Research paper

Visual-motor functional connectivity in preschool children emerges after handwriting experience

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ABSTRACT

Handwriting letters has been shown to increase Blood Oxygen Level Dependent (BOLD) signal during letter perception in visual and motor brain regions relative to other types of training in preschool children. However, co-activation in these regions speaks neither to the presence of functional connections between them nor to the experiences by which such connections might be established. We investigated functional connectivity by applying generalized psychophysiological interactions analysis to BOLD data obtained from 4 to 6 year-old children after learning symbols through handwriting, tracing, or typing. Functional connections between (1) visual and parietal regions increased after all training conditions, (2) visual and ventral frontal regions increased after handwriting training with letters more than shapes, and (3) visual and dorsal frontal motor regions increased more after handwriting than typing letters. We conclude that visual-motor training creates functional connections among visual and motor brain regions that reflect different aspects of the handwriting experience.

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1. Introduction

One of the earliest emergent literacy skills important for subsequent literacy development is letter knowledge – visual letter recognition and translating written letterforms to their corresponding phonology [1]. The ability to identify letters visually in preschool is the single highest predictor of short- and long-term literacy success [2]. For this reason, learning to identify letters should be an important learning goal of preschool curricula. Additionally, several behavioral studies have shown that writing letters by hand in preschool significantly improves letter identification ability, both immediately and in subsequent years [3–11]. However, letter learning activities typically amount to less than 20 min of each day in preschools – only one minute of which is spent writing letters by hand [12].

Perhaps one reason why there is such little emphasis on handwriting in preschool is due to the lack of controlled experimental research demonstrating its efficacy in improving pre-literacy skills. For instance, one shortcoming of the behavioral research to date is that it often does not experimentally test the effects of handwriting on letter knowledge skills, but rather correlates early handwriting practice with letter knowledge skills and later literacy gains. To our knowledge, there are at least two exceptions. One study experimentally compared the efficacy of handwriting vs. typing practice on letter identification. The results of this study demonstrated that in older preschool children, handwriting letters facilitated letter recognition more than typing [6]. Another study in preschool children with a similar training period found that handwriting letters positively affected recognition at the word level more than typing [10]. Although both studies suggest that handwriting letters is particularly supportive of pre-literacy skill development, letter learning activities in preschool are increasingly relying upon digital learning devices – further reducing the amount of time spent writing letters by hand. As the prevalence of digital learning devices in preschool increases, the need to understand what it is about handwriting that is so effective as a letter learning activity is becoming increasingly important.

Controlled experimental research on the effects of handwriting on letter knowledge in preschool faces several shortcomings. First, it is difficult to control the amount of handwriting practice children receive outside of the laboratory or school setting. Second, controlled experiments performed in laboratory settings typically

suffer from somewhat low sample sizes and their sample populations are often biased towards middle- and upper-income households. In contrast, experiments performed in school settings often suffer from a lack of rigorous control. Third, preschool children often experience frustration when performing tasks that tap into pre-literacy skills, which often leads to boredom, random responses, and, ultimately, unreliable results. Thus, tasks must be designed that are age appropriate and enjoyable, which often leads to a lack of stimulus control (i.e., comparing letters to other stimuli that are less familiar, less complex, and/or less salient). A final shortcoming is the reliance of behavioral methods on measuring performance, as opposed to assessment of underlying mechanism. In other words, even if behavioral responses are the same for two groups of children at one point in time, they may be reaching this performance in different ways and, furthermore, this difference may be crucial for the development of later, more complex skills.

To circumvent some of these shortcomings in measuring overt behavior in preschool children, we can use brain activation as a dependent measure to assess the effects of experimental manipulations. Brain activation measurements from functional Magnetic Resonance Imaging (fMRI) provide us with important information regarding the mechanisms that underlie certain behaviors, thereby unveiling how and sometimes why behaviors happen. We have been performing controlled experimental research using functional neuroimaging of brain activation to better understand the mechanisms that underlie pre-literacy skills and how these might be affected by handwriting experience [13–15]. Through this method, we are able to bypass the difficulties inherent to measuring overt behavioral responses in experimental contexts with child participants.

