind of actions.

In the attempt to further delineating upper-limb movements and to explore this coordinative aspect of motor control in patients with VPD, in the present study we asked a group of patients with VPD to carry out reach-to-grasp movements in the direction of visual targets of different sizes. The performance of these patients was then compared with that of a matched group of patients with IPD and with a group of neurologically HC.

Because no previous reach-to-grasp kinematical analysis on patients with VPD has been performed, only tentative predictions are advanced. First, on the basis of previous reports of pyramidal slowing (that might qualify for the term bradykinesia), a slowness of movement might be foreseen (34). Second, assuming that VPD performance is in line with that of patients with IPD, a modification of the amplification of hand opening in relation to the size of the object might be expected. Third, given the difficulties expressed by patients with VPD in coordinating gait, a dysfunction in activating almost simultaneously motor plans might be evident and emerge also at the level of the coordination of the transport and prehension components of the reach-to-grasp movements. Other aspects of reach-to-grasp kinematic parameterization are estimated to be largely unaltered with respect to neurologically healthy participants (19, 24).

MATERIALS AND METHODS

PARTICIPANTS

Three groups of participants were recruited for the study. The first group ($N = 12$) was composed of patients with VPD. Demographic information, clinical data, vascular risk factors (35), and imaging details for these patients are outlined in **Table 1**. Participants in the second group ($N = 12$) were all diagnosed with IPD and were treated with dopaminergic drugs (**Table 2**). Patients with vascular lesions detected on magnetic resonance imaging (MRI) were excluded from the study with the exception of those with minimal evidence of small vessel disease considered normal for the patient's age and in areas other than the basal ganglia (36). An independent radiologist, blinded to the study design and modality, evaluated the scans. The severity of Parkinson's disease symptoms in both groups of patients studied was assessed by a board-certified neurologist using two different measures: the Hoehn and Yahr (37) severity scale and the Unified Parkinson's Disease Rating Scale (38). All of the patients with IPD and three patients with VPD were tested after they had taken their medication. The fact that levodopa was producing optimal therapeutic responses was provided by the UPDRS, which was administered to those patients prior to their respective experimental session. None of the participants showed therapy-related motor complications that could interfere with the study task. A third group ($N = 12$) was made up of healthy participants (HC) without neurological or skeletomotor dysfunctions. The Mini-Mental State Examination (MMSE) was used to provide an index of the patients' current global cognitive state (39). The scores of the patients with VPD and IPD ranged between 28 and 30 (**Tables 1** and **2**) while all the HC participants had a score of 30, all falling within a normal range of cognitive functioning. Mean age was not significantly different in the groups studied nor significant differences in terms of disease duration in the two groups of patients were highlighted. Both the IPD and VPD patients scored an average of 18 out of 20 on visual acuity test, while the participants in the HC scored 20 out of 20. All the participants showed right-handed dominance (40). The experimental session was individual and lasted an hour. Approved by the ethics committee of the University of Padova, this study was carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all of the participants.

Table 1 | Demographic data and clinical features of the patients with vascular parkinsonism (VPD) studied.

PD patient	Age (years)	Sex	Years since diagnosis	Most affected upper-limb	UPDRS (upper-limb)	UPSRR score	MMSE score	Clinical signs						
								T	R	B	A	P	O	F
1	66	F	3	L	4.4	35	30	–	–	–	–	–	–	–
2	68	F	3	L	3.3	37	30	–	–	–	–	–	–	–
3	68	F	2	L	6	31	30	–	–	+	–	–	–	–
4	69	F	4	L	4.8	34	30	R	–	+	–	–	–	–
5	69	F	1	L	3	33	29	–	–	+	–	–	–	–
6	70	F	3	R	8	36	29	L	–	+	–	–	–	–
7	72	F	2	L	3	35	28	R	+	+	–	–	–	–
8	68	M	2	L	6.2	32	29	L	–	–	–	–	–	–
9	66	M	4	R	5	36	30	L	+	+	+	–	–	–
10	67	M	2	L	10	34	29	R	–	+	+	–	–	–
11	69	M	3	L	4	37	30	–	–	L	–	–	–	–
12	71	M	2	L	8	35	30	–	–	+	+	–	–	–

Patient	Onset	Clinical features	MRI	Vascular risk factors	L-DOPA response
1	Insidious	Hemiparkinsonism following stroke, bradykinesia	DWML, PWML	Hypertension	Not tried
2	Insidious	Asymmetric parkinsonism with tremor, bradykinesia	DWML, PWML	Hypertension	Good
3	Acute	Hemiparkinsonism following stroke, bradykinesia	Lesion contralateral LN	Hypertension	Not tried
4	Acute	Asymmetric parkinsonism with tremor, bradykinesia	Bilateral GP lesion	Hypertension, stroke	Not tried
5	Acute	Hemiparkinsonism following stroke, bradykinesia	Lesion contralateral GP	Stroke	Not tried
6	Acute	Hemiparkinsonism following stroke, bradykinesia	Bilateral GP lesion	Hypertension, stroke	Poor
7	Insidious	Hemiparkinsonism following stroke, bradykinesia	DWML, PWML	Family history of stroke	Good
8	Acute	Hemiparkinsonism following stroke, bradykinesia	Bilateral GP lesion	Hypertension, diabetes	Not tried
9	Acute	Shuffling gate, bradykinesia	Lesion contralateral LN	Stroke	Not tried
10	Insidious	Lower body parkinsonism, bradykinesia	DWML, PWML	Family history of stroke	Good
11	Insidious	Shuffling gate, asymmetrical Parkinsonism with rest tremor, bradykinesia	DWML, PWML	Hypertension	Good
12	Acute	Hemiparkinsonism following stroke, bradykinesia	Lesion contralateral GP	Stroke	Not tried

STIMULI AND APPARATUS

The visual stimuli (i.e., to-be-grasped targets) consisted of two plastic spherical objects (small object = 4 cm diameter; large object = 8 cm diameter). At the beginning of the session, each individual was asked to place his/her right hand on a starting platform within which a pressure sensitive switch was embedded (i.e., starting switch). The platform was designed with slight convexities dictating a natural flexed posture of the fingers (**Figure 1**). The target object was placed on a second pressure sensitive switch (i.e., the ending switch) embedded within the working surface (**Figure 1**). To control vision, the participants were asked to wear spectacles fitted with liquid crystal lenses (Translucent Technologies Inc., Toronto, ON, Canada), able to change from opaque to transparent (**Figure 1**). Participants were told that pressing the starting switch, which would determine visual availability of the target (i.e., opening of the spectacles), should correspond to the onset of the reaching movement toward the target.

RECORDING TECHNIQUES

Hand kinematics was measured by means of a flex sensor glove (CyberGlove, Virtual Technologies, Palo Alto, CA, USA), worn on the participant's right hand (**Figure 1**). The sensors' linearity was 0.62% of maximum non-linearity over the full range of hand motion. The sensors' resolution was 0.5° remaining constant over the entire range of joint motion. The output of the transducers was sampled at 12-ms intervals.

PROCEDURES

At the beginning of the session, the participant was positioned with his/her elbow and wrist resting on a flat surface, the forearm horizontal, the arm was oriented in a natural parasagittal plane passing through the shoulder, and the right hand was placed in a pronated position with the palm toward the working surface on the starting switch. The target was aligned with the participant's body midline, located 33 cm from the hand starting position to the left of the participant's right shoulder (**Figure 1**). The sequence of events for each trial was the following: (1) once correctly positioned, the participant's vision was occluded while the target was being placed on the working surface; (2) 500 ms later an auditory signal was sounded; (3) participants were instructed to reach toward, grasp, and lift the target when they heard the tone. The participants were instructed to reach for the object at

Table 2 | Demographic data and clinical features of the patients with idiopathic Parkinson's disease (IPD) studied.

PD patient	Age (years)	Sex	Years since diagnosis	Stage of the disease	Most affected upper-limb	UPDRS (upper-limb)	UPSIT score	MMSE score	Dopaminergic medication	Clinical signs						
										T	R	B	A	P	O	F
1	65	F	4	II	L	4	18	30	0–0–0	–	+	+	–	–	–	–
2	66	F	1	II	L	9	15	30	0.5–0.5–0.5[b]	–	–	+	+	–	–	–
3	68	F	2	II	R	8	14	30	1–1–1[a]	–	–	+	+	–	–	–
4	68	F	3	I	R	5	15	29	0–0–0	–	–	R	L	–	–	–
5	71	F	1	I	R	6	14	30	0–0–0	–	+	R	–	–	–	–
6	71	F	2	II	L	12	13	30	1–1–1	R	R	+	+	–	–	–
7	66	M	3	II	L	2	17	28	0–0–0	–	–	+	+	–	–	–
8	66	M	3	II	L	10	17	29	1–1–1[a]	–	+	R	+	–	–	–
9	67	M	2	II	L	5	17	30	1–0–1[a]	–	+	+	+	–	–	–
10	68	M	2	I	L	3	15	30	1–1–1[a]	R	+	+	+	–	–	–
11	68	M	3	I	L	2	17	30	0–0–0	–	–	R	–	–	–	–
12	69	M	2	I	R	8	12	30	0–0–0	–	–	+	–	–	–	–

Medication: number of tablets, morning–midday–evening (dopaminergic medication, [a]50 mg; [b]125 mg). Clinical signs: signs when medicated, according to examination at time of testing and self report: T, resting and/or postural tremor; R, rigidity; B, bradykinesia; A, akinesia; P, problems with static and dynamic upright posture; O, on–off phenomenon; F, freezing; "+," both sides affected; "–," neither side noticeably affected; L, left side mainly affected; R, right side mainly affected; MMSE, Mini-Mental State Examination. Stage of the disease was determined on the basis of the Hoehn and Yahr's scale.

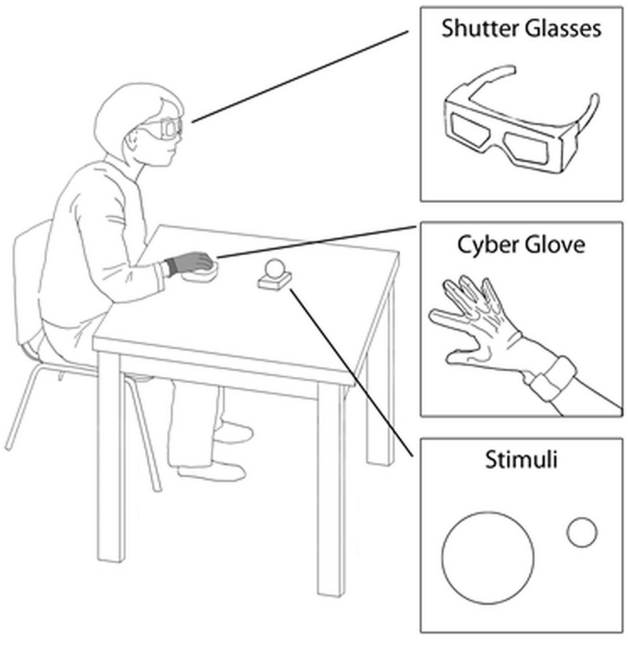

FIGURE 1 | Graphical representation of the experimental set-up. Legends indicate the relevant details.

a natural speed. An experimenter visually monitored all the trials to ensure that participants complied with instructions. The experimenter noted that the participants naturally grasped the small objects between the thumb and the index finger, at times also with the help of the middle fingers, while the large objects were grasped using the thumb and the rest of the fingers. The task was performed under two experimental conditions: (i) a reach-to-grasp movement toward the large target ("large" condition); and (ii) a reach-to-grasp movement toward the small target. Each participant took part in a total of 48 trials (24 for each experimental condition), which were presented in randomized order.

DEPENDENT MEASURES

In accordance with previous reports assessing the kinematics of reach-to-grasp movements in patients with IPD, the dependent variables specifically relevant to test our hypotheses were: (i) *movement time*, namely the time occurring from the release of the starting switch and the time at which the hand closed upon the object, to test for the slowness in movements in patients with Parkinson's disease; (ii) *maximum grip aperture*, or the amplitude of the maximum distance reached by the index finger and thumb in the transport phase, to test for hand opening alterations [hypometria; (41)]; and (iii) *delay*, or the interval between the beginning of the arm movement and the opening of the fingers, to test for impaired coordination of the reach and grasp components (19).

DATA ANALYSIS

For each dependent measure, a mixed analysis of variance (ANOVA) with "target size" (small, large) as within-subjects factor and "group" as between-subjects factor (VPD, IPD, HC) was performed. The main assumptions behind this statistical model (i.e., normality and sphericity) were checked before running the ANOVA. The Kolmogorov–Smirnov test showed that the normality assumption was satisfied (α-level: $p < 0.05$). The Mauchly test showed that the sphericity assumption was not violated. Results from the ANOVA performed on the slope absolute values were assessed through *post hoc* comparisons using *t*-tests. The

Bonferroni's correction was applied whenever required (α-level: $p < 0.05$).

RESULTS

MOVEMENT TIME

The main effect of "target size" was significant for movement duration [$F(1, 11) = 388.92$, $p < 0.0001$, $\eta p^2 = 0.972$]. For all groups movements toward the small stimulus were longer than those toward the large stimulus (1385 ± 180 vs. 1322 ± 109 ms). The main effect of "group" was significant for movement duration [$F(2, 11) = 159.76$, $p < 0.0001$, $\eta p^2 = 0.936$; **Figure 2A**]. *Post hoc* contrasts indicate that movement duration for the VPD group (1581 ± 45 ms) was comparable to that of the IPD group (1593 ± 38 ms), and both longer than for the HC group (887 ± 52 ms; $ps < 0.05$). No significant two-way interaction "target size" by "group" was found ($ps > 0.05$).

MAXIMUM GRIP APERTURE

The main effect of "target size" was significant [$F(1, 11) = 919.96$, $p < 0.0001$, $\eta p^2 = 0.988$]. Participants' maximum grip aperture was larger for the large target as compared to the small target (100 ± 9 vs. 63 ± 4 mm). Significant differences across groups were also evident [$F(2, 11) = 78.11$, $p < 0.0001$, $\eta p^2 = 0.877$; **Figure 2B**]. *Post hoc* contrasts revealed that the amplitude of maximum grip aperture was significantly larger for the HC participants (87 ± 31 mm) than for both the VPD (78 ± 24 mm) and the IPD groups (78 ± 24 mm; $ps > 0.05$). A significant two-way interaction "target size" by "group" was found [$F(2, 11) = 72.99$, $p < 0.0001$, $\eta_p^2 = 0.869$]. For the large target, maximum grip aperture was larger for HC participants (111 ± 2 mm) than for both the VPD (95 ± 5 mm) and the IPD groups (95 ± 5 mm; $ps > 0.05$). Moreover, for the small target, maximum grip aperture was larger for HC participants (67 ± 3 mm) than for the IPD group (61 ± 6 mm; $p > 0.05$).

DELAY

The main effect of "target size" was not significant for the delay ($p > 0.05$). The main effect of group was found to be significant [$F(2, 11) = 555.19$, $p < 0.0001$, $\eta p^2 = 0.981$; **Figure 2C**]. The delay was longer for the VPD when compared to HC participants (85 ± 12 vs. 73 ± 8 ms; $p < 0.05$). But it was shorter when compared to that exhibited by the IPD group (246 ± 23 ms; $p < 0.05$). When comparing the IPD and the HC groups a significant difference did emerge (246 ± 23 vs. 73 ± 8 ms; $p < 0.05$). No significant two-way interaction "target size" by "group" was found ($p > 0.05$).

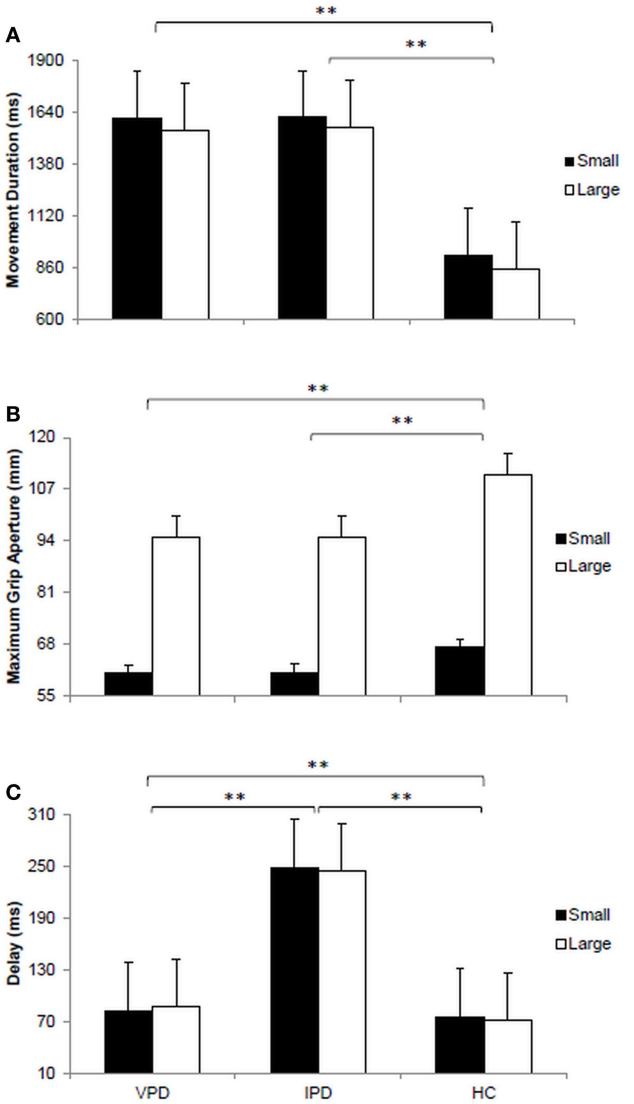

FIGURE 2 | Visual presentation of the dependent variables measured for each of the groups tested. **(A)** Bar plot represents the movement duration expressed in milliseconds (ms). **(B)** Bar plot shows the maximum grip aperture measured in millimeters (mm). **(C)** Bar plot demonstrates the delay between the beginning of the arm movement towards the target object and the opening of the fingers to grasp it. VPD, vascular parkinsonism; IPD, idiopathic Parkinson's disease; HC, healthy controls.

DISCUSSION

The aim of this study was to compare the kinematic patterning of patients diagnosed with VPD and IPD during a reach-to-grasp task. The results indicate that patients with VPD showed similar movement durations and hand-grip conformation to patients with IPD, but longer movement duration and smaller hand opening than controls. Furthermore, for patients with VPD the onset of the grasping component was delayed with respect to the onset of the transport component when compared to the performance of controls. Although this pattern has been retrieved also for the patients with IPD, the VPD group showed a significantly shorter delay. Interestingly, the standard prehension task provides a simple and natural opportunity to examine whether the organization of upper-limb movements is somewhat dysfunctional in patients with VPD. The nature of this task, composed of a proximal transport component and a distinct but inter-related distal manipulation constituent, makes it a potentially good candidate for the exploration of the motor consequences of the disorder. This view is also supported by empirical evidence suggesting that subthalamic nucleus and internal pallidum overactivity is responsible for motor-related deficit in VPD (42) and that in primates the pallidal output of the basal ganglia is directed toward the ventrolateral

thalamus, which selectively innervates the hand representation in the primary motor cortex (43, 44). Nevertheless this assumption should be taken with a certain degree of caution, given that the putative pathophysiology of VPD varies according to the type of evidence found and the behavioral manifestations observed can be linked to lesions at any level of the cortico-subcortical motor loops (45).

Zooming on the results of the kinematic analysis, significantly different patterns were found for the two target sizes in all the groups studied. The movement time was longer and the maximum grip aperture was reduced for smaller as compared to larger targets in both groups of patients (19, 24) as well as in the neurologically healthy participants (8–10). Thus, patients with VPD, as for the other groups, were able to modify the spatiotemporal characteristics of the grasping pattern in response to experimentally imposed changes in the size of the object. Patients with VPD showed longer movement duration for actions requiring greater accuracy such as when reaching for smaller objects (8–10). And they were able to scale hand opening in relation to the size of the object to be grasped (8–10). It appears, therefore, that VPD does not necessarily lead to any significant impairment of the central processes involved in organizing the reach-to-grasp movement.

Patients with VPD took longer to complete the movement and reached a smaller peak aperture than age-matched control participants. Similarly, and as previously demonstrated, patients with IPD demonstrated that their reach-to-grasp movements were slower (19, 24) and their maximum grip aperture smaller (41) with respect to control participants. Thus, VPD patients do show bradykinesia and hand hypometria, which limits the speed of movement execution and affects the modulation of hand aperture, respectively. This suggests that, as reported for patients with IPD, patients with VPD might have problems modulating movement speed and the command related to the opening/closing phases of the hand.