Studying the development of literacy skills by studying pre-literacy in young children is one approach to understanding how and, perhaps, why handwriting experience influences performance on letter knowledge tasks. However, using fMRI to study pre-literacy development in young children also comes with some shortcomings. For example, the activation measurements are sensitive to movement and young children are notoriously prone to movement. Consequently, many fMRI studies on letter processing have focused on understanding how the literate adult brain supports letter processing. Studying the adult state can provide valuable information. For instance, it can provide a snapshot of the 'end point' of a developmental trajectory. However, knowing how the adult brain processes letters provides little to no information about how the establishment of those mechanisms was achieved, a crucial step in truly understanding what it is about handwriting during pre-literacy that is so beneficial for letter learning and later literacy gains. Nonetheless, to provide the 'end point' of the mechanisms supporting literacy, we first outline the research to date on letter perception and production in adults, before turning to a summary of developmental work.

An abundance of research has shown that the neural response associated with efficient letter recognition abilities in literate adults is characterized by heightened activation in the ventral-temporal cortex during letter perception, particularly in the anterior fusiform gyri [13–24]. As part of the Lateral Occipital Complex [25], the ventral-temporal cortex is comprised of regions that respond to a differential degree during the visual presentation of some objects relative to others. Both left and right fusiform gyri are well-documented regions within the Lateral Occipital Cortex that increase their responses during visual presentation of letters relative to other written forms. An anterior portion of the left fusiform gyrus (L FuG), in particular, responds more during letter perception than shape perception in the literate adult [13,16–24]. Further, recent work has demonstrated that the activation of this region in literate adults can be influenced by handwriting experience [15,23]. On the other hand, activation in the anterior right FuG during visual perception can be influenced by any experience with any symbol [14,22]. Taken together, the left and right anterior FuG respond more to symbols than other types of forms with the left FuG responding more to letters and being affected by handwriting experience in the adult.

However, the stereotypical literate adult brain response during letter perception also includes a more extended set of regions than originally thought, including not only the left and right FuG visual regions, but also spanning the frontal motor and premotor regions as well as parietal regions [21,26]. There is some evidence that one reason that both visual and motor regions respond during letter perception is that early letter learning is typically supported through visual-motor practice, such as handwriting or tracing. The practice of producing letters motorically continues beyond this initial stage such that literate adults typically acquire a lengthy history of visual-motor experience with letters. For instance, James and Gauthier [21] showed that the set of regions activated during letter perception is very similar to the set that is activated during letter writing. When adults wrote letters without seeing them, they found activation in the L FuG, the left dorsal precentral gyrus (L dPrG), the left inferior frontal gyrus (L IFG), the left middle frontal gyrus (L MFG), and the left cuneus [21]. Importantly, when the same adults simply viewed letters without writing, they found activation in the same regions – that is, both perceiving and producing letters recruits both visual and motor systems. Longcamp et al. [27] found similar regions when adults printed letters with visual feedback. Furthermore, the laterality of the frontal motor and premotor activations during letter perception is affected by handedness and, therefore, it has been suggested that this response may be best thought of as reactivation of motor regions associated with the experience of handwriting letters [28]. In addition, adults who were trained on novel pseudoletters (forms constructed from the features of letters that are re-arranged) through handwriting, typing, or visual-only training showed a similar effect after two relatively brief training sessions: only handwriting training resulted in a difference between trained and untrained forms in the L FuG and in the L dPrG [22]. In this study, participants learned pseudoletters through twelve exposures at each training session, for a total of twenty-four exposures over the course of two days. The results showed that both visual and motor brain regions responded to the pseudoletters trained through handwriting significantly more than they did pre-training and this difference was greater than for pseudoletters trained through typing or visual-only [22]. Interestingly, a similar effect was found in the R FuG for pseudoletters learned through typing: only typing training resulted in a difference between trained and untrained forms in the R FuG and, although typing training did not increase the L dPrG response more than handwriting training, it did increase the response more than visual-only training [22]. These studies demonstrate that both visual and motor brain regions respond preferentially to visually presented letterforms that are learned through motor production and that this effect can occur after considerably brief experiences with letters.