The kinematic analysis of the reach-to-grasp task allows examining the hypothesis that Parkinson's disease leads to a problem with concurrent execution of functionally independent motor programs with the same limb (19, 46). In this respect, significant grasp-transport coordination impairments have been observed (19, 47). On average, IPD patients tended to start distancing the index finger and the thumb later than control subjects, relative to the onset of the transport movement (i.e., delay). It appears, therefore, that IPD does lead to a significant impairment of the central processes involved in organizing the concurrent execution of functionally independent motor programs, which are executed by the same effector system. It is possible that the disease affects the well-established motor programs controlling the coordination of subcomponents in the performance of everyday actions such as reaching and grasping.

Here, we found that also patients with VPD started to open the hand later than controls. A point worth noting, however, is that the extent of the delay between the transport and the manipulation components was less for the patients with VPD than for patients with IPD, resembling the delay exhibited by the control participants. Nevertheless, this effect but might be the result of the same mechanism, namely the difficult coordination of movements with a motor output system – disrupted by pathological descending signals – which significantly limit the ability to assemble movement components. Tentatively, we suggest that lesions linked to VPD motor outcomes may affect the responsiveness of cortical areas to activation – defined as the readiness to the elaboration of triggers not originating from the basal ganglia – and result in an inadequate cortical preparation of the movement. If this lack of cortical responsiveness was confined to a specific neural channel (e.g., reach or grasping), this would explain why a movement shows a delay of activation. The different pattern of results might indicate that the more focal pathophysiology resulting in VPD less affects this cortical readiness phenomenon. The ultimate reason why this is so, still remains to be determined.

We are fully aware that the present study has some limitations. Indeed, VPD encompasses a heterogeneous set of conditions and the extent of the spectrum of VPD remains quite imprecise. However, given the promising results in finding markers differentiating VPD and IPD kinematical profiles, further work should address a full characterization of the unfolding of the reach-to-grasp movement in this population.

In conclusion, the present study provides the first attempt to compare the kinematic patterning of reach-to-grasp movements in VPD with respect to the better characterized IPD, in the effort of unveiling possible upper-limb dysfunctions in this population. The results indicate that the basic pattern of performance is similar across the two groups of patients. They both show bradykinesia, hypometria, and loss of coordination between the reach and the grasp components. However, the dysfunction in the concurrent execution of the coordinated motor plans, patients with VPD appear to be much less compromised than patients with IPD. With a certain degree of caution, we contend that this kinematical landmark might be a useful tool for distinguishing across different parkinsonian syndromes.

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The Irvine, Beatties, and Bresnahan (IBB) forelimb recovery scale: an assessment of reliability and validity

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The IBB scale is a recently developed forelimb scale for the assessment of fine control of the forelimb and digits after cervical spinal cord injury [SCI; (1)]. The present paper describes the assessment of inter-rater reliability and face, concurrent and construct validity of this scale following SCI. It demonstrates that the IBB is a reliable and valid scale that is sensitive to severity of SCI and to recovery over time. In addition, the IBB correlates with other outcome measures and is highly predictive of biological measures of tissue pathology. Multivariate analysis using principal component analysis (PCA) demonstrates that the IBB is highly predictive of the syndromic outcome after SCI (2), and is among the best predictors of bio-behavioral function, based on strong construct validity. Altogether, the data suggest that the IBB, especially in concert with other measures, is a reliable and valid tool for assessing neurological deficits in fine motor control of the distal forelimb, and represents a powerful addition to multivariate outcome batteries aimed at documenting recovery of function after cervical SCI in rats.

Keywords: spinal cord injury, recovery of function, forelimb functional task, reliability, validity

INTRODUCTION

Motor function loss is a major consequence of spinal cord injury (SCI) and has been the focus of experimental studies for over a century. Most studies have used thoracic injury models and assessed locomotor function as the primary outcome measure. A number of cervical injury models have been developed (3–9), and are being used more frequently due to the understanding that the majority of SCI occurs at this level in the human population (10). Individuals with cervical injuries are reported to be most interested in the reinstatement of hand function (11), and hence outcome measures focused on recovery of forelimb use are becoming more commonplace.

In our attempts to model cervical SCI, we chose to use unilateral injuries to reduce the burden of neurological deficits, including bladder dysfunction and quadriplegia. Prior work (4) had shown the feasibility of this approach. We used the well-established MASCIS injury device for the early studies (6), but are now using the IH device (2, 12) due to its currently widespread use in the SCI research community. We selected outcome measures that evaluated spontaneously expressed behaviors, thus reducing training requirements and food deprivation since weight loss is a consistent consequence of SCI. In our initial studies (6), we measured paw placement during vertical exploration as originally described by Schallert et al. (13) for assessing forebrain injuries, grooming as originally described by Bertelli and Mira (14) for assessing brachial plexus injuries, over-ground locomotion in an open field and on the Catwalk apparatus (Noldus Information Technology, Sterling, VA, USA), and locomotion on a horizontal ladder (4, 15, 16). Performance on most of these measures reflected graded injury effects, and using principle components analysis (PCA), these behavioral outcomes were seen to co-vary with biomechanical and anatomical descriptors of the lesion (2). However, what was missing in this battery of tests was an assessment of distal forelimb and digit function.

Food retrieval and manipulation for consumption is a critical behavior that is spontaneously expressed in all individuals across mammalian species, and requires involvement of both proximal and distal forelimb. A novel task involving food manipulation was described by Allred et al. (17) and was based on the observations of Whishaw and Coles (18). In this task, pasta is presented to rats for eating and forelimb use is assessed during consumption. This test was sensitive to a number of forebrain injuries. In our initial attempts to use this test with spinal cord injured animals, we discovered that our rats were not particularly interested in eating pasta but would readily consume sugared cereal, which is available in a variety of shapes of consistent size. The manipulation of

these cereal pieces was observed to involve detailed movements of the forelimbs and digits as the rats rotated the cereal pieces and somewhat systematically bit off small chunks to eat. Therefore, we attempted to evaluate the movements that were used to manipulate these food items while recovering from unilateral cervical contusion injuries. The first attempt to establish a recovery scale was presented in a video and manuscript (1) describing the methods, and termed the "IBB." The scale was generated by characterizing the movements made during cereal eating over the post-SCI recovery period, and assigning an ascending series of numbers for each functional set, and adjusting the scale until it reflected a sequential representation of the recovery (1). This procedure was based on our prior experience in developing and testing the Basso–Beattie–Bresnahan (BBB) locomotor rating scale (19). In that effort, we used an iterative process to construct an ordinal scale that withstood the test of inter-rater reliability (IRR) and construct validity (20, 21). The usefulness and metric properties of motor outcome scales are not always tested or considered in the SCI literature. But in response to suggestions made as more and more laboratories adopted the BBB and more data became available, this scale was modified in light of a growing body of data that suggested the metric properties were not optimized (22). A similar approach has been taken in the construction of scales for walking in human SCI patients (23). Similarly, in the present paper, we describe modifications to the original IBB scale based on our iterative evaluation of its usefulness and attempt to establish its validity and reliability. In addition, using the syndromics approach described recently for cervical SCI (2), we are now able to evaluate the relationship of this new outcome scale to other forelimb functional tests currently in use in our laboratory and in the field.

We first provide a brief history of the scale and metric properties analysis that guided its initial development. We then present results of IRR testing across a group of 9–10 novice and expert raters, and propose some minor revisions that improve reliability. Finally, we address the issue of validity (face, concurrent, predictive, external, and construct validity) for the IBB scale.

The results demonstrate that the IBB is a reliable and valid scale that is sensitive to injury severity and recovery over time. In addition, the IBB correlates with other outcome measures and is highly predictive of biological measures of tissue pathology. Multivariate analysis using PCA demonstrates that the IBB is highly predictive of the syndromic outcome after SCI, and is among the best predictors of bio-behavioral function, that is, there is good evidence of construct validity. Altogether, the data suggest that the IBB, especially in concert with other measures, is a reliable and valid tool for assessing neurological deficits in fine motor control of the distal forelimb, and represents a powerful addition to multivariate outcome batteries aimed at documenting recovery of function after cervical SCI in rats. Further, the similarities of "hand function" across rodents and primates may make such measures as this especially important in translating therapeutic strategies from rodent studies to clinical studies in man.

MATERIALS AND METHODS

ANIMALS

Long Evans and Sprague Dawley rats aged 77–87 days at the time of injury were used in the initial scale development and validity testing ($N = 70$). All experiments adhered to the National Institutes of Health Guide for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California San Francisco (UCSF). For many of the subjects, the primary data on non-IBB outcomes have been presented elsewhere as part of recently published papers (2, 24). These data are re-plotted here (with permission) for the purposes of comparative (concurrent) validity testing of the IBB.

SURGICAL PROCEDURES FOR CERVICAL SCI

All surgical procedures were performed aseptically as described previously (6). Briefly, animals were anesthetized with Ketamine HCL (80 mg/kg, Abbott Laboratories, North Chicago, IL, USA) and Xylazine (20 mg/kg, TraquidVed, Vedco Inc., St Joseph, MO, USA) intraperitoneally (ip) or with isoflurane before surgery. A dorsal, midline skin incision was made, the skin dissected, and the trapezius muscle was cut just lateral to the midline from C2 to T2. Spinous processes from C4 to T1 were exposed and a C5 dorsal laminectomy was performed to expose the entire right side and most of the left side of the underlying spinal cord. Contusion injuries were produced using the Infinite Horizon Impactor (Precision Systems and Instrumentation LLC, Fairfax, VA, USA) with a modified impactor tip 2 mm in diameter, with a force of 75 (mild) or 100 (moderate) kdynes. Cord hemisections were performed in a separate group of animals at the same vertebral level by inserting the tip of a #11 blade at the midline and sweeping laterally to cut all fibers of the hemi-cord. The sham group of animals underwent the laminectomy without SCI. The wound was closed in anatomical layers. The analgesic, buprenorphine (0.05 mg/kg, Buprenex, Hospira, IL, USA), and the antibiotic, Cefazolin (50 mg/kg, Henry Schein, Melville, NY, USA) were administered, and the animal recovered overnight in an incubator (Thermocare®, Intensive Care Unit with Dome Cover; Thermocare, Incline Village, NV, USA). All animals were inspected daily for wound healing, weight loss, dehydration, autophagia, and discomfort. Appropriate veterinary care was provided when needed.

SURGICAL PROCEDURES FOR TRAUMATIC BRAIN INJURY

A controlled cortical contusion injury (CCI) was produced using a device that has been described in detail elsewhere (25). Briefly, rats were mounted in a Kopf stereotaxic frame under isoflurane anesthesia. A unilateral craniectomy (6.0 mm diameter) between 3.0 mm posterior and 3.0 mm anterior to bregma, and between 1.0 and 7.0 mm lateral to bregma was produced using a high-speed drill. CCI was produced using a 5.0 mm diameter impactor with a convex tip (Custom Design & Fabrication, Inc., Sandston, VA, USA), oriented perpendicular to the cortical surface. The cortex was compressed to a depth of 2.0 mm at 4.0 m/s velocity with a dwell time of 150 ms. Sham animals received the craniectomy only. During the surgical procedure, heart rate and blood oxygenation were monitored with a Mouse Ox™ pulse-oximeter (Torrington, CT, USA); temperature was monitored and maintained at 37.5°C. The injury sites were closed and the animals were recovered in an incubator (Thermocare®, Intensive Care Unit with Dome Cover; Thermocare, Incline Village, NV, USA).

COMBINED SCI + TBI

In animals with both traumatic brain injury (TBI) and SCI, both surgical sites were prepared and then the TBI was performed followed by the SCI. All other aspects of the procedure were as described above and previously (24).

BEHAVIORAL TESTING

All behavioral testing for the IRR and validity testing was performed by raters who were blind to the experimental condition. Testing was typically performed pre-operatively and on post-operative days 2, 7, 14, 21, 28, 35, and 42 after injury.

Forelimb testing using the Irvine, Beattie, and Bresnahan (IBB) Scale

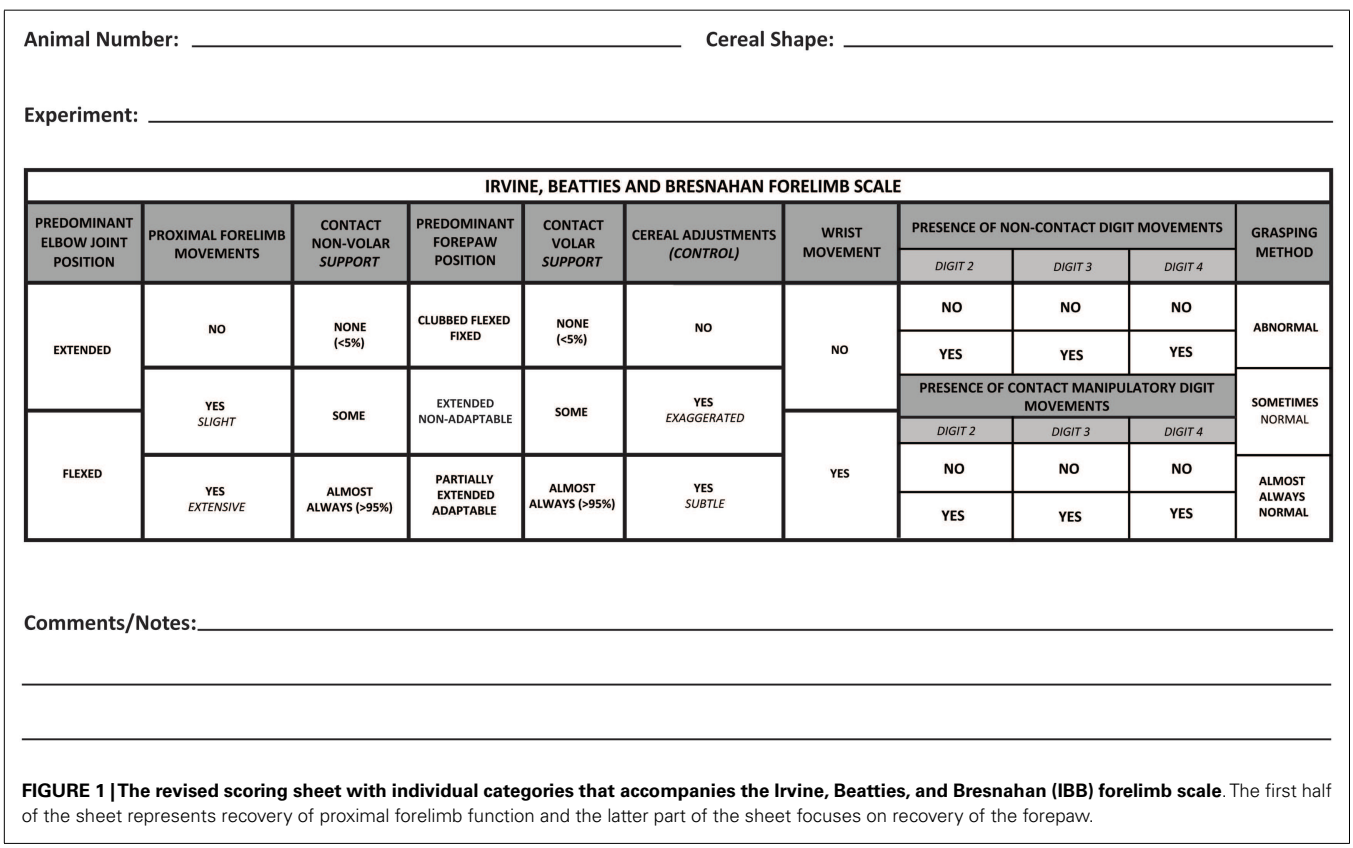
Rats were given pieces of cereal in their home cage twice daily beginning as soon as they entered the lab. Forelimb function was assessed while rats were eating cereal as described previously (1). Briefly, rats were individually placed in a Plexiglas cylinder (diameter = 20 cm; height = 46 cm) or in their home cage and given spherical- and donut-shaped pieces of cereal ("Reeses Puffs™," The Hershey Co., and "Froot Loops™," Kellogg's Co.) that were of a consistent size and shape prior to the initiation of eating. Rats were not scored when eating cereal pieces that were broken prior to the initiation of testing. Each trial was recorded to allow slow motion HD playback and evaluation of forelimb use. Videos of animals eating the cereal were evaluated using a standardized scoring sheet (**Figure 1**) to record observations of forelimb behaviors, including joint position, object support, wrist and digit movement, and grasping method used while consuming both cereal shapes. An IBB score was assigned using the 10-point (0–9) ordinal scale for each shape, and the highest score reflecting the greatest amount of forelimb recovery, was assigned.

Grooming test

Forelimb grooming function was assessed using a scoring system described previously (6). Cool tap water was applied to the animal's head and back with soft gauze, and the animal was placed in a clear plastic cylinder (diameter = 20 cm; height = 46 cm) or in their home cage. Grooming activity was recorded with a video camera from the onset of grooming through at least two stereotypical grooming sequences (~2 min). A score was assigned depending on the highest region touched by the hand as follows: 0, no contact with the head; 1, contact with the mouth only; 2 contact with the snout below the eyes; 3, contact with the face from the eye level to below the ears; 4, contact with the ears; 5, contact with the head behind the ears. Slow motion video playback was used to score each forelimb independently by the maximal contact made while initiating any part of the grooming sequence. The animals were tested on day 2 post-operatively, and then at least weekly until sacrifice.

Forelimb use during vertical exploration: forelimb asymmetry or cylinder test

Animals were placed in a clear plastic cylinder and spontaneous exploratory behavior was recorded for 5 min. Slow motion video playback was used to determine the number of times the animal placed its left, right, or both hands against the side of the



Animal Number: _____ Cereal Shape: _____

Experiment: _____

IRVINE, BEATTIES AND BRESNAHAN FORELIMB SCALE

PREDOMINANT ELBOW JOINT POSITION	PROXIMAL FORELIMB MOVEMENTS	CONTACT NON-VOLAR *SUPPORT*	PREDOMINANT FOREPAW POSITION	CONTACT VOLAR *SUPPORT*	CEREAL ADJUSTMENTS *(CONTROL)*	WRIST MOVEMENT	PRESENCE OF NON-CONTACT DIGIT MOVEMENTS			GRASPING METHOD
							DIGIT 2	*DIGIT 3*	*DIGIT 4*	
EXTENDED	NO	NONE (<5%)	CLUBBED FLEXED FIXED	NONE (<5%)	NO	NO	NO	NO	NO	ABNORMAL
							YES	YES	YES	
	YES *SLIGHT*	SOME	EXTENDED NON-ADAPTABLE	SOME	YES *EXAGGERATED*		PRESENCE OF CONTACT MANIPULATORY DIGIT MOVEMENTS			SOMETIMES NORMAL
FLEXED						YES	*DIGIT 2*	*DIGIT 3*	*DIGIT 4*	
	YES *EXTENSIVE*	ALMOST ALWAYS (>95%)	PARTIALLY EXTENDED ADAPTABLE	ALMOST ALWAYS (>95%)	YES *SUBTLE*		NO	NO	NO	ALMOST ALWAYS NORMAL
							YES	YES	YES	

Comments/Notes: _____

FIGURE 1 | The revised scoring sheet with individual categories that accompanies the Irvine, Beatties, and Bresnahan (IBB) forelimb scale. The first half of the sheet represents recovery of proximal forelimb function and the latter part of the sheet focuses on recovery of the forepaw.

cylinder during weight-supported movements according to previously published criteria (26). Individual placements were scored as either "left" or "right" when 0.5 s or more passed without the other limb contacting the side of the cylinder. If both hands were used for weight-supported movements within 0.5 s of each other, a score of "both" was given. Results are reported as a percentage of contralateral limb use versus total placements and reported as the "paw preference" outcome.

Over-ground locomotion

Forelimb use during over-ground locomotion was assessed in an open field. Limb use for stepping was assessed using a simple four-point scale: 0, no use of the forelimb; 1, stepping on the dorsal surface of the paw; 2, stepping on both the dorsal and plantar surface of the paw; 3, stepping on the plantar surface only.

CatWalk

The walkway and CatWalk analysis program was used to measure forelimb function during gait as described previously (27). Briefly, animals were trained to cross a glass walkway (120 cm long) with black Plexiglass walls and ceiling. Light transmitted through the walkway floor revealed foot contacts which were captured and collected by a digital video camera placed underneath the runway (for details, see **Figure 9**). A digital file for each run across the middle 90 cm of the walkway was analyzed using the CatWalk program (version 7). Measurements for locomotion included stride length, print area during maximal contact, and the distribution of total steps among the four limbs. During training, animals were gently guided to make complete passes across the walkway and were reinforced with sugared cereal or access to the home cage. Data were gathered pre-operatively (baseline), and then at 2–3 week-intervals post-operatively. Data were averaged across five runs in which the animal maintained a constant speed across the middle 90 cm of the CatWalk runway.

Inter-rater reliability testing protocol

Inter-rater reliability was assessed by measuring means and standard deviations of ratings of the same 10 rat videos chosen to represent all parts of the IBB scale, across multiple raters similar to that described for the BBB (21). In the first IRR, nine participants were given an initial IBB training session in which videos of the pattern of recovery in rats with cervical unilateral SCI were shown and the method of scoring using the IBB was explained. The rating of individual rats was then practiced with concurrent discussions, followed by individuals silently rating, and then comparing and discussing scores with those of the trainers. Then each participant was given a CD with ten videos of rats performing at all levels of recovery; each CD presented the videos in a different, randomized order. Also provided to each rater were a set of data recording sheets (**Figure 1**), a copy of the originally published IBB manuscript and video instructions (1), a set of frequently asked questions with answers, and a score determination guide for ease of assigning scores (**Figure 2** shows the revised version). All participants then independently

	Predominant Elbow Position	Proximal Forelimb Movements	Contact Non-Volar Support	Predominant Forepaw Position	Contact Volar Support	Cereal Adjustments	Wrist Movements	Non-Contact Digit Movements	Contact Manipulatory Digit Movements	Grasping Method
ZERO	Extended	No/Slight	None							
ONE	Flexed	Slight/ Extensive	Some	Clubbed Fixed, Flexed						
TWO	Flexed	Extensive	Almost Always	Clubbed Fixed, Flexed						
THREE	Flexed	Extensive		Extended, Non-Adaptable	None/ Slight	None/ Exaggerated				
FOUR	Flexed	Extensive		Extended, Non-Adaptable	Some	Exaggerated	Yes/No	Digit 2		
FIVE	Flexed	Extensive		Extended, Non-Adaptable	Almost Always	Subtle	Yes		Digit 2	
SIX	Flexed	Extensive		Extended, Non-Adaptable	Almost Always	Subtle	Yes	Digit 3	Digit 2	Abnormal
SEVEN	Flexed	Extensive		Partially Extended, Adaptable	Almost Always	Subtle	Yes	Digit 4	Digit 2, 3	Sometimes Normal
EIGHT	Flexed	Extensive		Partially Extended, Adaptable	Almost Always	Subtle	Yes		Digit 2, 3, 4	Sometimes Normal
NINE	Flexed	Extensive		Partially Extended, Adaptable	Almost Always	Subtle	Yes		Digit 2, 3, 4	Predominantly Normal

FIGURE 2 | The score determination guide. This guide can be used to aid in the selection of the correct IBB score after viewing the video and filling out the IBB score sheet.

evaluated the 10 videos and assigned IBB scores based on the descriptions provided in Ref. (1). Data sheets were then collected, analyzed, and compared to a consensus score for each rat, arrived at by the original scale developers viewing, discussing, and arriving at a consensus score for each video. This consensus score was determined after all raters (including the experienced raters) had completed and submitted their independent ratings of the videos. The initial IRR test results then were discussed with the participants and problems in recognizing behavioral elements and in assigning scores were identified. Choices, definitions, and the score sheet were then revised to overcome the identified issues for the purpose of improving clarity and consistency in score assignment. Subsequently, a second IRR test was performed approximately 3 months later, with 10 raters most of whom participated in the first IRR test described above, and using the newly revised definitions and the modified score sheet. Consensus scores were determined as in test 1 and individual scores were again assessed for variation from the consensus score as in the first IRR test.

HISTOLOGICAL PREPARATION AND MORPHOLOGICAL ANALYSIS

Animals were perfused through the left ventricle of the heart with 4% paraformaldehyde under deep anesthesia with pentobarbital or ketamine–xylazine. The cords were removed and post-fixed in 4% paraformaldehyde for 2 h and then cryoprotected in PBS containing 30% sucrose. A 2 mm block containing the lesion epicenter was then incubated in 100% OCT for 1 h and then mounted in a cryomold (filled with OCT) in coronal orientation and rapidly frozen using dry ice. The blocks were stored at −80°C until sectioning. The cords were cut coronally at 10 μm and every section was retained and mounted. Sections were stained with Luxol fast blue or eriochrome cyanine for myelin/white matter integrity and counterstained with Cresyl violet or neutral red for cell body assessment.

Sparing at lesion epicenter

A camera lucida drawing of the section with the largest lesion extent (i.e., the lesion epicenter) was made outlining intact gray and white matter, and the lesion. Pixel counts from digitized drawings in Adobe Photoshop 5.5 (Adobe Systems Inc., San Jose, CA, USA) were used to determine the area of spared tissue for both hemi-cords at the lesion epicenter. The percent sparing for the ipsilateral hemi-cord was determined by dividing the total spared ipsilateral tissue area, spared white matter tissue area, or spared gray matter tissue area, by the same measure from the contralateral hemi-cord [(ipsilateral spared tissue area/contralateral spared tissue area) × 100]. Quantifying pathology in this manner normalized tissue sparing within subjects and corrected for any biological differences in spinal cord size or tissue preparation. Motor neuron counts through the lesion region were performed as in Ferguson et al. (28).

STATISTICAL ANALYSIS

All analyses were performed using SPSS v.19 (IBM) using base, regression, advanced models, and missing values packages. All graphs were generated in Graphpad Prism.

Inter-rater reliability assessment

Comparisons across raters were analyzed by assessing individual rater deviations from the "gold standard" or experienced rater-derived consensus scores on the same set of behavioral videos, using the formulas

$$Difference = \sum_{i,j} \left| X_j - \mu_j \right| \quad (1)$$

and the mean difference score (MDS) is represented by

$$\text{MDS} = \frac{Difference}{n_{i,j}} \quad (2)$$

where i = individual rater, j = individual rat, X_{ij} = observed score on rat j by rater i, μ_j = consensus score on rat j, n_{ij} = total number of observations by all raters for all rats.

Separate *MDS* values were calculated for expert and novice raters. In addition, MDS values for the novice and expert raters were regressed onto the consensus scores to assess the degree of linear correlation of assessments across raters.

Validity assessment

Internal and face validity were examined by testing whether the IBB responded to the impact of graded injury and recovery over time using two-way mixed analysis of variance (ANOVA). In addition, we assessed sensitivity/propriety of applying parametric statistics (e.g., ANOVA) to the IBB by assessing variance-explained (eta squared). Concurrent validity was assessed by correlating the IBB with other more established behavioral measures used by the SCI research community. Predictive validity was assessed by correlating IBB scores with terminal histology. Construct validity was assessed at a multivariate level using exploratory factor analysis using the principal component analysis (PCA) extraction method (2, 29, 30).

RESULTS

INITIAL SCALING

Based on general observations of rats with SCI while consuming cereal, we first divided the behaviors into different categories (posture, proximal forelimb joint movement, contact with the food object, digital clubbing, wrist movements, digital movements, and grasping method). These categories were further subdivided into ranks (e.g., no, yes but abnormal, yes but normal) and operational definitions were developed to describe the categories and attributes. Categories were loosely arranged to reflect the sequence of recovery, and scores were assigned (0, 1, 2) to reflect the rank-ordered attributes. Initial scaling involved summation of these ranked features and then the resulting 55-point scale was subjected to evaluation of the metric properties such as score frequency distribution, ordinality, discontinuities, and interval properties (22). This analysis revealed that certain features did not progress in an ordered sequence and further reanalysis revealed problems with reliability and sensitivity that increased measurement error and reduced ordinality. Through this process, we improved the operational definitions of observed behaviors and switched from a summation-based scale

to an ordinal scale with fixed definitions of each point. Ultimately, scores were winnowed down to a 10-point (0–9) scale that was published in video format (1). In the present paper, further modifications to the operational definitions are reported to correct for inconsistencies and interpretational difficulties identified during the formal IRR testing analysis as presented below.

DATA RECORD SHEET

An initial scoring sheet was developed to use with the IBB for ease of recording observations while viewing subjects eating cereal, and was provided in the original IBB manuscript and video (1). The data sheet was organized from left to right to reflect the course of recovery after SCI, with the earliest behaviors to recover being positioned on the left and the later behaviors on the right. The individual subcategories were organized from top to bottom to reflect less to more recovery. This data sheet was revised to reflect changes resulting from the current analysis as described below; the revised data sheet is now shown in **Figure 1**.

INTER-RATER RELIABILITY

Inter-rater reliability test 1

The results of the first IRR test (nine raters; three experienced, six novice) are shown in **Figure 3** and present the MDS (i.e., the absolute value of the difference between the assigned score and the consensus or "gold standard" score) for ratings of performance shown in the 10 videos. Experienced raters scored within <1 point of the consensus score (0.8 ± 0.36) while novice raters scored within an average of 1.5 ± 0.5 points of the consensus score. This suggests that experienced raters independently assigning scores for the 10 videos are more accurate than novice raters, but novice raters could clearly get in the range of experienced raters with only a one-day training session. Correlational analysis of the separate expert inter-rater scores revealed significant reliability (all r values >0.9, $p < 0.0001$).

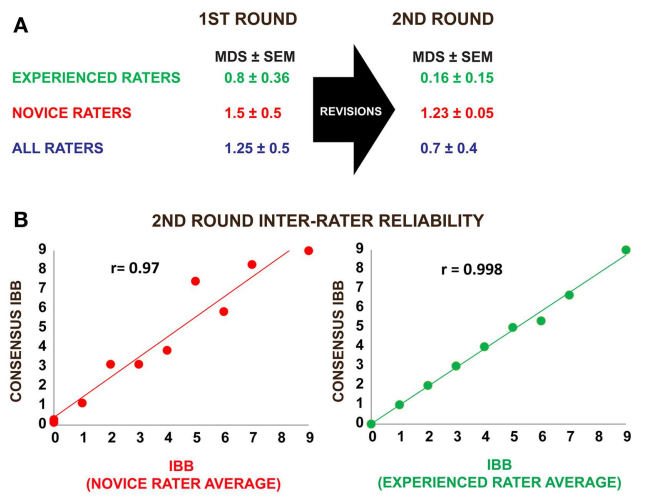


FIGURE 3 | Results of inter-rater reliability testing using a standardized set of rat behavioral videos before and after revision of the IBB operational definitions and score sheet. **(A)** Three experienced raters and six novice raters participated in the first round of inter-rater reliability testing. Mean difference scores (MDS) from a "gold-standard" consensus score were calculated as described in the methods. Following score-sheet revisions, a second round of inter-rater reliability testing was performed by three experienced and seven novice raters. Note that the MDS values as well as their standard errors (SE) were reduced after the revisions, indicating an increase in inter-rater reliability. **(B)** Pearson correlations between the mean IBB score and the consensus score suggest a high degree of agreement with consensus in both novice and experienced raters, providing strong evidence that the IBB has high inter-rater reliability that improves with practice.

On review of the results by the group, a number of issues were identified that caused problems for the raters. These were:

1. The original scale rated the **Predominant Elbow Joint Position** as "extended, partially flexed, or fully flexed." Discrimination between partially and fully flexed appeared to be problematic, and perhaps irrelevant in more recovered animals. Therefore, the predominant position subcategories were reduced to "extended" or "flexed" (**Figure 4**).
2. The definition for **Proximal Forelimb Movements** was initially defined only by the range of the movement; consideration of frequency of movements was identified as a feature that also reflected recovery and was deemed important to add to the operational definition. For example, many raters did not observe extensive movements in more well-recovered animals and thus scored the rat as 0 or 1, even though the rat was exhibiting a lot of recovery (**Figure 5**). Experienced raters appeared to ignore this aspect, so better clarification was warranted.
3. The explanation of the subcategory for Predominant Forepaw Position, **"Extended, Non-Adaptable,"** was unclear and needed more explanation. Participants also recommended that the designation of "Partially **Flexed** Adaptable" be changed to "Partially **Extended** Adaptable," so the emphasis is on the recovery of extension (**Figure 6**).
4. The subcategories of **"Cereal Adjustments,"** "Exaggerated Movements," and "Subtle Movements" needed further clarification as a distinction between these two levels was difficult. Momentary loss of contact, if the movement does contribute to proper cereal adjustment, was added to the explanation to increase discriminability (**Figure 7**).
5. Digit 5 was rarely visible. Elimination of the documentation of Digit 5 was recommended as it could not be consistently observed and scored.
6. A review of the participants' data sheets revealed errors in score assignment. These errors were typically due to either ignoring a feature marked on the score sheet, or missing a feature required for a particular score. It was recommended that double-checking score assignments for accuracy be performed. The score determination guide also was revised to make scoring easier (**Figure 2**).

The revised IBB scale and definitions are shown in **Table 1**; the changes from that provided in Irvine et al. (1), are indicated by *italics* and underlining.

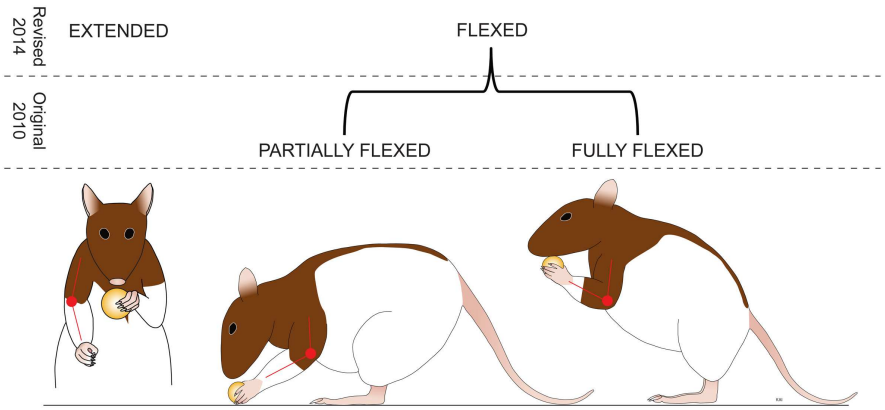


FIGURE 4 | Amendment: predominant elbow position. The rat is assessed for the most common position (more than 50% of the time) assumed by the elbow during eating. Extended is when the elbow is held straight with an angle of more than 160°. *Flexed – The elbow is flexed with an angle of less than 160°*. (Revisions of the IBB scale from the JoVE 2010 version are highlighted in italics.)

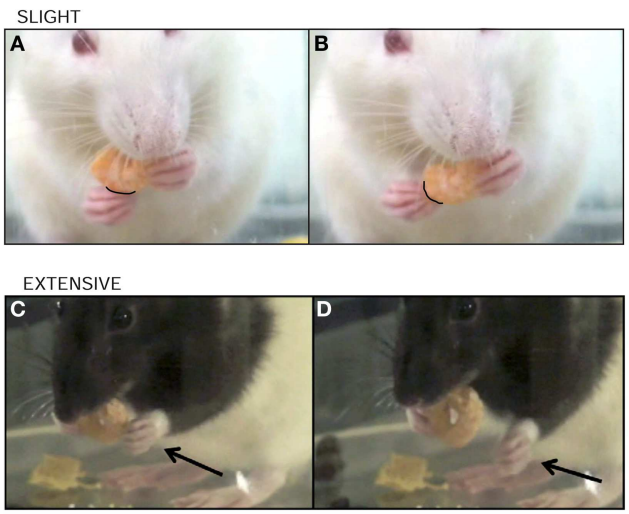


FIGURE 5 | Amendment: proximal forelimb movements. The rat is assessed for movements made by the shoulder and/or elbow of the impaired forelimb that may or may not result in contact of the forelimb with the cereal. These proximal forelimb movements are defined as either: none – there are no shoulder and/or elbow movements of the impaired forelimb. Slight **(A,B)** is defined as *infrequent movements (<5% of the time)* through less than third the range of the shoulder and/or elbow joint; twitches and shrugs fall into this category. Extensive is defined as *frequent movements (>5% of the time)* by the impaired forelimb *OR movements* **(C,D)** *that are more than third the range of the shoulder and/or elbow joint.* In early recovery, these movements can be numerous and erratic. (Revisions of the IBB scale from the JoVE 2010 version are highlighted in italics.)

Inter-rater reliability test 2

After the changes were made, a second IRR test (three experienced, seven novice raters) was performed to determine if the changes increased clarity and thus accuracy. As shown in **Figure 3**, following the revisions, experienced raters had a mean difference from consensus score of 0.16 ± 0.15 points and novice raters had a MDS of 1.23 ± 0.05. Experienced observers continued to show more accurate ratings, but all raters increased accuracy. The revisions not only increased accuracy, but also reduced variability in score assignment and improved IRR as reflected by a reduction in the overall variability in score assignments. Improved accuracy is revealed by the reduction in deviation from the consensus score. In addition, Pearson correlations between each rater and the gold standard were consistently high (**Figure 3B**).

VALIDITY

Internal and face validity

To assess internal and face validity of the IBB, we tested its sensitivity to a well-established experimental manipulation: graded SCI. We assessed sensitivity using a mixed repeated measures ANOVA (F-test) as well as effect size calculations (eta squared, η^2). To assess the IBB's sensitivity to recovery we performed repeated IBB testing over the post-injury interval. As shown in **Figure 8A**, the IBB was highly sensitive to the main effect of injury [sham, 75, 100 kdynes, or hemisection; $F(3,24) = 120.89$, $p < 0.00001$]. Effect size calculations indicated a very large effect of injury on IBB ($\eta^2 = 0.94$), over six times higher than the classical definition of "large" effect size (0.14) (31). This indicates that the IBB was highly sensitive to the effect of SCI. The IBB also performed very well as a measure of recovery over time, $F(3,72) = 27.52$, $p < 0.00001$, $\eta^2 = 0.53$. In addition, the IBB was highly sensitive to the injury × time interaction, $F(9,72) = 7.20$, $p < 0.00001$, $\eta^2 = 0.47$. The interaction term, in particular, indicates that the IBB is highly sensitive to the variable patterns of recovery produced by different SCI gradations. In addition, as shown in **Figure 8A** (inset), the IBB correlated very highly with the observed ("actual") injury force biomechanical read-out from the IH device force transducer ($r = -0.96$; $r^2 = 0.93$), providing strong evidence of face validity. Altogether these findings indicate that the IBB is an internally valid measure for assessment of recovery after SCI.

Concurrent validity: relationship to other functional tests

To assess concurrent validity, we compared the IBB to other established tests of outcome after SCI performed within the same

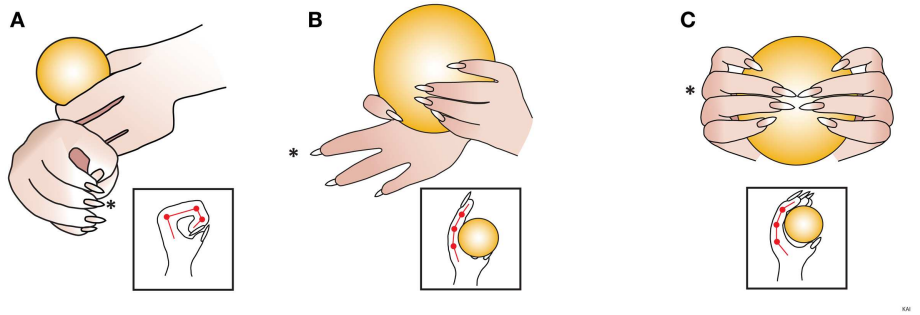


FIGURE 6 | Amendment: predominant forepaw position. The rat is assessed for the most common position (more than 50% of the time) assumed by the digits. Scored as either **(A)** *clubbed flexed fixed – the digits are flexed and held in a fist with joint angles of about 90°*. **(B)** *Extended, non-adaptable – One or more of the digits are partially extended with joint angles between 180° and 160°; in addition, these digits DO NOT CONFORM to the shape of the cereal.* **(C)** *Partially extended, adaptable – digits are partially extended with joint angles between 160° and 90°; in addition, these digits CONFORM to the shape of the cereal.* Diagrams within the squares are observing the impaired forepaw, depicting digits 1 and 3 (*), from above. (Revisions of the IBB scale from the JoVE 2010 version are highlighted in italics.)