Similarly, both visual and motor regions begin to increase their response when presented with trained letterforms when compared to other untrained stimuli, such as shapes, in preliterate children after considerably brief experiences with letters [13–15]. However, this change in response only occurs after children learn letters through handwriting (printing) practice, and does not occur after visual-auditory, typing or tracing practice. In one such study, the L FuG response to letters increased after handwriting training with each letter once per week for four weeks and its post-training response for letters was greater than for untrained stimuli [13]. The same amount of training did not evoke such neural changes when it involved seeing and hearing the letter names instead of

printing, Interestingly, neural changes after handwriting training emerged in the absence of significant behavioral changes in letter knowledge from pre- to post-scan, although there was a trend toward a training by scan day interaction [13]. Another study with a larger sample found significant behavioral changes after a three week training paradigm in which each letter was practiced only once per training session [6]. Finally, Kersey and James [15] found differences in the neural response to letterforms trained through handwriting after only one training session in which each form was practiced ten times each. Another study found similar training effects after one training session in which each form was practiced eight times each [14].

Although the L FuG response to letters in visual cortex may be found early and achieved through visual practice alone [29,30], the response in both visual and motor brain regions in preliterate children has consistently been found to be greater for letters learned through handwriting [13–15]. Increases in L FuG activity in preliterate children for letters is greater for letters learned through handwriting than for letters learned through tracing [14], typing [14], visual-only practice [13], or passively watching an experimenter write letters by hand [15], indicating that active motor engagement with a letter increases the L FuG response during letter perception. Furthermore, handwriting practice not only increases the response of the L FuG in preliterate children during letter perception relative to visual practice alone [13], it also increases the response of the L dPrG and left intraparietal sulcus (L IPS) relative to tracing practice [14]. Similarly, the L IFG response is heightened for letters trained through either handwriting or tracing relative to letters trained through typing [14]. That is, there is mounting evidence from developmental neuroimaging studies that suggests that the response of both visual and motor brain regions during passive visual perception may become more adult-like through closed-loop visual-motor movements – movements that produce real time visual feedback – such as handwriting.

To date, we know that the literate adult recruits a set of visual and motor brain regions during letter perception and production that overlap substantially and that these regions begin to respond in a letter-sensitive manner after handwriting experience in the preliterate child. Additionally, we know that the fusiform gyri, particularly the L FuG, appear to be crucial for letter processing, but that they may also be used to process other learned symbols after handwriting experience [22]. Thus, these putative visual regions appear to be highly affected by visual-motor experience. We propose that these effects are driven by the emergence of a functional network comprised of the visual and motor systems that are used during handwriting and that this network is functionally active during subsequent letter perception. Visual-motor training activities in which motor movements are more closely tied to their perceptual output, as in the case of handwriting letters, may facilitate the emergence of visual-motor communication pathways more than other visual-motor training activities, such as typing. Writing by hand requires the formation of the letter shape using fine-motor skills and visual guidance, whereas typing requires a single button press to produce the complete desired form. With this in mind, it might be expected that the response of the motor system will be stronger for letterforms with which the observer has had stimulus specific visual-motor training experience. Indeed, neural responses in L dPrG have been shown to be more closely associated with the perception of handwritten letterforms than typed letterforms in studies using TMS [31,32] and fMRI [26,33]. We suspect that these putative functional connections between visual regions and parietal and frontal motor regions become evident early in pre-literacy development as a function of handwriting experience.

The logic is as follows: If handwriting experience serves to create functional communication pathways among visual and motor brain regions that come to support letter perception, then functional connectivity among these areas will be evident in pre-literate children during letter perception, but only after handwriting practice. Furthermore, this effect will be strongest for letters when compared to other symbols. This pattern of results would indicate that handwriting practice with letters couples visual and motor brain regions and that these regions work together to support the subsequent perception of those letters. Alternatively, if there is no evidence of functional connectivity among these regions during letter perception, then the recruitment of both visual and motor regions during letter perception reflects co-activation due to chance, or experimental variables such as increased attention, heart rate, or responses to the physical environment of MRI. That is, to demonstrate that regions that activate in response to the same task are indeed functionally connected, one must be cautious in the type of analysis used and the subsequent interpretation of results. A robust method that provides information about task-based correlated functioning between regions that is present in addition to mere task-based co-activation is generalized psychophysiological interactions analysis [34], or gPPI, and therefore, we used this method as described below.

Our goal in the present work, was to take the first step in directly addressing the issue of functional connectivity among visual and motor brain regions during letter perception in the young preliterate child. To this end, we test five specific hypotheses: First, overall, visual-motor training, in general (handwriting, tracing, typing), will result in greater functional connectivity between the fusiform gyri in ventral temporal cortex and widespread motor regions, including parietal and left frontal motor regions. Second, visual-motor training, in general, will result in different functional connectivity patterns for letters than shapes. Third, different training conditions with letters will result in different functional connectivity patterns. Fourth, functional connections between visual and frontal motor regions after handwriting training specifically will reflect the stimulus category: handwriting letters will result in different functional connectivity patterns than drawing shapes. Fifth, functional connections between visual and frontal motor regions with the stimulus category of letters specifically will reflect the method of visual-motor training: handwriting training will result in greater functional connectivity among visual and frontal motor regions than tracing and typing.

2. Materials and methods

All analyses were performed on a pre-existing data set that will be described here briefly and is presented in more detail in James and Engelhardt [14].

2.1. Participants

Twenty pre-literate 5-year-old children (8 females; ages 4:2 to 5:0) participated in this study; however, five children voluntarily withdrew before entering into the actual MRI environment, leaving the final sample size at 15 children. Per parental reports, all children were right-handed, preliterate, native English speakers with normal vision and normal neurological histories, free of any neurological trauma, developmental disorders, and ongoing medication. In the gPPI analyses, data from five additional participants could not be used, resulting in data from 11 (6 females; ages 4:2 to 5:0) participants used in the present analysis.

2.2. Procedure

Participants were trained on different sets of letters and shapes

Fig. 1. An example of the experimental conditions. Each child was exposed to different sets of letters and shapes through different sensorimotor training activities. An additional group of non-trained letters and non-trained shapes were also presented to them during the fMRI scanning session. For a particular child, a particular letter or shape appeared in only once of the four conditions.

through different types of visual-motor activity and then passively perceived those letters and shapes in a blocked design during fMRI scanning. The training, neuroimaging, and post-neuroimaging assessments all took place on the same day as part of the same visit.

2.2.1. Training

All children received the same three training conditions with letters and shapes. Children were asked to draw 4 letters and 4 shapes, 8 times each. The same children were then asked to trace a different set of 4 letters and 4 shapes, 8 times each. Finally, the children were asked to type a third set of 4 letters and 4 shapes, 8 times each (Fig. 1). Letters included were 16 upper-case manuscript letters, presented in simple font style: B, F, G, A, Y, U, D, T, S, W, P, L, C, H, R, and K.

Four letters were pseudo-randomly selected and distributed across three training conditions and one untrained condition. Shapes included flower, crescent, circle, parallelogram, leaf, rectangle, semicircle, triangle, star, raindrop, arrow, prism, clover, cylinder, pentagon, and 'snowman'. The experimenter did not name any of the letters or shapes during any of the training conditions.

2.2.2. Neuroimaging

After training, all children were first familiarized with the MRI environment by watching a cartoon in a full-sized mock scanner. Children then advanced to the actual MRI environment if both parent and child indicated that they were comfortable in doing so.

2.2.2.1. Stimulation protocol.
All children underwent fMRI scanning while viewing the six different sets of trained stimuli and two additional sets of 4 letters and 4 shapes on which they were not trained (Fig. 1). The children passively viewed these stimuli in a block design with each of the resulting 8 conditions being presented once per run. Each of the 8 blocks consisted of 16 stimuli from one of the conditions in a randomized order; thus, each stimulus was repeated 4 times within a block. Each stimulus was presented for 1 s with 0.5 s between stimulus presentations, resulting in 24 s blocks. Blocks were separated by a 10 s interval during which only a fixation cross was in view. The same fixation cross was also visible for 20 s at the beginning and end of each run. Each run was 4 min and 55 s long and 1–3 runs were collected per

child depending upon their comfort level.

2.2.2.2. Scanning parameters.
Neuroimaging was performed at the Indiana University Imaging Research Facility, housed within the Department of Psychological and Brain Sciences with a Siemens Magnetom TIM TRIO 3-T whole-body MRI system. A 12-channel phased array head coil was used. Children viewed the stimuli through a mirror in the bore of the scanner onto which a Mitsubishi XL30 projector displayed the stimuli. High-resolution T1-weighted anatomical volumes were acquired first using a Turbo-flash 3-D sequence: TI=1100 ms, TE=2.86 ms, TR=1700 ms, flip angle=12°, with 120 sagittal slices of 1.5 mm thickness and 0% slice gap, a field of view of 240×240 mm, and an isometric voxel size of 1.5 mm^3. For functional images, the field of view was 192×192 mm and 33 axial slices of 4.0 mm thickness were acquired with an in-plane matrix of 64×64 mm and 0% slice gap, producing a voxel size of $3.0 \times 3.0 \times 4.0$ mm. Functional images were acquired using a gradient echo EPI sequence with interleaved slice order: TE=30 ms, TR=2000 ms, flip angle=90° for blood-oxygen-level-dependent (BOLD) imaging.