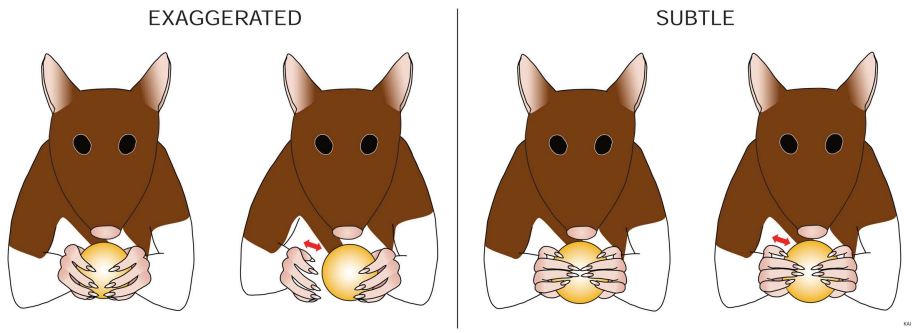


FIGURE 7 | Amendment: cereal adjustments (control). The rat is assessed for movements made by the impaired forelimb that are synchronized in time with successful manipulatory movements of the unimpaired forelimb, and that contribute to the proper manipulation of the cereal. These cereal adjustments can be defined as either: none – there are NO cereal adjustments made by the impaired forelimb. *Exaggerated – movements by the shoulder and/or elbow and/or wrist of the impaired forelimb that cause a loss of contact between the volar surface of the impaired forepaw and the cereal, which DO NOT adjust (control) the cereal position or DO NOT contribute to the proper manipulation of the cereal by the volar surface of the forepaws. Subtle – movements by the shoulder, and/or elbow, and/or wrist of the impaired forelimb that may or may not momentarily cause a loss of contact between the volar surface of the impaired forepaw and the cereal, which DO adjust (control) the cereal position or DO contribute to the proper manipulation of the cereal by the volar surface of the forepaws.* [If animals show both exaggerated and subtle proximal forelimb movements during eating, they are scored as having exaggerated movements, as these disappear with further recovery.] (Revisions of the IBB scale from the JoVE 2010 version are highlighted in italics.)

subjects, i.e., the grooming task, paw placement in a cylinder, CatWalk, and forelimb use for over-ground locomotion in the open field (**Figures 8B-D**; **Figure 9**). The IBB demonstrated a similar overall pattern of recovery as other measures, however, with mild injuries (75 kdynes) it appeared to show less of an asymptotic performance ceiling in later recovery stages, suggesting that it may have greater sensitivity to continued recovery in high-functioning individuals. In addition, the IBB significantly correlated with paw preference asymmetry in the cylinder (**Figure 8B**, $r = -0.87$; $r^2 = 0.75$), forelimb grooming test (**Figure 8C**, $r = 0.85$; $r^2 = 0.73$), and forelimb open-field (**Figure 8D**, $r = 0.66$; $r^2 = 0.43$). Comparisons to the CatWalk yielded less robust correlations (**Figure 9**), with significance reached ($r_{crit} = 0.317$) for the correlation with left (contralateral) forelimb print area ($r = 0.32$; $r^2 = 0.10$), right (ipsilateral) forelimb step distribution ($r = 0.55$; $r^2 = 0.31$), and right forelimb stride length ($r = 0.37$; $r^2 = 0.14$). This reinforces prior work suggesting that only a subset of CatWalk measures are sensitive to the effects of unilateral cervical contusion injuries (2, 6). Altogether, the analytics reveal that the IBB has high concurrent validity.

Predictive validity: relationship to terminal histology

To assess the predictive validity of the IBB test, we assessed its ability to predict postmortem histology (**Figure 10**). The IBB scores were averaged over the 42-day recovery interval and the binned IBB scores were correlated with postmortem histopathological assessment of total tissue sparing, white matter sparing, and gray matter sparing and motor neuron counts. The results revealed significant correlations for each of these measures ($r = 0.93$, $r^2 = 0.87$; $r = 0.89$, $r^2 = 0.79$; $r = 0.88$, $r^2 = 0.77$; $r = 0.68$, $r^2 = 0.46$, respectively; **Figure 10**, insets). Together, these results suggest that the IBB is highly predictive

Table 1 | *Revised* IBB Forelimb Recovery Scale.

0: The predominant elbow position is EXTENDED, with NO or SLIGHT proximal forelimb movements and/or NO non-volar support by the forelimb ipsilateral to the injury site.

1: The predominant elbow position is *FLEXED,* with SLIGHT proximal forelimb movements and SOME non-volar support by the forelimb ipsilateral to the injury site. The predominant forepaw position is CLUBBED, FIXED, and FLEXED.

2: The predominant elbow position is FLEXED, with EXTENSIVE proximal forelimb movements and ALMOST ALWAYS non-volar support by the forelimb ipsilateral to the injury site. The predominant forepaw position is CLUBBED, FIXED, and FLEXED.

3: The predominant elbow position is FLEXED, with EXTENSIVE proximal forelimb movements and NONE or SOME volar support by the forelimb ipsilateral to the injury. NONE or EXAGGERATED cereal adjustments are present. The predominant forepaw position is EXTENDED, NON-ADAPTABLE.

4: The predominant elbow position is FLEXED, with EXTENSIVE proximal forelimb movements and SOME volar support by the forelimb ipsilateral to the injury site. EXAGGERATED cereal adjustments are present with NON-CONTACT movements of DIGIT 2 and possible wrist movements. The predominant forepaw position is EXTENDED, NON-ADAPTABLE.

5: The predominant elbow position is FLEXED, with EXTENSIVE proximal forelimb movements and ALMOST ALWAYS volar support by the forelimb ipsilateral to the injury site. SUBTLE cereal adjustments are present with CONTACT MANIPULATORY movements of DIGIT 2 and possible wrist movements. The predominant forepaw position is EXTENDED, NON-ADAPTABLE.

6: The predominant elbow position is FLEXED, with EXTENSIVE proximal forelimb movements and ALMOST ALWAYS volar support by the forelimb ipsilateral to the injury site. Wrist movements and SUBTLE cereal adjustments are present with CONTACT MANIPULATORY movements of DIGIT 2 and NON-CONTACT movements of DIGIT 3. The predominant forepaw position is EXTENDED, NON-ADAPTABLE with an ABNORMAL grasping method.

7: The predominant elbow position is FLEXED, with EXTENSIVE proximal forelimb movements and ALMOST ALWAYS volar support by the forelimb ipsilateral to the injury site. Wrist movements and SUBTLE cereal adjustments are present with CONTACT MANIPULATORY movements of DIGIT 2 and 3 and NON-CONTACT movements of DIGIT 4. The predominant forepaw position is PARTIALLY *EXTENDED* but ADAPTABLE with a SOMETIMES NORMAL grasping method.

8: The predominant elbow position is FLEXED, with EXTENSIVE proximal limb movements and ALMOST ALWAYS volar support by the forelimb ipsilateral to the injury site. Wrist movements and SUBTLE cereal adjustments are present with CONTACT MANIPULATORY movements of DIGITS 2, 3, and 4. The predominant forepaw position is PARTIALLY EXTENDED, ADAPTABLE with a SOMETIMES NORMAL grasping method.

9: The predominant elbow position is FLEXED, with EXTENSIVE proximal limb movements and ALMOST ALWAYS volar support by the forelimb ipsilateral to the injury site. Wrist movements and SUBTLE cereal adjustments are present with CONTACT MANIPULATORY movements of DIGITS 2, 3, and 4. The predominant forepaw position is PARTIALLY EXTENDED, ADAPTABLE with an ALMOST ALWAYS NORMAL grasping method.

REVISED IBB DEFINITIONS

Predominant elbow joint position:

The rat is assessed for the most common position (more than 50% of the time).

EXTENDED: The elbow is held straight with an angle *of >160°.*

FLEXED: The elbow is flexed with an angle of *<160°.*

Proximal forelimb movements:

The rat is assessed for movements made by the shoulder and/or elbow of the impaired forelimb that may or may not result in contact of the forelimb with the cereal.

NONE: There are no shoulder and/or elbow movements of the impaired forelimb.

SLIGHT: *Infrequent* movements *(<5% of the time)* by the impaired forelimb through less than a third of the range of the shoulder and/or elbow. (Twitches and shrugs fall into this category.)

EXTENSIVE: *Frequent* movements *(>5% of the time)* by the impaired forelimb *OR movements* that are greater than one-third of the range of the shoulder and/or elbow. In early recovery, these movements can be numerous and erratic.

Note: If animals show both slight and extensive proximal forelimb movements during eating they are scored as having extensive movements.

(Continued)

Table 1 | Continued

Contact non-volar support:
The rat is assessed for its ability to use the non-volar surface of the impaired forelimb to stabilize the cereal piece and in doing so, maintaining it in a position to aid eating. (Areas of the forelimb that may act as supports are the forearm above the wrist, the wrist or the back of digits.)

NONE: No non-volar support by the forelimb during eating (<5% of the time).

SOME: Non-volar support of the object does occur during eating but not always.

ALMOST ALWAYS: Non-volar support of the object occurs nearly always or always during eating (>95% of the time).

Predominant forepaw position:
The rat is assessed for the most common position (more than 50% of the time) assumed by the digits, from flexed to extended, during eating.

CLUBBED, FLEXED, AND FIXED: Digits are flexed with joint angles greater than 90° and are held in a fist.

EXTENDED, NON-ADAPTABLE: *One or more of* the digits are partially extended with joint angles between 180° and 160°; *in addition,* these digits *do not conform* to the shape of the cereal.

PARTIALLY *EXTENDED,* ADAPTABLE: Digits are partially extended with joint angles between *160° and 90°; in addition,* these digits conform to the shape of the cereal.

Contact volar support:
The rat is assessed for its ability to use the volar (palmar) surface of the impaired forepaw to stabilize the cereal and, in doing so, maintains a position to aid eating.

NONE: No volar support by the forelimb during eating (<5% of the time).

SOME: Volar support of the object does occur during eating but not always.

ALMOST ALWAYS: Volar support of the object occurs nearly always or always during eating (>95% of the time).

Cereal adjustments (Control):
The rat is assessed for movements made by the shoulder and/or elbow and or/wrist of the impaired forelimb that are synchronized (in time) with successful manipulatory movements of the unimpaired forelimb, and that contribute to the proper adjustment (control) of the cereal position by the volar surface of both forepaws.

NONE: There are NO manipulatory movements made by the volar surface of the impaired forepaw.

EXAGGERATED: Hypermetric movements of the shoulder and/or elbow and/or wrist of the impaired forelimb that:

Cause a loss of contact between the volar surface of the impaired forepaw and the cereal, ***and***
DO NOT adjust (control) the cereal position or DO NOT contribute to the proper manipulation of the cereal by the volar surface of the forepaws.

SUBTLE: Tiny movements of the shoulder and/or elbow and/or wrist of the impaired forelimb that:

May or may not momentarily cause a loss of contact between the volar surface of the impaired forepaw and the cereal, ***and***

DO adjust (control) the cereal position or DO contribute to the proper manipulation of the cereal by the volar surface of the forepaws.

Note: If animals show both exaggerated and subtle proximal forelimb movements during eating, they are scored as having exaggerated movements, as these disappear with further recovery.

Wrist movements:
The rat is assessed for the presence of wrist movements of the impaired forepaw during eating, once volar support has been established. Movements of the wrist that occur in the absence of contact between the impaired forepaw and the cereal are *not* scored. These movements can occur in any direction, e.g., a dorsal (towards the back) to ventral (down towards the stomach) direction or medial (in towards the body midline) to lateral (away from the body midline) direction:

YES
NO

Presence of digit movements:
The rat is assessed for the presence of movements made by the individual digits during eating.

NON-CONTACT, YES or NO: Movements of the digits occur but these movements *do not* result in volar contact with the cereal.

CONTACT MANIPULATORY, YES or NO: Movements of the digits occur that *do* result in volar contact of the digit with the object and, in doing so, contribute to manipulation of the cereal.

(Continued)

Table 1 | Continued

Grasping method:

The rat is assessed for the most common (more than 50% of the time) grasping technique used during the eating phase. Several grasping methods exist but the most common are the "pincer," the "hook," and the "whole" grasp. The grasping techniques used by the rat are stereotypical depending on the size and shape of the cereal piece.

ABNORMAL: Consistent use of an alternative method of grasping to the method used prior to injury to support and control the cereal piece during the eating phase.

SOMETIMES NORMAL: Inconsistent use of the grasping method used prior to injury to support and control the cereal piece during the eating phase.

ALMOST ALWAYS NORMAL: Consistent use of the grasping method used prior to injury to support and control the cereal piece during the eating phase.

The changes from that provided in Ref. (1), are indicated by italics and underlining.

of histological changes after SCI, providing strong support for its use as a behavioral biomarker for SCI outcome assessment.

Correlations of individual variables with the IBB score were done using all animals including the shams. The reason for this was that we wanted the entire range of behavior and anatomy to be represented (i.e., from most injured with no function to no injury and normal function). An alternative approach is to ask if the scale is sensitive within the range of injury and partial function, i.e., without the shams. **Table 2** presents the correlations figured both ways. Pearson correlations (r) and shared variance (r^2) deflated without shams, indicating a smaller but often still significant dynamic range within different injury conditions. This suggests that the IBB has sensitivity across a wide dynamic range of injury conditions. Note that $r_{crit} = 0.31$ for $p < 0.05$.

External validity: responsiveness to other types of neurological injuries

To assess whether the IBB has external validity, we tested a new population of subjects and also assessed its sensitivity to alternative forms of neurological injury in the context of a model-development effort for central nervous system (CNS) polytrauma (SCI + TBI; (24)). IBB was assessed in subjects receiving either a unilateral cervical SCI alone (75 kdynes), TBI alone, or SCI + TBI combined injuries (with the TBI either ipsilateral or contralateral to the SCI). If the IBB has high external validity then it should show graded sensitivity in this new population of subjects. The results are shown in **Figure 11**, and demonstrate that IBB was highly sensitive to the impact of injury condition, $F(4,37) = 15.74$, $p < 0.00001$. The sensitivity of the IBB to CNS injury was reinforced with a very large effect size $\eta^2 = 0.63$, over four times higher than the classical cut off for "large" effect size [$\eta^2 = 0.14$; (31)]. Together, the results indicate that the IBB has high external validity for the combinatorial effect of SCI + TBI. Note, that the IBB was selectively sensitive to the impact of TBI contralateral to the SCI, but little impacted by TBI alone. This suggests that the IBB, like the grooming test, is somewhat selective for the effects of SCI, and perhaps, selectively sensitive to anatomical substrates through which contralateral cortical contusion impacts SCI recovery [see Ref. (24), and "Discussion" section for further review).

Construct validity: multidimensional syndromic assessment

Spinal cord injury is an intrinsically multifaceted syndrome that can be conceptualized within a multivariate, big-data analytic framework (2, 32–37). In this context, we can assess construct validity of SCI outcome batteries by borrowing well-established methods from the educational and neuropsychiatric testing fields. Namely, we can apply multivariate exploratory factor analysis on the full set of multi-trait multi-method outcomes to derive the underlying latent structure of the SCI syndromic space (2, 29, 38, 39). This approach is a realization of classical arguments about strong inference and the need to leverage full-information to deal with complexity in biology and neuroscience (40).

To assess the relationship of the IBB to multidimensional SCI, we performed exploratory factor analysis using the extraction method of PCA. PCA integrates the full bivariate cross-correlation matrix of all biological and functional outcomes through multivariate pattern detection coupled with dimension-reduction ((2, 29); **Figure 12**). In essence, PCA reduces the total number of observed variables down to a small number of principal components (PCs; or "latent variables") that concisely summarize the overall set of observations within the dataset. We performed PCA on the full set of outcome variables presented (in univariate form) in **Figures 8–11**. PCA revealed three latent multivariables (PC 1–3) that together accounted for 81.4% of the variance in outcome (**Figures 12A–C**). To understand how individual outcome metrics relate to the PC syndromic patterns, we plotted the correlation (so called "loadings") of each outcome metric on the PC patterns. Significant loadings above 0.45 are represented as arrows where arrow size indicates magnitude and heat represents valence (positive vs. inverse relationships). Note that IBB loads very highly on PC1, indicating that it is a highly de-noised measure of the latent construct represented by PC1. As in prior work (2), the PC1 loading pattern suggests that it represents the relationship between tissue sparing and recovery of function – the multidimensional target for neuroprotective therapies. The fact that the IBB is the highest loading variable on PC1, suggests that it is a powerful surrogate biomarker for the set of variables represented by PC1. In addition, note that IBB does not load on PC2 or PC3, which are both devoid of histological loadings. This suggests that the IBB is a highly selective detector of the histopathology–behavior

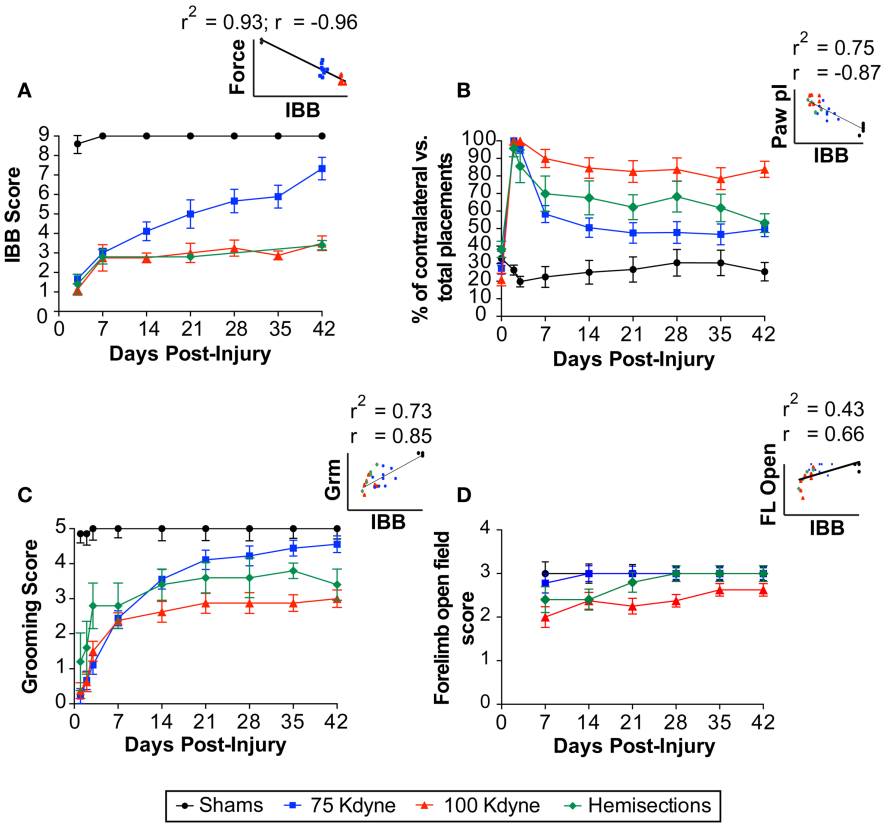


FIGURE 8 | Face, internal, and concurrent validity of the IBB score. **(A)** Face and internal validity of the IBB score is provided by responsiveness to experimentally graded spinal cord injuries as well as the correlation (inset) with a biomechanical measurement of tissue displacement at the time of contusion injury. Concurrent validity is provided by comparisons with other established outcomes including **(B)** paw placement, **(C)** grooming score, and **(D)** forelimb open field. Insets reflect the scatterplot and regression line between the IBB and each of the established tests. The Pearson correlation (r) and the shared variance (r^2) for each appear above the scatterplot; group identity for each point is color coded.

relationship. Combined with the univariate validity testing, the multivariate results provide strong validation of the IBB as a measure of recovery of function following cervical SCI.

DISCUSSION

DEVELOPMENT OF THE IBB

A major goal of preclinical modeling for SCI is to identify methods that can be used to evaluate treatments for translation to clinical trials. Our prior work on cervical SCI (6) used a variety of tasks to measure forelimb function including the grooming task, paw placement in a cylinder, CatWalk, and forelimb open-field locomotion. It is noteworthy that these tasks largely assessed proximal forelimb movements with some limited information about hand use. None of these tests focused on digit function, which we consider to be important to assess for the translational relevance of our preclinical outcome testing. A number of tasks that assess distal forelimb movements in rodents have been described especially by Whishaw and colleagues, and many have focused on the "reach-to-grasp task" [reviewed in Ref. (41)]. This task however, requires extensive training and food deprivation. We also considered an alternative task, pasta eating, that required hand use to accommodate a variety of food shapes (17, 18) and was sensitive to forebrain injuries. However, during the process of trying to acclimate rats to a variety of food items, we noticed that acutely injured subjects demonstrated movements of the affected limb during eating that did not contribute to food manipulation. The hand was fixed in a fisted position preventing the digits from grasping the food, and the forelimb was only used to support the food item. In contrast, the contralateral limb showed fine digital movements. Allred and colleagues (17) had made similar observations in their description of the "Vermicelli handling task," in which rats are filmed eating pieces of thin pasta and manipulation of the pasta was compared to pre-injury handling methods. However, the juxtaposition of the digits during pasta eating made it difficult to discern movement of individual digits, and only movements with physical contact with the pasta were described and assessed. We considered that this strategy would ignore the rats' attempts to use the forepaw ipsilateral to the SCI, and its continued improvement over time.

We therefore explored developing a formal observational scale to rate recovery of both proximal and distal forelimb movements in the affected limb during food manipulation, including fine digital control. Using a high-definition camera, we filmed subjects eating consistently sized cereal pieces in a Plexiglas cylinder surrounded by mirrors to enable 360° viewing of the movements.

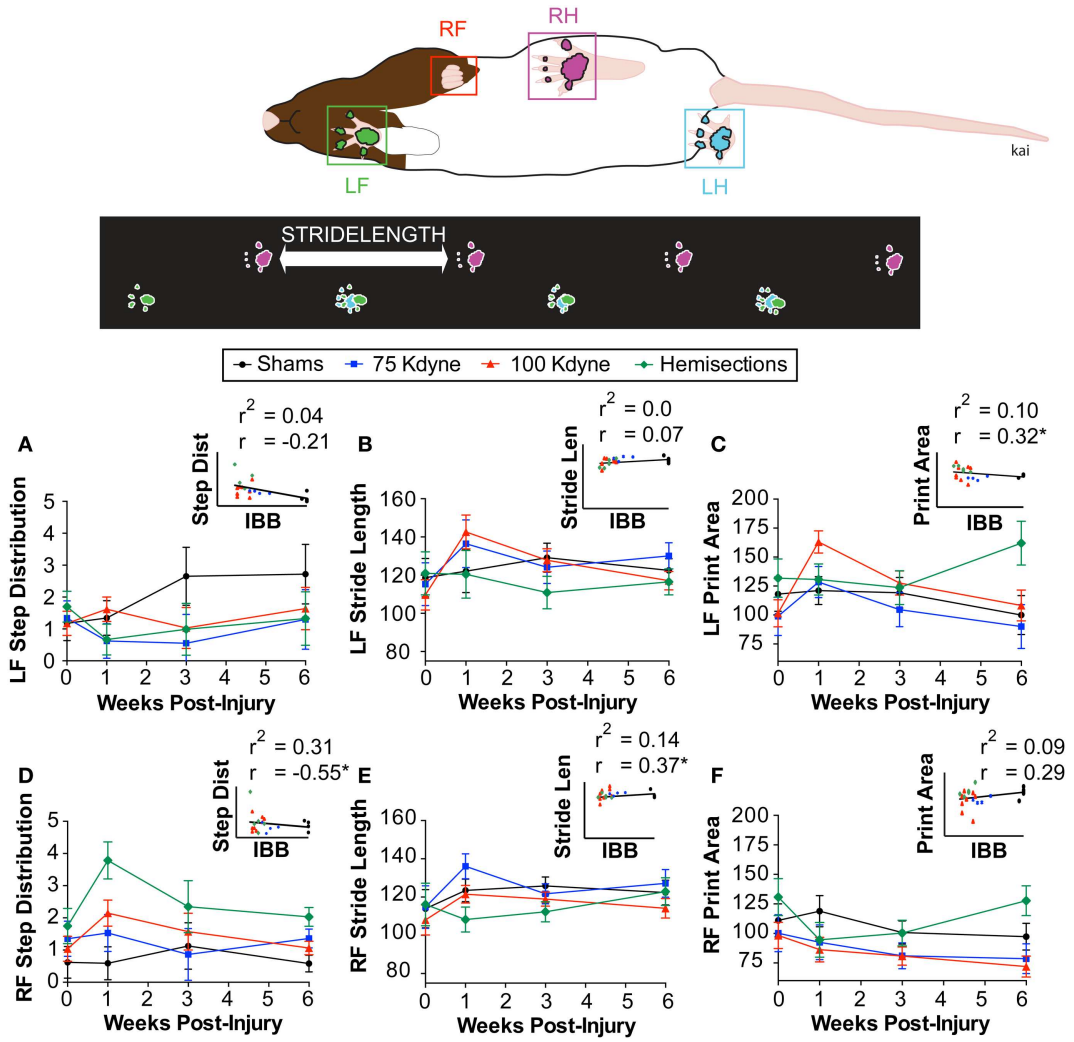


FIGURE 9 | Concurrent validity of the IBB with respect to automated gait analysis on the CatWalk. **(A)** Left forelimb step distribution. **(B)** Left forelimb stride length. **(C)** Left forelimb print area. **(D)** Right forelimb step distribution. **(E)** Right forelimb stride length. **(F)** Right forelimb print area. Insets reflect the scatterplot and regression line between the IBB and each of the CatWalk outcomes. The Pearson correlation (r) and the shared variance (r^2) appear above each scatterplot; group identity for each point is color coded. * Indicates significant correlation above $r_{crit} = 0.317$.

Both uninjured subjects and subjects with a range of unilateral cervical injuries produced by the IH device were examined over 6 weeks. Initial observations were unconstrained notes based loosely on the structured note-taking scheme of the BBB locomotor rating scale (19). Like the BBB, attention was first given to gross position of the joints in the affected limb and then to more refined features of movement. We also noted differences in the grasping techniques across different cereal shapes, largely inspired by work of Whishaw and colleagues. The result of this analysis, termed the "IBB," was described in Irvine et al. (1).

In the current paper, we have assessed this method for both reliability and validity. These are distinct but related issues in the field of testing theory. IRR deals with the issue of consistent scoring of observations whereas validity deals with the issue of whether a measurement assesses what it purports to assess. These issues will be discussed separately below.

INTER-RATER RELIABILITY

Inter-rater reliability deals with whether an assessment tool is consistent from rater to rater. To assess IRR, we used an approach similar to that used during the development of the BBB Locomotor Rating Scale (21). This approach relied on assessing deviations from a gold-standard consensus score that is derived by expert raters working together as a team. The current study used a consistent set of videos to assess IRR. This provided some advantages over the live-rating strategies used to assess the BBB scale. First, it ensured that there was only one view of the behavior, providing a more direct assessment of inter-rater variability. Second, we could randomize the presentation of the exact same behavior allowing us to control for sequence effects in raters. We found that there was a high concurrence of score assignment for both experienced and novice raters, and that concurrence was improved after some minor adjustments to the scale definitions and procedures.

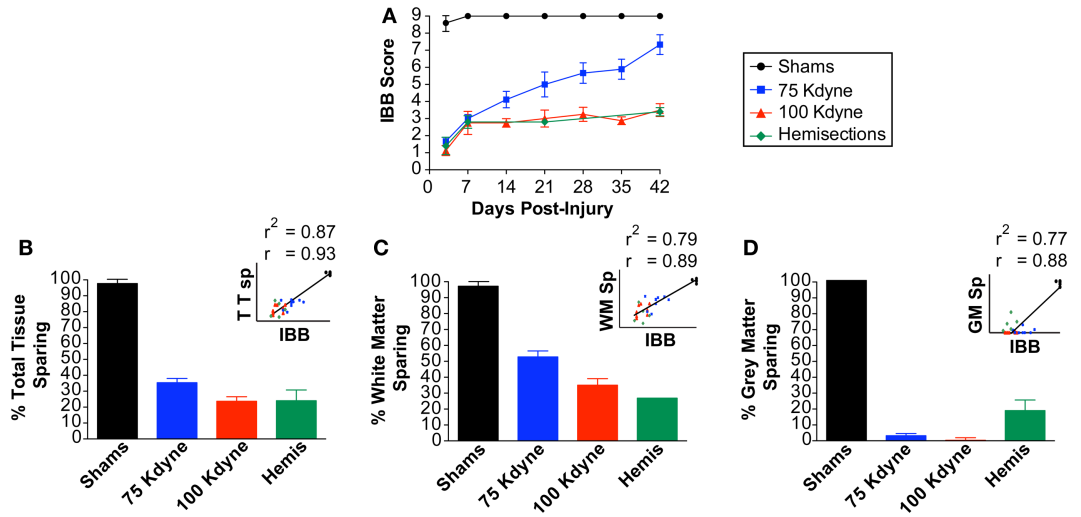


FIGURE 10 | Predictive validity of the IBB score with respect to histological outcome after spinal cord injury. **(A)** IBB score. **(B)** Total tissue sparing at lesion epicenter. **(C)** White matter sparing at lesion epicenter. **(D)** Gray matter sparing at lesion epicenter. Insets reflect the scatterplot and regression line between the IBB (averaged over time) and each of the established tests. The Pearson correlation (*r*) and the shared variance (r^2) appear above each scatterplot; group identity for each point is color coded.

Table 2 | Correlations of individual variables with IBB score.

Variable	*r* (all subjects)	r^2 (all subjects)	*r* (no shams)	r^2 (no shams)
Actual force	−0.96	0.93	−0.75	0.56
Tissue displacement	−0.83	0.70	−0.09	0.01
Abnormal paw PL	−0.87	0.75	−0.69	0.48
Grooming	0.85	0.73	0.47	0.22
Forelimb open field	0.66	0.43	0.67	0.45
LF step distribution	−0.21	0.04	−0.31	0.10
LF stride length	0.07	0.00	0.34	0.12
LF print area	0.32	0.10	0.42	0.17
RF step distribution	−0.55	0.31	−0.27	0.08
RF stride length	0.37	0.14	0.67	0.45
RF print area	0.29	0.09	0.03	0.00
Total sparing	0.93	0.87	0.55	0.30
WM sparing	0.89	0.79	0.61	0.37
GM sparing	0.88	0.77	0.06	0.00
Motorneuron sparing	0.68	0.46	0.27	0.07

Note that separate correlations were calculated for all injury conditions (all subjects) and excluding shams (no shams). Note that Pearson correlations (r) and shared variance (r^2) deflated without shams, indicating a smaller but often still significant dynamic range within different injury conditions. This suggests that the IBB has sensitivity across a wide dynamic range of injury conditions. Note: $r_{crit} = 0.31$ for $p < 0.05$.

We also found that experience improves consistency and accuracy of score assignment [as was observed with the BBB; Ref. (21)]. Novice raters could be trained to identify the behavioral features for rating within a single day, and were able to identify definitional issues that, when changed, improved accuracy for both novice and experienced raters. The full set of IRR assessment videos and materials are available to qualified neurobiological researchers upon request. Given that the videos are identical, researchers should be able to match their results to those presented in the current paper.

INTERNAL/FACE VALIDITY

The internal or face validity of this measure is reflected in its ability to detect differences in the degree of injury to the nervous system. Performance in cohorts of animals with 75 and 100 kdyne unilateral contusion SCI, lateral hemisection, and combined SCI with TBI showed that the IBB was sensitive to varying damage to the spinal cord and cortex, both individually and in combination. Graded SCI produced differential recovery (**Figure 8A**). Interestingly, TBI alone produced a mild initial deficit which quickly

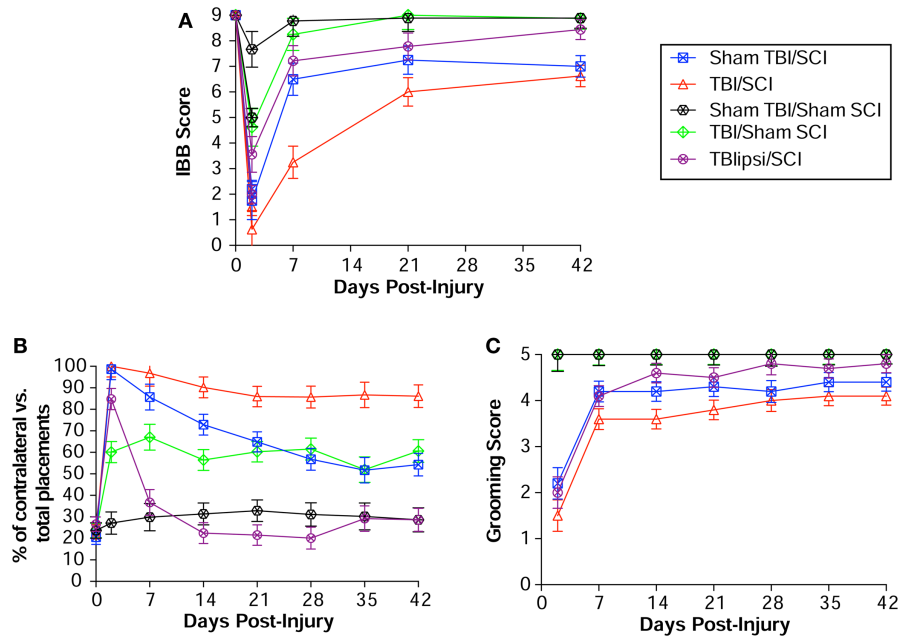


FIGURE 11 | External validity of the IBB Score. **(A)** The IBB was performed in an independent cohort of subjects as part of a model-development project for spinal cord injury (SCI) with concomitant traumatic brain injury (TBI). Note that the IBB was sensitive to the impact of SCI as well as the additive effect of SCI+TBI. **(B)** Paw placement and **(C)** grooming in the same subject cohort for comparative purposes. Reprinted with permission from Ref. (24).

recovered (by 1 week post-TBI; **Figure 11**, green line). Whishaw et al. (42) showed that cortical lesions did not affect the ability of rats to pick up food with their mouth and transfer it to their hands for manipulation, but did observe that cortical injuries produced difficulty with pronation and supination. This type of deficit could be reflected in the early mild suppression of the IBB score after the cortical injury alone. Interestingly, the addition of a cortical injury contralateral to an SCI, produced a significant depression of IBB scores over the SCI alone, suggesting that the contralateral cortex was involved in the recovery from the SCI. A TBI placed ipsilaterally to the SCI, did not show the same effect as the contralaterally placed TBI, and in fact slightly, but not significantly, improved outcome on this measure. The dual lesions' effect on the circuitry supporting paw use is complex and a multivariate approach to determining the output shows that this is indeed the case (35) but is beyond the scope of the present discussion.

CONCURRENT VALIDITY

Concurrent validity asks how performance on this test relates to performance on other tests used to assess recovery after unilateral SCI [e.g., Ref. (4, 6, 9)]. The current study found that IBB scores correlate very highly with paw placement and grooming scores, and less highly, but still significantly, with forelimb use for locomotion in the open field and on the Catwalk (although only on some of the Catwalk measures). These tests evaluate hand use during vertical exploration, during grooming of the face and head, and for locomotion respectively. Other tests which evaluate hand use during grasp and retrieval [e.g., Ref. (42–44)] were not tested. The IBB test focuses on a different aspect of forelimb use than the reach and grasp tasks. The IBB represents an assessment of hand use during food manipulation for consumption as opposed to reaching and grasping tasks, which involve forelimb use for retrieval of items distal to the animal (41, 45). During reaching tasks, animals are required to extend their arm through a slot to reach a food object. The hand is then brought over the food pellet using a stereotyped arpeggio movement and the pellet is grasped, followed by bringing the food to the mouth. For the IBB, animals first locate the food on the floor of the cage using at least olfaction and somatosensory input via the vibrissae, they pick the food up with their mouth and then bring the forelimbs to the mouth to support and manipulate the food, especially if the item is large. The food is then rotated and positioned for biting with both hands. The reach and grasp tasks do not focus on this proximal manipulation during consumption. In this sense the IBB is complementary to reach and grasp tasks.

Whishaw has pointed out that "reach and grasp" is a highly evolutionarily conserved function that is similar across the mammalian class, and thus is likely to be a useful tool for translational modeling (41). While the ability to use fine digital movements increases and individuates as one "ascends" the class from rodents to primates, the basic organization of the neural systems underling these behaviors are likely to be similar. Therefore, attempts to develop outcome measures with similar features across species that can be combined to develop batteries of tests evaluating different substrates for recovery, would seem to increase the probability of translation from rodent injury models to the human clinical situation. In this sense, the IBB represents an important addition to a complete battery of tests that can be used to assess recovery

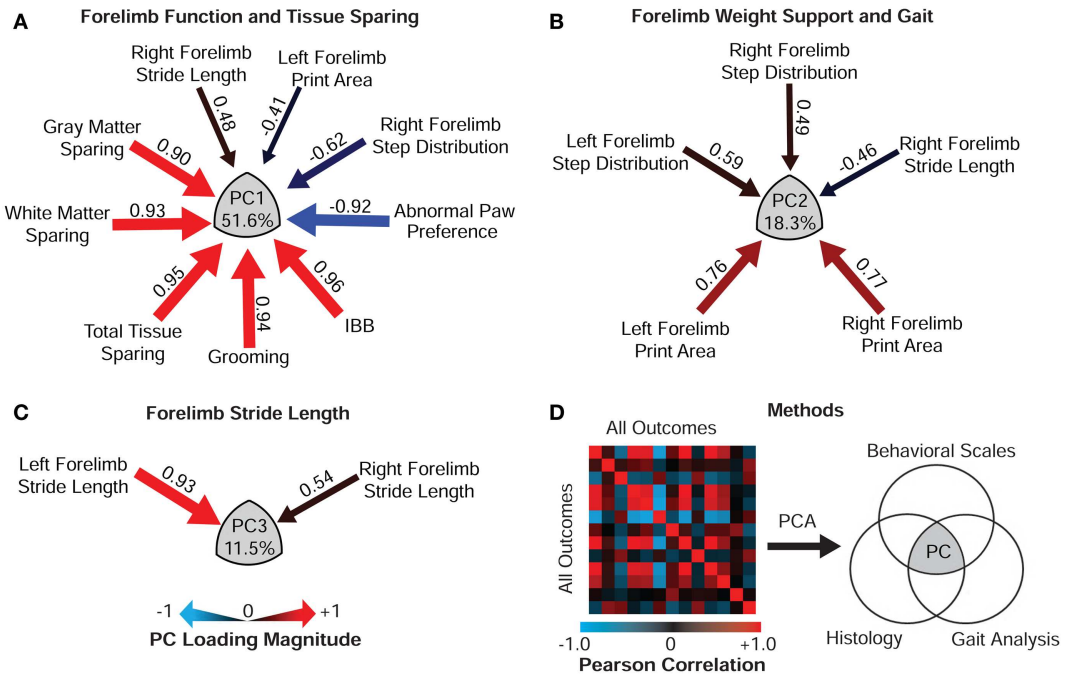


FIGURE 12 | Construct validity of the IBB Score. Principal component analysis (PCA) extracted three orthogonal multivariable principal component (PC) clusters that together accounted for 81.4% of the variance in outcome after SCI. **(A)** PC1, the largest cluster of variance (51.6%) reflects the relationship between forelimb function and histological outcome. Note the IBB score is the highest loading variable on PC1, providing evidence of construct validity. **(B)** PC2 (18.3% variance) reflected the relationship of forelimb weight support and gait. **(C)** PC3 (11.5% variance) reflects forelimb stride length. **(D)** PCA extracts the PCs through eigenvalue decomposition of the bivariate correlation matrix of all outcomes, here represented as a heat map of Pearson values. PCs are reflected as the Venn intersection (gray) across outcome domains and the PC loading values (correlation between each variable and the PC cluster) are represented as arrows where gage represents loading magnitude and heat reflects direction (red positive relationship, blue inverse relationship).

of function after cervical SCI. By combining data from multiple tests, we will have a better, more holistic view of recovery after neurological injury.

PREDICTIVE AND EXTERNAL VALIDITY

To test the predictive validity of the IBB, we examined the relationship with the underlying tissue damage in the spinal cord. We found that the IBB scores were highly and significantly correlated with the amount of tissue sparing at the SCI lesion site. How the IBB predicts SCI severity in comparison to other tests is discussed in the multivariate section below. The IBB was minimally sensitive to the impact of TBI alone, but as mentioned above, showed a similar sensitivity to combined SCI + TBI as the paw placement test (24). In a recent report from Speck et al. (46), the IBB was also shown to be sensitive to recovery from peripheral nerve injuries in mice.

CONSTRUCT VALIDITY: MULTIVARIATE ASSESSMENT OF FUNCTION

Findings from multifaceted outcome batteries applied to the same subject ultimately need to be integrated in some manner to derive a complete picture of forelimb recovery. Multivariate statistical pattern detectors such as PCA and the related approach of exploratory factor analysis provide quantitative means to perform this integration across outcomes (29, 39). This approach has classically been applied in the human assessment literature as a tool to gauge construct validity: the degree to which an individual test measures or "taps into" an underlying trait of interest [e.g., intelligence, executive function, memory etc.; Ref. (39)]. Indeed, this application of multivariate statistics is the underlying basis for most modern, standardized human achievement and neuropsychological tests. However, PCA has rarely been applied in preclinical research studies to assess the validity of scales used in animal models of neurobiological disorders. In the present paper we applied PCA to, (1) integrate outcome across multiple assessment tools, and (2) to assess the construct validity of the IBB. Based on prior work, we knew that PCA has the capacity to detect specific neurobiological substrates for forelimb recovery after SCI, specifically tapping into the relationship between tissue sparing and multifaceted forelimb function on the first principal component (PC1) (2, 32, 33, 37). The question in the current paper was, "does the IBB predict (or "load onto") the established forelimb neurobehavioral recovery construct outcome set?" The results indicated that not only did the IBB predict the forelimb neurobehavioral recovery construct (PC1), but it actually had the highest loading of all of the outcome variables assessed, providing strong evidence of construct validity for the IBB.