2.2.3. Post-neuroimaging assessment

After scanning, children were tested to ensure their ability to identify the letters and shapes. Children also completed Phonemic Awareness, Letter Identification, and Visual Word Discrimination subtests of the Bader Reading and Language Inventory [35] along with the Beery-Buktenica Developmental Test of Visual-motor Integration [36] to ensure equal pre-literacy among participants and characterize visual-motor skills, respectively.

2.3. Analyses

2.3.1. Functional connectivity analysis

To assess functional connectivity, we employed a generalized psychophysiological interactions (gPPI) analysis [34]. To perform a gPPI analysis, a design matrix is created that contains standard task predictors (psychological predictors), a predictor that is the activity of the seed region (physiological predictor), and predictors for the interactions of task and seed (gPPI predictors). These predictors are entered into a mass univariate GLM model and applied to the functional data. One model is constructed for each seed

region of interest. Therefore, one psychological predictor is entered for each task, one physiological predictor is entered for the seed, and one gPPI predictor is entered for each task. The psychological and physiological predictors are treated as predictors of no interest; their role as covariates is to account for changes in the BOLD signal that are purely driven by task or correlations between voxels that are purely driven by physiological noise. This is the key to the utility of the gPPI method to infer functional connectivity from brain activation measurements.

Each predictor type plays a very important role in the model. A psychological predictor exists for each task and these predictors absorb variance that is best explained by each task, which is similar to a standard GLM used to detect task-based activation. The physiological predictor, then, will absorb variance that is best explained by the activity of the seed region and, therefore, effectively represents the strength of correlation with the seed. However, the beta-weight assigned to the physiological predictor will correspond to the strength of the correlation with the seed that is non-task-specific, because the main task-based activation is already accounted for by the psychological predictors. Although important, the psychological and physiological predictors are essentially predictors of no interest. What is interpreted are the beta-weights assigned to each gPPI predictor, which, due to the presence of the psychological and physiological predictors in the model, represents the strength of the correlation with the seed that is task-specific. In other words, the gPPI predictors will receive beta-weights that are reflective of the amount of variance that can be explained by the task and correlated activity with the seed region – a task-based correlation with the seed. Thus, with the main effects of external stimulation (task-based activation) and physiology (seed region activity) regressed out of the functional data, the gPPI predictors provide a clear picture of the task-based correlation between the seed region and all other voxels that exists above and beyond what would be expected from the task and non-task-specific physiological influences alone..

2.3.1.1. Preprocessing. All preprocessing steps were performed in BrainVoyager QX™ version 2.8.0. Preprocessing of functional data included slice scan time correction, 3-D motion correction using trilinear/sinc interpolation, and 3D Gaussian spatial blurring with a full-width-at-half-maximum of 6 mm. Temporal high-pass filtering was done using a voxel-wise GLM with predictors that include a Fourier basis set with a cut-off value of 2 sine/cosine pairs, which included a linear trend predictor. Individual anatomical volumes were normalized to Talairach space[37]. During normalization, functional data were re-sampled to an isometric voxel size of 3.0 mm³. Coregistration of functional volumes to anatomical volumes was performed using a rigid body transformation.

2.3.1.2. Motion correction. Measures of functional connectivity have been shown to be sensitive to head motion, being particularly problematic for between-groups analyses [38–42]. Therefore, several additional steps were taken in the present analysis to properly correct for motion. As a first step, we visually inspected the rigid body motion estimates for each run with the intention of discarding runs with extreme motion; however, no runs included in the gPPI analysis reached this criteria and no runs were discarded. Second, local effects of head motion typically result in large and immediate signal magnitude changes, or spikes, that are qualitatively different than the slower BOLD signal response and temporally coupled to the head movement itself [43]. If any one of the six rigid body motion parameters indicated that head movement exceeded 5 mm, the influence of those volumes was corrected for by using 'soft removal'. Problematic volumes were soft-removed by way of setting the predictor value for that time point in the design matrix to zero, as opposed to the addition of a