It is noteworthy that the IBB did not correlate as well with CatWalk measures of gait during locomotion. This suggests that the CatWalk assesses different neurobiological substrates than the IBB. This is consistent with prior work showing that the CatWalk

outcome metrics do not have high construct validity with respect to multivariate tissue sparing in contusive SCI (PC1) but do tap into orthogonal variance (PC2, PC3) related to hemisection injuries (2). This indicates that the CatWalk may reflect tissue changes not captured by crude measures of histological sparing after unilateral cervical SCI. This could account for the observation that hemisection injuries impact CatWalk, a model in which white matter and gray matter sparing at the lesion epicenter are relatively consistent. This dissociation between CatWalk and tissue sparing is reminiscent of the pattern observed in prior analyses that have included the horizontal ladder test after cervical SCI (6, 47). The horizontal ladder, the CatWalk and forelimb locomotor function clustered together as a coherent functional assessment construct (PC2); however, this outcome cluster did not correlate with histological sparing (47). We have argued that this indicates that CatWalk and horizontal ladder reflect fine-details of locomotor recovery that are organized by more subtle neurobiological changes (perhaps due to sprouting and plasticity), not reflected by gross gray and white matter sparing metrics *per se* (2, 37).

FORELIMB OBJECT MANIPULATION AS A TRANSLATIONAL TOOL

Our group has begun developing a primate analog to the IBB to facilitate cross-species translation of SCI research findings (34, 48, 49). Early work suggests that the IBB can be scaled up into an analogous object manipulation task in a non-human primate (NHP) model of cervical SCI in the rhesus macaque (48, 49). The primate version of the task shows strong sensitivity for loss and recovery of function after cervical lateral hemisection injuries. In addition, early cross-species testing of construct validity suggests that the rodent IBB and primate object manipulation task co-load along with tissue sparing on PC1, enabling consistent assessment of translational features of forelimb recovery (34, 48, 49).

Of course, the utility of object manipulation as a translational outcome measure may depend on the neurobiological substrates under study. It is often assumed that much of the loss and recovery of fine digital movement, and reach and grasp, in humans after CNS damage or degeneration is due to loss of cortico-spinal tract (CST) function. The classic work of Lawrence and Kuypers (50–52) indeed points to the pyramidal tract as a critical mediator of forelimb and especially fine digital control in primates. However, attempts to assign specific roles to the multitude of descending tracts and intra-spinal circuits in experimental models of SCI have proven to be difficult, and recent work suggests that there may be considerable redundancy in the organization of forelimb motor function. For example, Fouad and colleagues tested performance on a single pellet reaching task after various lesions of the dorsal and lateral funiculi, and found little correlation between lesion size and performance in the rat (53). In a related study, Morris et al. (54) found that lesions restricted to the dorsolateral funiculus where the rubrospinal tract is located, only affected the "arpeggio" movement, and not other aspects of reach and grasp.

It seems clear that more flexibility and individuation of movement might be supported by the development of the cortical system mediated through the CST as the primate CST developed, and that the ability of primates to produce highly accurate ballistic movements in space and to produce individual finger movements is extraordinary. However, recent work from several laboratories using NHPs suggests that recovery of fine digital control can be accomplished via reorganization of descending reticular systems impinging upon interneurons in the cervical cord. This raises the issue of how much of the forelimb control is mediated by cortical brainstem circuits versus those organized intrinsically within the cervical cord. In the case of the IBB scale, the results of our CCI studies suggest that the circuits in the sensorimotor cortex are involved in recovery of forelimb and fine digital movements, but that certainly much of this circuitry is organized at the spinal level, at least in the rodent.

Comparative studies of the neurobiology of forelimb recovery after rodent and primate SCI are a major focus of ongoing studies (55, 56). Object manipulation tasks such as the IBB will play an important role in making these cross-species comparisons to unravel the neurobiological substrates of forelimb recovery in the context of translational therapeutic testing.

CONCLUSION

The IBB is a recently developed forelimb scale for the assessment of fine control of the digits after damage to the nervous system (1). The present paper suggests that the IBB has strong IRR and validity (face, concurrent, and construct). Thus, the IBB may be useful in conjunction with, and in comparison to, other measures of forelimb and fine digital control in other mammalian species including primates. And, it may be a valuable adjunct to the armamentarium of translational tools for assessing recovery after nervous system damage and degeneration.

AUTHOR CONTRIBUTIONS

Karen-Amanda Irvine, Stephanie B. Beattie, Jacqueline C. Bresnahan, Michael S. Beattie provided the testing concept, Karen-Amanda Irvine, Stephanie B. Beattie, Jacqueline C. Bresnahan, Adam R. Ferguson, Michael S. Beattie designed the studies; all authors participated in the data collection, IRR testing (except Stephanie B. Beattie), and the interpretation of the results; Adam R. Ferguson supervised the data analysis; Karen-Amanda Irvine implemented the figures with input from Adam R. Ferguson, Jacqueline C. Bresnahan, and Michael S. Beattie; Karen-Amanda Irvine, Adam R. Ferguson, Jacqueline C. Bresnahan, and Michael S. Beattie generated the manuscript draft, and all authors critically reviewed and revised the manuscript.

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Time reproduction and numerosity interaction in the parietal cortex: some missing links

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A commentary on

Interaction of numerosity and time in prefrontal and parietal cortex
by Hayashi, M. J., Kanai, R., Tanabe, H. C., Yoshida, Y., Carlson, S., Walsh, V., and Sadato, N. (2013). J. Neurosci. 33, 883–893.

The interaction of time and numerical representation in the human brain is currently object of debate in the field of cognitive neuroscience. A wide amount of research based on "A theory of Magnitude (ATOM)" (Walsh, 2003), a model which proposes common cortical metric for the representation of space, time, and numbers, has provided extensive evidence in support of this suggestion (Dormal et al., 2006; Xuan et al., 2007; Oliveri et al., 2008; Vicario et al., 2008, 2012; Lu et al., 2009; Vicario, 2011). For instance, it has been shown that merely looking at numbers causes a bias in a time-bisection task that depends on its magnitude (Vicario, 2011). Nevertheless, there are also works which contrast with such proposal as it seems that these two dimensions refer to at least partially separated neural segregations (for instance, see Dormal et al., 2008; Agrillo et al., 2010). Thus, we are only beginning to understand the complex neuronal mechanisms underlying the interaction of these magnitudes in the human brain.

In a recent issue of the *Journal of Neuroscience*, Hayashi et al. (2013) added new insight into the current debate on the interaction of time and numerosity by exploring their neural correlates. The use of transcranial magnetic stimulation (TMS) for the investigation of this issue in the human brain is not new in the literature, as an attempt was previously made by Dormal et al. (2008). However, in the current work Hayashi et al. (2013) have used a clever methodology for testing their hypothesis as they applied TMS upon the neural regions which resulted in a joint activation during the execution of time and quantity processing tasks. Moreover, they used two different timing tasks (time discrimination vs. time reproduction) with the purpose of exploring the time numerosity interaction in the presence and in the context of a reduced involvement of categorical magnitude judgment. Remarkably, this work shows that TMS upon the right inferior frontal gyrus (IFG) impairs categorical duration discrimination, but in contrast, it has no effect on time estimation in the duration reproduction task. On the other hand, the TMS upon the right intraparietal cortex (IPC) modulates the degree of influence of numerosity on time reproduction and impairs precise time estimation. This evidence is striking insofar as it provides clear evidence of a common neural origin for the processing of time and numerosity in a precise region of the parietal cortex. The provided results lead the authors to propose a two-stage model of numerosity–time interactions, in which it is stated that the categorical decision takes place in the frontal cortex, whereas the interaction of numerosity information on perception of time occurs within the parietal cortex. Nevertheless, in the context of the present discussion there are two aspects worthy of receiving attention: the "*motor*" nature of the time reproduction task and the involvement of hand/fingers body part for its execution.

Previous studies have shown neural activity in the parietal cortex for particular visuomotor actions involving right hand/fingers representation such as grasping (Baumann et al., 2009), and the go/no go task (Sugawara et al., 2013). Interestingly, this parietal activation occurred after the Go stimulus, which can be considered the equivalent of the stimulus disappearance in the time reproduction task, of Hayashi et al. (2013). In fact, in this task participants were instructed to start the time reproduction action once the stimulus (digit or dot arrays) disappeared from the computer screen. It is also interesting to note that the parietal cortex of the right hemisphere is directly involved in motor tasks performed with the right hand. For instance, Hinkley et al. (2009) have recently reported that two regions of the right posterior parietal cortex were active in all the experimental subjects asked to perform a grasping task with their right hand. Thus, in consideration of this evidence, it is not possible to exclude that TMS upon the right IPC might affect performance by modulating the accuracy with which participants performed the required movement with their right hand, rather than their timing ability. In fact, by using a time reproduction it is not possible to disentangle the effect played by TMS on the motor and temporal dimensions of this task.

In strict connection with this argument is the close relationship between the representation of numerosity and hand/fingers in the parietal cortex, as showed by the common parietal activation for finger movements (Sugawara et al., 2013) and numerical cognition (Dormal et al., 2008). Moreover, previous works have extensively shown that, in both forced (Dehaene et al., 1990) and free-response (Daar and Pratt, 2008; Vicario, 2012) paradigms, perceiving numbers affects the execution of fingers action. The relationship between fingers and numerosity is also supported by the study of Costa et al. (2011) showing that deficits in fingers gnosia were found in association to mathematical difficulties. Interestingly, the authors provide argument that the deficits in fingers gnosia could not be attributed to a shortage in working memory capacity but rather to a specific inability to use fingers

to transiently represent magnitudes. All these works provide further support to the argument of a close relationship between hand/fingers and numerical magnitude through different experimental designs. Although the studies above mentioned have explored mechanisms qualitatively different from those investigated by Hayashi et al. (2013), the involvement of the hand/fingers at motor and/or representational level represents the *trait d'union* among all of these studies.

Taken together these results suggest that there may be a limit related with the use of the time reproduction task for testing the interaction of time and numerosity since it becomes difficult to establish: (i) if numerosity modulates performance by affecting the execution of the hand/fingers movements or the temporal representation of participants; (ii) if the effect played by TMS on the current timing task is due to its modulatory effect on hand/fingers motor planning rather than on timing performance. To overcome these concerns a good control would have been to subject participants to a non-timing motor task which involves the hand also (as in the case of the current time reproduction task), to ensure that the reported effect is not related to the execution of the action and/or to the hand/fingers involvement. Perspective works devoted to dissociate the interaction between time and numerosity might focus on experimental paradigms that are able to control the factors mentioned above (action and hand/fingers representation). The use of a *verbal time estimation* task (Hurks and Hendriksem, 2011) could represent a valid solution to clear these limits. In fact, this task does not require the involvement of hand/fingers movements.

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Effect of auditory constraints on motor performance depends on stage of recovery post-stroke

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In order to develop evidence-based rehabilitation protocols post-stroke, one must first reconcile the vast heterogeneity in the post-stroke population and develop protocols to facilitate motor learning in the various subgroups. The main purpose of this study is to show that auditory constraints interact with the stage of recovery post-stroke to influence motor learning. We characterized the stages of upper limb recovery using task-based kinematic measures in 20 subjects with chronic hemiparesis. We used a bimanual wrist extension task, performed with a custom-made wrist trainer, to facilitate learning of wrist extension in the paretic hand under four auditory conditions: (1) without auditory cueing; (2) to non-musical happy sounds; (3) to self-selected music; and (4) to a metronome beat set at a comfortable tempo. Two bimanual trials (15 s each) were followed by one unimanual trial with the paretic hand over six cycles under each condition. Clinical metrics, wrist and arm kinematics, and electromyographic activity were recorded. Hierarchical cluster analysis with the Mahalanobis metric based on baseline speed and extent of wrist movement stratified subjects into three distinct groups, which reflected their stage of recovery: spastic paresis, spastic co-contraction, and minimal paresis. In spastic paresis, the metronome beat increased wrist extension, but also increased muscle co-activation across the wrist. In contrast, in spastic co-contraction, no auditory stimulation increased wrist extension and reduced co-activation. In minimal paresis, wrist extension did not improve under any condition. The results suggest that auditory task constraints interact with stage of recovery during motor learning after stroke, perhaps due to recruitment of distinct neural substrates over the course of recovery. The findings advance our understanding of the mechanisms of progression of motor recovery and lay the foundation for personalized treatment algorithms post-stroke.

Keywords: bimanual movements, upper extremity, rehabilitation, motor learning/training, electromyography, task specificity, cerebrovascular disorders

INTRODUCTION

Stroke strikes one in six people worldwide. It is the leading cause of disability in the United States (1) and Europe (2), and the second leading cause of disability in the world (3). Hemiparesis is the most common reason for stroke-related disability, and the majority of individuals with hemiparesis have persistent deficits in hand function (4). There has been a recent surge in the availability of new rehabilitation strategies post-stroke. However, several large randomized controlled trials have failed to show the benefit of any one intervention over conventional treatment (5), and there remains a lack of understanding about how to select an appropriate treatment strategy for a given individual. While it is now accepted that task-specific training is an important aspect of a rehabilitation intervention, the constraints under which the task(s) should be practiced to be optimally therapeutic are not known. A constraint may be defined as the specific conditions under which a task is performed, for example, with one hand or both, with auditory/visual/multi-sensory feedback or without, etc. Task constraints are important because they regulate the information that is processed and assimilated by the nervous system, and the selection of constraints for any specific task may depend on the integrity and/or capacity to recruit specific neural substrates that facilitate processing of the relevant movement-related information. The stage of motor recovery, as measured by the level of motor impairment, may provide an indication for the type of task-specific constraints that are useful during practice for a given individual.

Fortunately, recovery of motor function after a hemiplegic stroke has been shown to follow a predictable pattern. Twitchell (6), Brunnstrom (7), and Fugl-Meyer et al. (8) described a hierarchical progression of recovery of patients who initially present with flaccid paralysis on one side of the body with areflexia. The

reflex activity returns next and becomes heightened as spasticity emerges, and voluntary movements occur in stereotypical flexor and extensor synergy patterns. Spasticity then reaches its maximum level, producing characteristic patterns of stretch-sensitive responses such as spastic co-contraction. Eventually, the synergy patterns start to break up and spasticity begins to reduce as normal patterns of voluntary movement are restored. The emergence and disappearance of spasticity are thus important milestones in motor recovery (9, 10), although the severity of spasticity may vary considerably and temporary arrests in recovery or "plateaus" can occur at any stage (6).

Recent imaging studies further show how recovery processes unfold after a stroke [see Ref. (11) for review]. Early in recovery, the undamaged contralesional hemisphere shows increased activation (12–15), but eventually normal sensorimotor lateralization is restored in the stroke-affected hemisphere (16–18). Importantly, increases in neural activity in the contralesional motor areas in the first weeks after stroke correlate with better motor recovery in humans (19, 20) and monkeys (15), although persistent activation of the motor and non-motor areas in the contralesional hemisphere is noted in patients with poor motor outcome (18, 21). A recent longitudinal case study of a patient's recovery over 21 months revealed continuous change in activation in the contralesional hemisphere with concomitant improvement in motor performance, whereas the ipsilesional hemisphere demonstrated significant change only toward the end of the study period (22). Taken together, these studies suggest that (1) redundant homologous pathways in the intact hemisphere can facilitate re-organization of the central nervous system, particularly in the earlier stages of recovery, and (2) that motor recovery occurs over a protracted and variable time period post-stroke. Hence the time since stroke may not reflect where an individual is in his or her recovery process.

Two kinds of bimanual training protocols have been developed to capitalize on contralesional cortical activity post-stroke. In active bimanual training, both arms move independently and simultaneously, requiring that individuals have at least some active movement on the paretic side. Active bimanual arm training combined with rhythmic auditory stimulation (BATRAC protocol) led to increased recruitment in the contralesional and ipsilesional hemispheres with concomitant improvement in performance of the paretic hand (23, 24). These data suggest that there may be a synergistic effect of bimanual and auditory constraints, but their individual contribution to performance improvement has not been ascertained. Rhythmic auditory stimulation by itself has also been found to be a useful adjunct to post-stroke rehabilitation (25–28). In active–passive bimanual training, the non-paretic arm drives movements of the paretic arm and leads to simultaneous mirror movements of both arms. Here, bimanual training occurred without auditory stimulation, was used to prime the ipsilesional motor cortex for subsequent training with the paretic arm, and also led to significant gains in arm function (29–32). An advantage of the active–passive approach is that it requires little active movement in the paretic arm and can therefore be used in individuals with significant paresis. Furthermore, the active–passive approach may be used to probe subsequent motor learning with the paretic arm. We have previously shown that motor learning is often impaired with the paretic hand, but may be temporarily restored after prior practice with the non-paretic hand (33).

In this study, we sought to determine the effect of various auditory constraints on bimanual-to-unimanual (paretic hand) learning in individuals at different stages of motor recovery post-stroke. Rhythmic stimulation with a metronome has been shown to improve spatiotemporal control of arm movements, perhaps via activation of brainstem–cerebellar networks (34, 35). However, several lines of evidence suggest that emotional drive via activation of limbic networks may also be an important predictor of motor performance (36) and post-stroke motor recovery (37). Music has been shown to activate a bilateral network of mesolimbic structures involved in processing emotions and reward information (38), and affective vocalizations have been shown to modulate attention via activation of pre-frontal–limbic networks (39). It is not clear when over the course of recovery one type of auditory stimulation versus another or no auditory stimulation will be beneficial. Hence, the objectives of this study were to: (1) characterize the stage of recovery in a disparate group of subjects with post-stroke hemiparesis using task-based kinematic measures, and (2) to examine how various types of auditory constraints interact with stage of recovery to facilitate learning with the paretic limb on a bimanual-to-unimanual learning task. Since voluntary wrist extension is frequently compromised post-stroke (40) and active wrist extension ability is predictive of hand function (41), we focused our task on training of wrist extension in the paretic hand. We hypothesized that auditory constraints that enhance emotional drive would facilitate learning of wrist extension with the paretic arm particularly in the early stages of recovery post-stroke.

MATERIALS AND METHODS

SUBJECTS

Twenty subjects with chronic post-stroke hemiparesis (at least 6 months prior to enrollment) were recruited through referrals from physicians at the Rusk Institute of Rehabilitation Medicine and through public advertisement. Subjects provided informed consent in accordance with the Institutional Review Board of the New York University School of Medicine. All subjects had at least 15° of passive and 5° of active wrist extension on the paretic side to perform unimanual movements, and they were screened to rule out hearing deficits prior to participation.

PROTOCOL

The clinical assessments and experimental protocols were administered by well-trained research staff at the Motor Recovery Research Laboratory in the Rusk Institute of Rehabilitation Medicine. At the first visit, the Fugl-Meyer Scale (8) was used to assess upper extremity motor impairment; the Modified Ashworth Scale (42) assessed spasticity in the affected shoulder, elbow, wrist, and finger joints; active and passive range-of-motion at shoulder, elbow, wrist, and finger joints were measured using a goniometer (43), and the threshold for joint proprioception was also assessed. Depression and mood were assessed using the 15-item Geriatric Depression Scale, which has been recommended for

FIGURE 1 | Custom-made wrist extension trainer.

the assessment of post-stroke depression in adults of all ages (44, 45), and the Brunel Mood Scale (BRUMS) (36), respectively. An appropriate tempo for the metronome beat was then determined by asking subjects to flex and extend their paretic wrist at a comfortable pace using a custom-made wrist trainer (**Figure 1**) for 15 s. Subjects then selected three familiar songs from public media to increase their feeling of vigor, happiness, and calmness. An up-tempo major key song that matched their metronome speed, or was in multiples of their metronome speed, was chosen to induce a positive mood-state. The BRUMS Scale was repeated after the subjects listened to their self-selected song to verify improvement in mood-states (**Figure 2**).

At the second visit, subjects performed repeated bimanual and unimanual (with the paretic hand) wrist flexion-extension movements using a custom-made wrist trainer. The device was designed to constrain movement of the wrist in the sagittal plane, and limit compensatory movement of the forearm and arm. The height of the chair was adjusted to keep the shoulders level and maintain proper alignment of the trunk. Table height and the position of the wrist trainer were maintained across all task conditions for each subject. Before the start of the experiment, subjects were informed that the goal of training was to facilitate wrist extension. Electromagnetic motion sensors (trakSTAR, Ascension Technology Corporation, Shelburne, VT, USA) affixed to the limb segments on each side measured wrist kinematics. Bipolar surface electrodes (DE 2.1, Delsys Inc., Natick, MA, USA) affixed over the flexor carpi ulnaris (FCU) and extensor carpi radialis longus (ECRL) muscles on each limb recorded electromyographic (EMG) signals. Video, kinematic, and EMG data were captured synchronously using The Motion Monitor (Innovative Sports Training Inc., Chicago, IL, USA), and analysis was performed offline using Spike 2 (Cambridge Electronic Design, Cambridge, England).

Wrist movements were performed under four different auditory conditions: (1) at baseline without auditory cueing; (2) to positively valenced affective "happy" sounds (baby's laughter) recorded for 11 s and looped continuously, providing non-musical and non-rhythmic auditory stimulation; (3) to the self-selected up-tempo major key song chosen during visit 1; and (4) to a metronome beat set at each individual's comfortable tempo. The subject was required to complete one cycle of wrist extension and flexion to each beat. Each condition consisted of 18 15-s trials of wrist flexion and extension, where subjects performed two bimanual trials followed by one unimanual trial with the paretic hand. A 20-s rest break was provided between each trial to prevent fatigue. The order of the conditions was counterbalanced across subjects, and subjects rated their fatigue levels after the completion of all trials for each condition.

DATA ANALYSIS

Kinematic data were sampled at 120 Hz and EMG data were sampled at 1206 Hz. The kinematic data were low pass filtered at 6 Hz and up-scaled to 1206 Hz using linear interpolation. The EMG data were filtered using a dual band pass filter (10–52.5 and 67.5–500 Hz) and the root mean square (RMS) of the signal was obtained for wrist flexion and extension phases of the movement separately. The EMG signals were normalized to the maximum amplitude recorded for each muscle across all trials and conditions (46, 47) to facilitate within- and between-subject comparisons. This method was chosen after extensive reliability testing of different methods of normalization (by Ying Lu). Movement speed, amplitude of wrist extension, wrist extensor activation (RMS of agonist, ECRL), wrist flexor activation (RMS of antagonist, FCU) during extension, and co-activation (defined as RMS of antagonist, FCU/RMS of agonist, ECRL) were the variables used in the analyses. Recognizing that the subjects may present at various stages of recovery at the time of the study, we used hierarchical cluster analysis with the Mahalanobis metric (48) based on baseline wrist kinematics to stratify subjects into groups. The stratification scheme corresponded well with recovery characterized by the Fugl-Meyer Scale as shown in the results below. We then fit linear mixed effect models with group interactions and individual random effects to assess: (1) differences among subject clusters, and (2) learning rates across repeated unimanual trials with the paretic hand after bimanual priming with the four auditory conditions. Learning rate on unimanual trials was defined as the slope of the linear trend fit over the six unimanual trials. All the statistical analyses were conducted using R (v. 2.15.1). The R package "lme 4" was used for the mixed effect model estimation. To control for multiple comparisons but preserve statistical power (due to low sample size in the subgroups), we present all results but choose to interpret results with marginal statistical significance ($0.01 < p < 0.05$) with caution.

RESULTS

Our first objective was to characterize the stages of recovery across a disparate group of patients with post-stroke hemiparesis. Since wrist kinematics provide direct, objective, and reliable measures of movement ability in the paretic hand, we used the movement speed and extent of wrist extension from the first trial with the paretic hand under the baseline condition (no auditory cueing) to perform hierarchical cluster analysis (48), which stratified subjects into three distinct groups (**Figure 3**).

Clinical metrics (**Table 1**) showed clear differences across the three groups. The Fugl-Meyer scores were lowest in group 1, followed by group 2, and then group 3 ($p = 0.047$). Active wrist motion, measured using goniometry separately from the wrist extension task, showed that both wrist flexion and extension were surprisingly lowest in group 2, intermediate in group 1, and highest in group 3 ($p < 0.001$). Spasticity at the wrist flexors was, however,

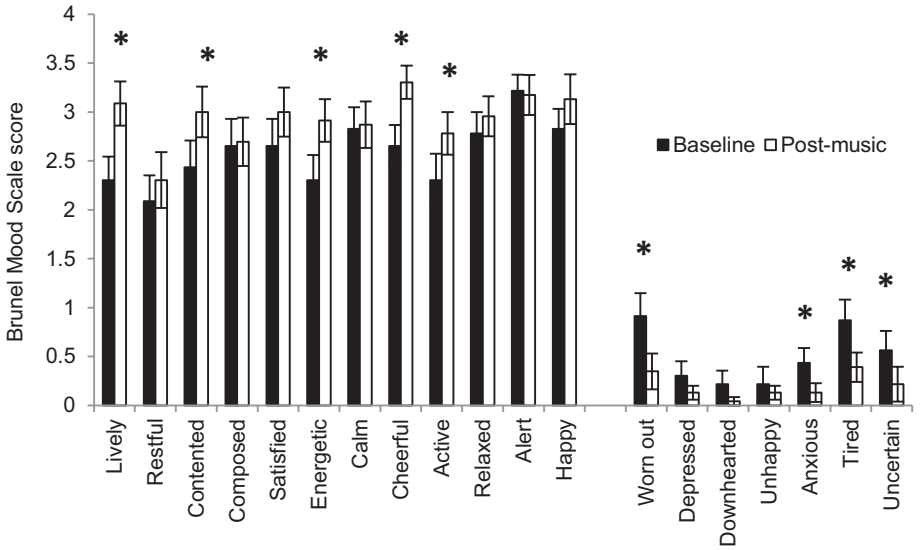

FIGURE 2 | Mean Brunel Mood Scale scores at baseline and after listening to self-selected music. Error bars represent the standard error. *Represents statistically significant differences at $p < 0.05$.

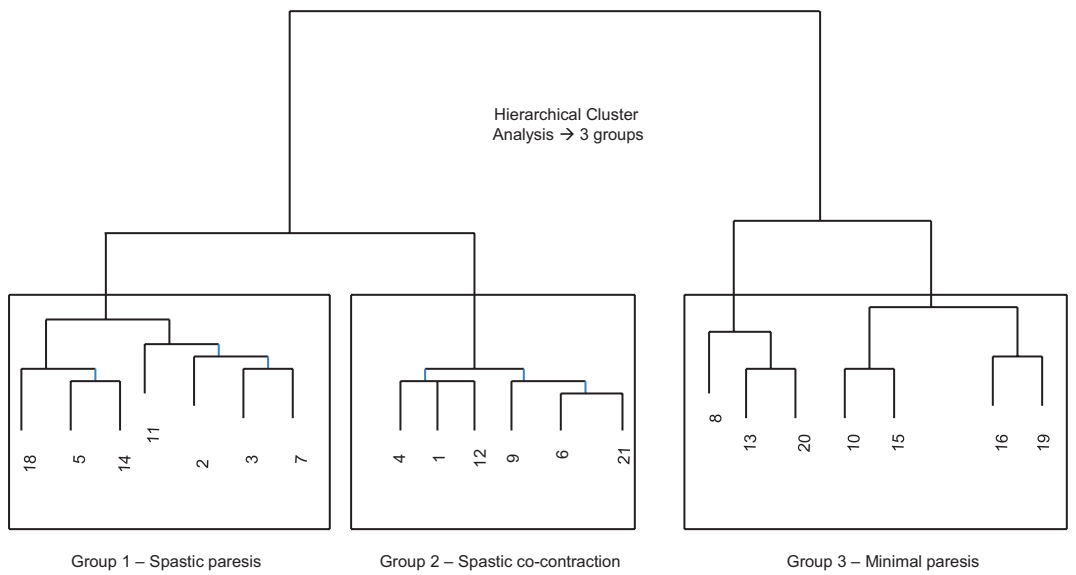

FIGURE 3 | Cluster dendrogram from hierarchical cluster analysis using the Mahalanobis metric based on speed and amplitude of wrist extension on the first trial with the paretic hand at baseline (without auditory stimulation). Three distinct groups emerged.

highest in group 1, and similar in groups 2 and 3 ($p = 0.066$). There were no significant differences among the three groups in joint proprioception at the wrist, depression scores, mood scores, tempo of the metronome beat or selected song, or fatigue levels. Note that the mean time since stroke was also not different across the three groups ($p = 0.89$).

Baseline performance metrics on the wrist extension task also showed clear differences across the three groups. Movement speed was higher in group 3 compared to groups 1 and 2 ($p < 0.001$, **Figure 4A**). Extent of wrist extension was lowest in group 2 (where attempted wrist extension produced paradoxical flexion), intermediate in group 1, and highest in group 3 ($p < 0.001$, **Figure 4B**). Wrist extensor muscle (ECRL) activation was also lowest in group 2, intermediate in group 1, and highest in group 3 ($p = 0.047$, **Figure 4C**), whereas wrist flexor muscle (FCU) activation was not differentiated in the three groups ($p = 0.877$, **Figure 4D**). Co-activation between wrist extensor and flexor muscles was highest in group 2, intermediate in group

Table 1 | Clinical characteristics of subjects: [a]Sub, subjects in each group (see also Figure 2); [b]age, in years; [c]H/H, handedness/hemiparesis; [d]TSS, time since stroke in months; [e]FMS, Fugl-Meyer score, values represent total upper extremity scores out of a maximum of 66/hand and wrist score out of a maximum of 30; [f]AROM, active range-of-motion in degrees at the wrist measured with a goniometer; [g]MAS, modified Ashworth Scale measured at the wrist. Lesion location and stroke subtype obtained from: [h]radiology reports and [i]medical history narrative from subject.

		Sub[a]	Age/sex[b]	H/H[c]	Stroke location/subtype	TSS[d]	FMS[e]	AROM[f] Flexion	AROM Extension	MAS[g] Flexors	MAS Extensors
Group 1	Spastic paresis	2	43/F	R/R	L frontal hge[h]	8	**21/8**	**20**	**15**	**3**	1
		3	43/M	L/R	L subcortical hge[i]	N/A	**35/20**	**20**	**15**	**3**	1
		5	62/F	R/L	R parietal hge[h]	71	**48/22**	**15**	**15**	**3**	2
		7	36/M	R/R	L MCA infarcts with hge[h]	37	**38/20**	**20**	**20**	**1**	1
		11	65/M	R/R	L BG infarct[h]	50	**51/21**	**33**	**35**	**2**	1
		14	52/M	R/R	L IC occlusion[i]	123	**28/10**	**20**	**15**	**3**	1+
		18	60/F	R/L	R cerebral hge[i]	84	**33/18**	**20**	**10**	**2**	2
		Mean	51.6			62.2	**36.3/17**	**21.1**	**17.9**	**2.4**	1.4
		(SD)	(11.2)			(39.9)	**(10.6/5.6)**	**(5.6)**	**(8.1)**	**(0.8)**	(0.5)
Group 2	Spastic co-contraction	1	28/F	R/R	L MCA infarct[h]	44	**51/21**	**10**	**5**	**1**	0
		4	46/F	R/L	R MCA hge[i]	77	**42/20**	**20**	**10**	**2**	2
		6	61/M	R/R	L cerebral hge[i]	18	**37/21**	**15**	**15**	**3**	3
		9	54/M	R/R	L lacunar infarct[h]	49	**54/27**	**20**	**10**	**1**	1
		12	56/M	R/R	L lacunar infarct[h]	51	**62/27**	**10**	**5**	**1+**	1+
		21	87/F	R/L	R MCA infarct[h]	84	**25/5**	**15**	**5**	**1**	2
		Mean	55.3			53.8	**45.2/20.1**	**15.0**	**8.3**	**1.6**	1.6
		(SD)	(19.3)			(23.9)	**(13.3/8.0)**	**(4.5)**	**(4.1)**	**(0.8)**	(1.0)
Group 3	Minimal paresis	8	47/M	R/R	L IC occlusion[i]	71	**35/25**	**30**	**25**	**3**	1
		10	69/F	R/R	L thalamic infarct[h]	24	**58/25**	**50**	**55**	**1**	1
		13	42/F	R/L	R IC occlusion[h]	30	**40/20**	**50**	**70**	**2**	2
		15	71/M	R/L	R MCA infarct[h]	37	**55/20**	**50**	**45**	**1**	1
		16	41/M	R/R	L BG hge[h]	192	**65/29**	**60**	**50**	**1**	1
		19	59/M	R/R	L thalamic infarct[h]	36	**59/26**	**75**	**60**	**1**	1
		20	62/M	R/R	L MCA infarct[h]	69	**60/27**	**40**	**30**	**1**	1.1
		Mean	55.9			65.6	**53.1/24.5**	**50.7**	**47.9**	**1.4**	1.1
		(SD)	(12.5)			(58.8)	**(11.2/3.4)**	**(14.3)**	**(16.0)**	**(0.8)**	(0.4)
P-value across the three groups						0.89	**0.047**	**<0.001**	**<0.001**	**0.066**	0.502

Bolded variables showed statistically and/or clinically important differences across the three groups.

1, and lowest in group 3 ($p = 0.07$, **Figure 4E**). Taken together, the baseline performance and clinical metrics enabled characterization of recovery patterns into the three descriptive groups below.

GROUP 1 – SPASTIC PARESIS

In this group, performance on the paretic side (**Figure 4**, shown in blue) relative to the non-paretic side showed low movement speed (~10%), moderate wrist extension (~20%), moderate activation in the wrist extensor (~30%), and five times greater co-activation. Clinically, these subjects had the lowest Fugl-Meyer scores (range 21–51), but had 15–33° of active wrist flexion and 10–35° of active wrist extension. Spasticity was observed predominantly in the wrist flexors. Lesion location and stroke subtype (**Table 1**) suggest that these subjects had very severe strokes that were caused predominantly by intracerebral hemorrhage (subject #s 2, 3, 5, 18) or hemorrhagic transformation of ischemic infarcts (subject # 7).

GROUP 2 – SPASTIC CO-CONTRACTION

In this group, performance on the paretic side (**Figure 4**, shown in red) relative to the non-paretic side showed very slow movement speed (~7%), paradoxical wrist flexion on attempted wrist extension (−27%), minimal activation of the wrist extensor muscle (~10%), and ~10 times greater co-activation. Clinically, these subjects had higher Fugl-Meyer scores (range 25–62) than those in group 1. However, they had only 10–20° of active wrist flexion and 5–15 of active wrist extension. Spasticity was distributed equally in both wrist flexors and extensors for the most part. Lesion location and stroke subtype (**Table 1**) suggest that these subjects had moderately severe strokes caused predominantly by infarcts in the MCA territory (subject #s 1, 9, 12, 21).

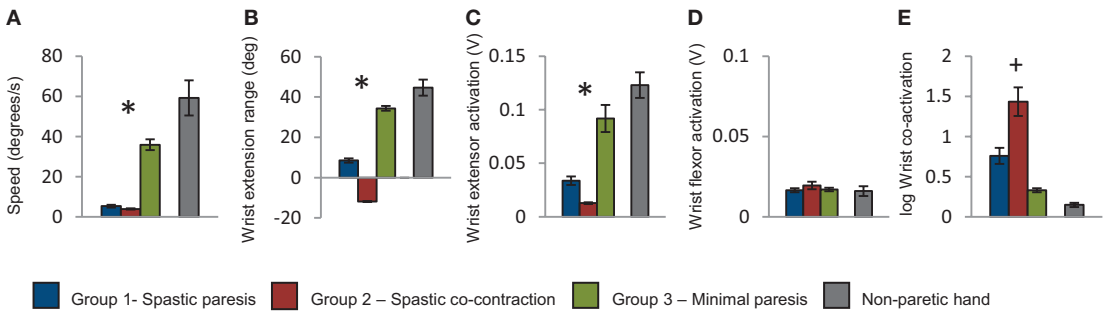

FIGURE 4 | Group means computed for the first trial with the paretic hand. **(A)** Speed of wrist extension in degrees per second; **(B)** extent of wrist extension in degrees; **(C)** root mean square of wrist extensor muscle activation during wrist extension; **(D)** root mean square of wrist flexor muscle activation during wrist extension; **(E)** log of wrist co-activation computed as ratio of wrist flexor to extensor muscle activation. The blue bars represent the group with spastic paresis, which had the lowest Fugl-Meyer scores, the red bars represent the spastic co-contraction group with intermediate Fugl-Meyer scores, and the green bars represent the minimal paresis group, which had the highest Fugl-Meyer scores. Values for the non-paretic hand are shown in gray for reference. Error bars represent the standard error. *Represents differences between the three groups at $p < 0.05$, and $^{+}$represents differences between the three groups at $p < 0.1$.

GROUP 3 – MINIMAL PARESIS

In this group, performance on the paretic side (**Figure 4**, shown in green) relative to the non-paretic side showed relatively high movement speed (~60%), substantial wrist extension (~77%), and wrist extensor activation (~75%), and twice the co-activation as the non-paretic side. Clinically, these subjects had the highest Fugl-Meyer scores (range 35–65) and the greatest range of active wrist flexion (30–75°) and extension (25–70°) of the three groups. Lesion location and stroke subtype (**Table 1**) suggest that these subjects had a mixed variety of strokes predominantly in the MCA territory.

Our second objective was to examine how different types of auditory stimuli interact with bimanual training to facilitate subsequent learning with the paretic limb in the three groups. Subjects performed six cycles of two bimanual trials followed by one unimanual trial with the paretic hand, where each trial consisted of multiple repeats of wrist flexion-extension over 15 s. We were interested in the changes in wrist extension, wrist extensor activation, wrist flexor activation, and co-activation over the six unimanual trials for each of the auditory conditions (represented by the different line patterns, see **Figure 5**). The mean level of the trend lines provides an indication of the amplitude of overall performance, whereas the slope of the trend lines quantifies the rate of learning on the paretic side. A positive slope suggests sustained improvement whereas a negative slope suggests reduced performance under that constraint. Subjects in the spastic paresis (**Figure 5A**) and spastic co-contraction (**Figure 5B**) groups started with low or negative wrist extension, but showed sustained improvements under certain auditory constraints. Subjects in the minimal paresis group (**Figure 5C**), showed good wrist extension at first, but did not improve much over the repeated trials.

The slope (unit change per trial) succinctly summarizes which auditory conditions couple with bimanual training for sustained improvement on the paretic side in the three groups (**Figure 6**). In the spastic paresis group (**Figure 6A**), wrist extension improved most with the metronome beat (slope $b = 0.86$, $p = 0.03$), even though it also increased wrist flexor activity ($b = 0.0021$, $p < 0.0001$) and co-activation ($b = 0.07$, $p = 0.004$). Self-selected music did not increase wrist extension, but marginally increased flexor muscle activity ($b = 0.0010$, $p = 0.04$). Thus rhythmic auditory constraints improved motor control in subjects with spastic paresis who were at an earlier stage in motor recovery post-stroke. In the spastic co-contraction group (**Figure 6B**), wrist extension improved most *without* any auditory cueing ($b = 1.83$, $p < 0.0001$), which increased wrist extensor muscle activation ($b = 0.004$, $p = 0.0002$) and decreased co-activation across the wrist joint ($b = -0.1$, $p = 0.0006$). In contrast, self-selected music increased co-activation ($b = 0.059$, $p = 0.04$) in this group. Thus practice without auditory constraints was most beneficial in subjects with spastic co-contraction. In the minimal paresis group (**Figure 6C**), there was no improvement in wrist extension across the auditory conditions. The slope for wrist extension was most negative with happy sounds ($b = -0.86$, $p = 0.03$), wrist extensor activation decreased with the metronome beat ($b = -0.0022$, $p = 0.02$), and wrist flexor activation increased without auditory stimulation ($b = 0.0012$, $p = 0.015$).

DISCUSSION

Neurological and behavioral differences between patients and within each patient over the course of post-stroke recovery can influence how learning occurs during task-specific interactions. Hence, it is necessary to reconcile the vast clinical and movement heterogeneity in the post-stroke population to develop evidence-based rehabilitation protocols directed toward more homogenous groups of patients. Toward this end, the purpose of this study was to: (1) stratify subjects with post-stroke hemiparesis according to their stage of recovery using task-based kinematic measures, and (2) to examine how various types of auditory constraints interact with stage of recovery to facilitate learning of a wrist extension task with the paretic limb. The subjects were stratified into three distinct groups based on their speed and extent of wrist extension. Differences in clinical metrics and task performance led to the characterization of stage of recovery into three groups: (1) the spastic paresis group showed weak extensor drive with flexor spasticity and moderate co-activation of the flexors and extensors, and higher level of motor impairment; (2) the

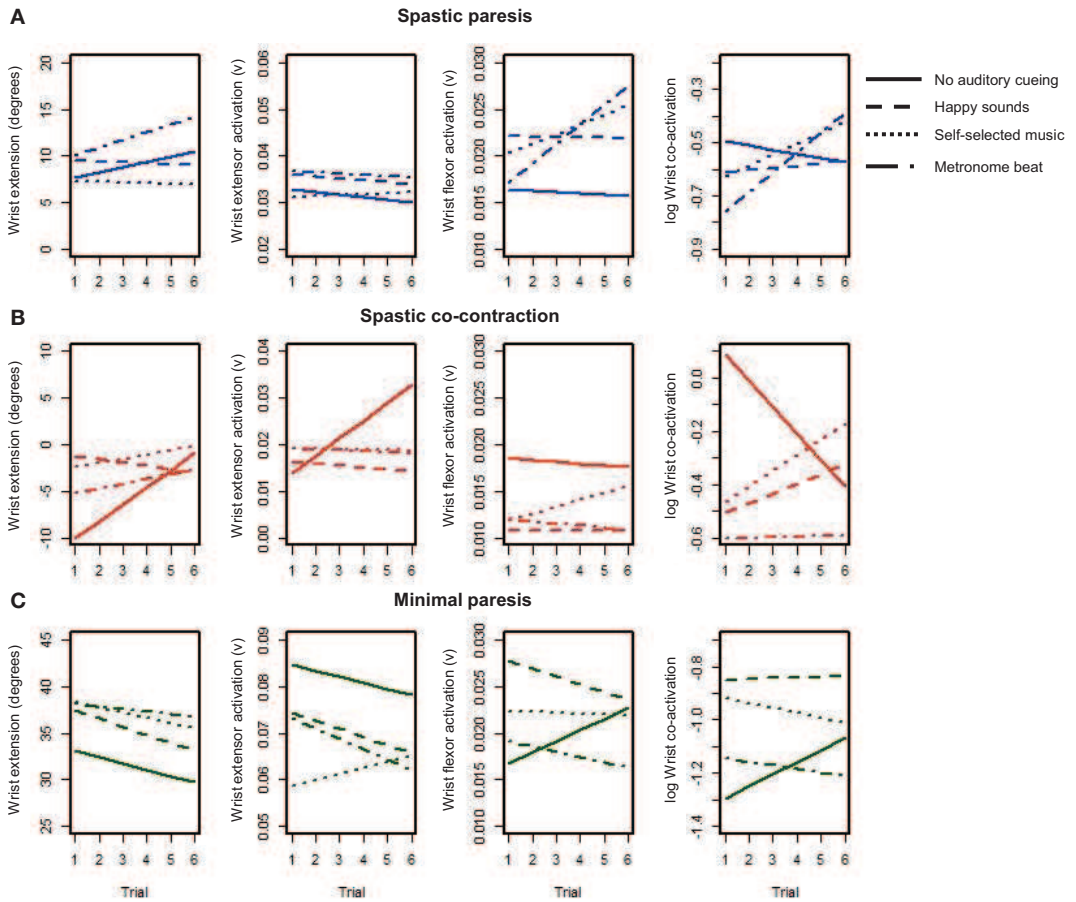

FIGURE 5 | Trendlines of wrist extension performance variables over six repeated trials with the paretic hand under each condition for the three groups: (A) spastic paresis (blue); (B) spastic co-contraction (red); (C) minimal paresis (green). The four conditions are represented by the different patterned lines.

spastic co-contraction group showed higher flexor activation relative to the extensor and excessive co-activation of the flexors and extensors, with moderate level of motor impairment; and (3) the minimal paresis group showed restored extensor drive, low levels of co-activation, and minimal level of motor impairment. The effect of auditory constraints on rate of learning with the paretic hand after bimanual training was measured by the slope of wrist extension, and wrist extensor and flexor muscle activation patterns. Auditory stimulation with a metronome beat increased the rate of learning of wrist extension in subjects with spastic paresis, even though it increased flexor activation and co-activation across the flexor and extensor muscles. In contrast, bimanual training *without* auditory stimulation produced the greatest improvement in subjects with spastic co-contraction, increased wrist extensor activation, and reduced co-activation. Auditory stimulation in subjects with minimal paresis did not improve wrist extension, but performance was sensitive to the effects of auditory stimulation in this group. These results suggest that altering auditory task constraints during the same task can have different and even opposite effects on motor performance and learning in individuals at different stages of recovery post-stroke. These results cannot be explained by differences in proprioceptive sensation, task difficulty, or fatigue across the groups or conditions. The results further our understanding of possible mechanisms underlying progression of recovery from one stage to the next after stroke.

STRATIFICATION OF SUBJECTS REFLECTS TEMPORAL STAGES IN POST-STROKE RECOVERY

Subjects with stroke have traditionally been classified based on the time elapsed since their stroke into acute (0–3 months), subacute (3–6 months), and chronic (6 months onward) categories. Recovery has been found to be most rapid in the acute and subacute periods (49), but recently compiled evidence shows that it continues well into the chronic period (50), although the trajectory of recovery may be punctuated by "plateaus" or temporary arrests in recovery. All the subjects in our study were in the chronic phase and may be considered to have plateaued. In longitudinal studies, increases in Fugl-Meyer scores suggest progression toward recovery. The Fugl-Meyer Scale is based on the observation of sequential recovery of motor function by Twitchell and Brunnstrom (6, 9, 10). It is the most widely used quantitative measure of motor recovery

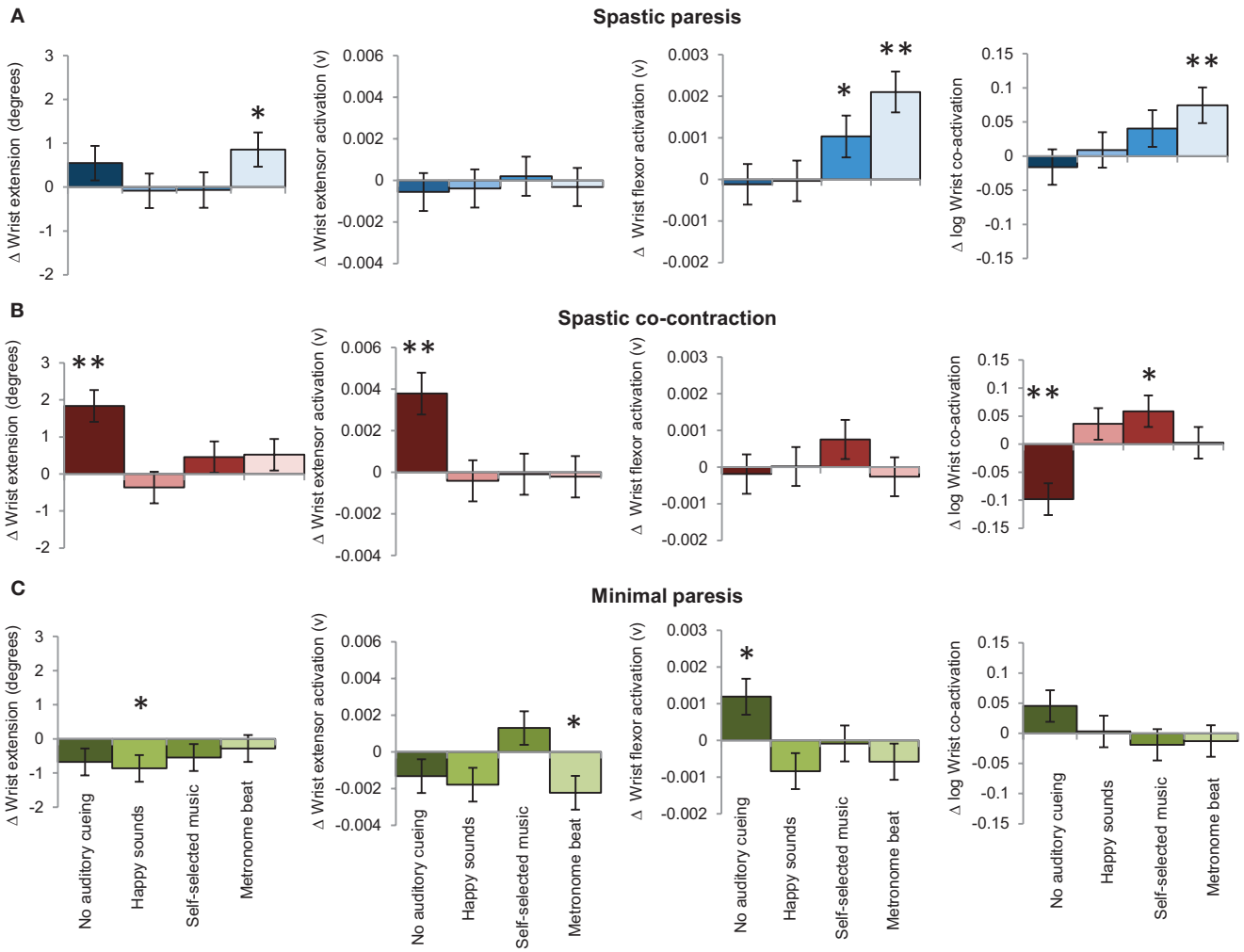

FIGURE 6 | The bars represent the mean slopes showing the effect of auditory stimulation on bimanual-to-unimanual learning for wrist extension performance variables in the three groups: (A) spastic paresis (blue); (B) spastic co-contraction (red); (C) minimal paresis (green). Error bars represent the standard error. **Represents differences between the three groups at $p < 0.01$, and *represents differences between the three groups at $p < 0.05$.

post-stroke (51, 52), and the scores have been shown to correlate with the extent of corticospinal tract damage (53). Hence, one can consider subjects with lower Fugl-Meyer scores as being more impaired or at an earlier stage in the recovery process compared to those with higher scores. In this study, subjects in the spastic paresis group had the lowest average Fugl-Meyer scores (both total and for the wrist and hand), which progressively increased in the spastic co-contraction and minimal paresis groups.

Fugl-Meyer scores have also been used to stratify subjects into groups (54, 55), but the cut-offs have been variable. Furthermore, the Fugl-Meyer Scale was constructed on the assumptions that recovery proceeds in a proximal-to-distal fashion and from synergistic-to-isolated movements (8, 51); however, both these assumptions have been contested recently (56–58). To circumvent the shortcomings of the Fugl-Meyer Scale in stratifying subjects, we used task-based kinematic measures, that is, speed and extent of wrist extension during the task, as direct, objective, and reliable measures of movement ability to stratify subjects into groups. Note that wrist movement amplitudes recorded during the task were lower than those recorded with goniometry prior to the task as would be expected due to the repetitive nature of the task.

We found that the spastic paresis group showed higher speed and amplitude of movement than the spastic co-contraction group, even though the Fugl-Meyer scores were higher in the spastic co-contraction group. This may seem surprising and contradictory to the notion of a linear improvement in movement ability over the course of post-stroke recovery. However, Twitchell observed that spasticity or tone continues to increase and reaches a peak before it starts to decrease (6). In this study, we measured spasticity clinically using the Modified Ashworth Scale, and by the extent of co-activation across the flexors and extensors during the task. We found that the spastic co-contraction group had equally increased tone in both the flexors and extensors, and 10 times greater co-activation on the paretic side than on the non-paretic

side. While some degree of co-activation between the agonist and antagonist muscles is normal during movement, excessive co-activation leads to reduced movement speed and amplitude (59). Therefore, it follows that a progression of recovery from spastic paresis would lead to a dip in movement ability due to increases in co-activation before it begins to improve again as seen across our three groups. Our results suggest that the processes underlying progression of recovery are non-linear, and predict that movement kinematics and muscle activation patterns may worsen as recovery progresses and then get better. These predictions should be confirmed by future longitudinal studies that measure kinematics and EMG over time.

Furthermore, our results show that auditory constraints increase movement amplitude but also increase muscle co-activation in subjects with spastic paresis, suggesting that individuals at earlier stages of motor recovery benefit from an excitatory drive. In contrast, in subjects with spastic co-contraction, who were at a later stage in recovery and showed excessive co-activation from excitatory overdrive, auditory constraints were not helpful. Instead, bimanual-to-unimanual training *without* auditory stimulation led to reduced muscle co-activation and increased agonist muscle activity, suggesting that an inhibitory drive may be more beneficial to transition from spastic co-contraction. These findings are discussed further in the sections below. Thus, we propose that stratification of subjects based on relatively simple kinematic parameters of speed and extent of movement into the groups: (1) spastic paresis, (2) spastic co-contraction, and (3) minimal paresis reflects temporal stages in the course of post-stroke recovery, and transition from each of these stages may be triggered by specific constraints imposed during training.

RHYTHMIC AUDITORY STIMULATION IMPROVES PERFORMANCE IN INDIVIDUALS WITH SPASTIC PARESIS

At baseline, subjects with spastic paresis had both weakness and spasticity, defined as velocity-dependent increase in muscle tone at rest (60), as measured by the Modified Ashworth Scale (61). The emergence of spasticity is thought to reflect re-organization of the descending brainstem pathways leading to diffuse and synergistic patterns of movement. Weakness predominates in the early stages of spasticity (62), hence, while subjects in the spastic paresis group could activate their wrist extensor muscle, their range of wrist extension was limited. Spasticity was greater in the flexor muscles, consistent with the emergence of a flexor synergy pattern (10). Co-activation across the flexors and extensors was increased, but not disabling, as it did not hinder wrist extension (63, 64). In this group, auditory stimulation with a metronome beat in conjunction with bimanual training led to increased wrist extension, while that with self-selected music and happy sounds did not. However, both the metronome beat and self-selected music increased wrist flexor activation.

Both the metronome beat and self-selected music have rhythmic components; the rhythm was even and constant with a metronome, but uneven and changing with music. Both even and uneven rhythmic stimulation have been shown to increase muscle co-activation (65). The underlying mechanism is thought to be increased excitability of spinal motor neurons via the reticulospinal pathway, with facilitation of the H-reflex response (66, 67). Using functional MRI and effective connectivity analyzes, it has been shown that listening to music relative to scrambled musical sounds, activates a bilateral network of mesolimbic structures including the nucleus accumbens and the ventral tegmental area (38) leading to dopamine release and arousal. The ventral tegmental area in turn forms part of the midbrain reticular formation where the reticulospinal tracts originate. Excitation of the reticular formation is known to increase spasticity via the reticulospinal projections to the spinal cord (68). Thus, both the metronome beat and stimulating music can increase muscle tone and co-activation that may be helpful in earlier stages of recovery from flaccid paralysis. Non-musical and non-rhythmic auditory stimulation, as in our happy sounds condition, does not produce this effect. Furthermore the type of music, whether stimulating or relaxing, can modulate the extent of arousal and may produce a different effect on muscle tone.

However, only auditory stimulation with a metronome beat in conjunction with bimanual training led to increased unimanual wrist extension, while that with self-selected music and happy sounds did not. Even rhythms have been shown to reduce the variability in EMG responses, whereas uneven rhythms increase the variability in healthy individuals (65). Patients with stroke show disordered motor unit recruitment on EMG (69–72), but training to even metronome beats has been shown to decrease EMG variability (73) and improve motor outcomes post-stroke (23, 25, 27, 73–75). More efficient motor unit recruitment and sensorimotor synchronization (28) to the even metronome beat can explain the increased wrist extension without a notable increase in extensor activation as seen in our subjects with spastic paresis. In contrast, the variable rhythms in music and subtle differences in the type of music chosen, the tempo of the song and its match to the individual's physical abilities may have influenced attention to the rhythmic component of music leading to a reduced peripheral synchronizing effect on wrist extension.

In healthy individuals, sensorimotor coupling to temporally structured auditory input has been shown to recruit a striato-thalamo-cortical-system involving basal ganglia, thalamus, premotor cortex (PMC), supplementary motor area (SMA), and dorsolateral prefrontal cortex [see Ref. (76) for review]. Simultaneous bimanual rhythmic movements involve interhemispheric coupling primarily in the PMC, posterior parietal cortex, and cerebellum (77), and switching from simultaneous bimanual synchronized movements to unimanual movements leads to a higher degree of interhemispheric connectivity involving the PMC, SMA, and sensorimotor areas (78). Furthermore, studying acallosal patients has shown that temporal coupling during rhythmic movements arises in large part from interactions between the two hemispheres (79). Taken together with these data, our results suggest that bimanual-to-unimanual movements synchronized to rhythmic auditory stimulation excites a bilateral distributed sensorimotor network, which may facilitate the progression of motor recovery in individuals with spastic paresis.

AUDITORY STIMULATION DOES NOT IMPROVE INHIBITORY CONTROL IN INDIVIDUALS WITH SPASTIC CO-CONTRACTION

When the threshold for reflex activity continues to reduce due to progressive re-organization of the supraspinal descending drive

to the spinal cord, peripheral structures of the muscle, muscle spindles, and fascia are further shortened and spasticity evolves into stretch-sensitive forms such as spastic co-contraction (63). Spastic co-contraction refers to inappropriate antagonist recruitment triggered by volitional command (64). Clinically, spastic co-contraction opposes voluntary movement and contributes to impairment in active function, which was seen clearly in our subjects in this group where attempted wrist extension produced paradoxical wrist flexion. While some degree of co-activation between the agonist and antagonist muscles is normal during movement and necessary for joint stability, better movement accuracy and energy efficiency during functional activities, it has been shown to decrease with skill training (80–82). However, its persistence post-stroke signals disrupted reciprocal inhibition of antagonist muscles (83). Sensory feedback from muscle afferents mediates reciprocal inhibition through both spinal and cortical mechanisms (84, 85). Cortical suppression of the antagonist muscle is initiated centrally during preparation of agonist muscle contraction (86, 87) and the degree of suppression is proportional to the amplitude of stretch of the muscle (88).

Bilateral synchronous mirror symmetric flexion-extension movements have been shown to modulate cortical inhibition in neurologically intact individuals (89) and subjects with stroke (30). Somatosensory and visual information from each side of the body is processed bilaterally (90–92), and interlimb coordination is mediated by motor representations in the parietal and premotor areas shared by both limbs (93). Transcallosal pathways between homotopic regions of the two hemispheres (94–96) may also facilitate transmission of accurate sensory information from the intact hemisphere (33). Passive wrist extension on the affected side (which was facilitated by linked movements with the unaffected hand in this study) in severely impaired patients has been shown to produce fMRI changes in contralesional secondary sensorimotor areas in the ventral premotor and parietal cortices (97), which play a crucial role in re-organization of motor output. Thus, in patients with spastic co-contraction, bimanual training *without* auditory stimulation may restore sensory feedback, and reinstate reciprocal control in the paretic hand, aiding progression to the next stage of post-stroke recovery. In contrast, self-selected music may have continued to potentiate the stretch reflex through facilitation of descending spinal pathways in this group as discussed above.

INDIVIDUALS WITH MINIMAL PARESIS SHOW VARIED RESPONSES TO AUDITORY STIMULATION

In subjects with minimal paresis, there was little change in wrist extension across the auditory conditions perhaps due to a ceiling effect. Later stages of recovery have been shown to be mediated by re-organization in the ipsilesional cortex (16–18). Thus, it is not surprising that subjects in this group, who were farther along in their recovery, did not benefit substantially from either bimanual-to-unimanual training or auditory stimulation at the wrist. These strategies would perhaps still be applicable for training of hand and finger control. Subjects with minimal paresis no longer had significant spasticity or co-contraction, but were clearly still impaired compared to the unaffected side. The challenge in these subjects is fine-tuning of muscular control and restoration of dexterity, which may require different types of task constraint.

CONCLUSION

This was a single-session study where bimanual-to-unimanual training of the paretic side was focused on improvement in performance and learning of a wrist extension task, as restoration of control at the wrist is especially challenging after stroke and necessary for hand function. The main purpose and novelty of this study is to show that auditory stimulation interacts with stage of recovery post-stroke to influence motor learning on a bimanual-to-unimanual wrist extension task. Several important conclusions may be drawn from this study. First, subjects in the chronic post-stroke period can be stratified based on simple movement kinematics to reflect their temporal stage of recovery, which may not be reflected by the time since stroke, and which in turn can inform the selection of strategies to drive subsequent progression of recovery post-stroke. Our data predict that during natural progression of post-stroke recovery, there could be a dip in movement ability due to increased co-contraction and then an increase in movement ability when co-contraction is inhibited. Second, our results show how different auditory constraints influence motor performance at various stages of recovery, perhaps through excitation and inhibition of distinct neural substrates. The effects of auditory constraints on muscle activation patterns provide insight into the mechanisms of transition across impairment levels, contributing to the understanding of how re-organization of CNS pathways may occur. Third, bimanual-to-unimanual learning can be a useful model to probe the rate of learning during single-session studies, providing an alternative to or a stratification tool prior to lengthy and expensive randomized control trials. We have recently found that long-term training locks-in the transient improvement seen during single-session bimanual-to-unimanual training (Preeti Raghavan, unpublished data). Together, the results lay the foundation for personalized protocols for post-stroke rehabilitation to advance the progression of recovery from one stage to the next, and hold significant implications for further research and clinical practice. Future work may confirm the effect of auditory constraints seen in our study on longitudinal progression of motor recovery in patients at different stages of recovery.

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