regressor into the design matrix with the predictor value of that time point set to one. This has been referred to as 'soft' removal of motion spikes [44] and was preferred to a hard removal of motion spikes through spike regression due to evidence that the spike regression technique may systematically bias results, especially in populations in which head motion is generally high, because it results in the removal of a substantial amount of data, thereby decreasing the degrees of freedom for some subjects relative to others [45,46]. Of the 1674 vol. used for gPPI analysis, only 8 vol. were corrected, with no more than 2 vol. corrected in any particular run and with volume corrections occurring in different conditions for each of the 4 subjects affected. Finally, the rigid body transformation parameters were included in the gPPI design matrix as predictors of no interest [47,48].

Inclusion of the rigid body motion parameters is an effective way of regressing out variance due to head motion and it is often accompanied by the inclusion of second- and third-derivatives to account for the temporally delayed magnetic susceptibility effects from the head motion [38,41,49,50]. However, because gPPI models already suffer from problems of co-linearity due to the high number of predictors [34], we opted to not include second- or third-order derivatives.

2.3.1.3. Seed selection for gPPI analysis. Because our primary interest was to assess functional connectivity among visual and motor regions during letter processing, we functionally localized peak regions in each individual that responded more to letters than to a rest condition. This contrast, $((dLet+trLet+tyLet) > 3fixation)$ at $p_{vox} < 0.05$, uncorrected (Table 1; Fig. 2) resulted in activation in an anterior location within the L FuG and R FuG in 11 of the 15 participants, resulting in a total of 29 runs used in the present analyses. Runs used to functionally localize seeds were discarded from further analyses, leaving 18 runs for gPPI analysis. Discarding runs used to localize seeds may not have been necessary; however, we know of no published research demonstrating that reusing these runs is an acceptable practice for gPPI analyses. Therefore, we elected to err on the side of caution, and ran the gPPI analysis using only the runs that were not used to localize the seed regions.

2.3.1.4. gPPI analysis. Separate gPPI analyses were performed for each seed region and within each individual. Time courses were extracted from each individual seed region and were demeaned, creating unique physiological predictors for the L FuG and R FuG seeds for each individual run. The gPPI predictors were created through an element-wise multiplication between each psychological predictor, one for each task, and a particular physiological predictor, creating unique gPPI predictors for each individual run. Performing gPPI at the individual level resulted in subject-specific voxel-wise beta-weights that corresponded to the strength of the correlation between the signal from that voxel and each of the unique psychological, physiological, and interaction predictors.

We then performed a series of whole brain contrasts at the group level targeted at determining differential functional connections associated with visual-motor training with letters. First, to test for effects of training overall, trained stimuli were compared to untrained stimuli. Second, to assess the effects of stimulus type within training, all trained letter conditions were contrasted against all trained shape conditions. Third, we performed contrasts to characterize the effects of the different training conditions within letters by contrasting each condition to untrained letters. We followed this contrast with a conjunction analysis to determine the degree to which the different training conditions influenced the same functional connections. Fourth, to assess the potential of stimulus-specific effects from self-production (handwriting and drawing) we compared letters trained through handwriting to shapes trained through drawing. Finally,

Table 1
Specifications of individual subject seed regions.

Seed	Subject	Cluster size (voxels)	Center (x, y, z)	Min x	Max x	Min y	Max y	Min z	Max z
L FuG	AB	596	(−36, −65, −13)	−41	−32	−68	−60	−18	−9
	BD	573	(−35, −41, −13)	−40	−31	−46	−37	−17	−9
	DS	220	(−26, −40, −12)	−30	−23	−44	−37	−15	−8
	EB	433	(−31, −55, −4)	−37	−28	−59	−51	−9	0
	HM	256	(−38, −44, −17)	−43	−34	−47	−38	−22	−14
	KJ	402	(−39, −42, −17)	−43	−34	−47	−38	−21	−12
	MM	139	(−42, −32, −17)	−46	−37	−33	−29	−19	−13
	PM	382	(−38, −31, −18)	−43	−34	−36	−28	−22	−15
	SS	145	(−45, −47, −9)	−49	−41	−50	−43	−11	−6
	TB	659	(−35, −55, −11)	−40	−31	−60	−51	−14	−6
	TM	849	(−34, −43, −11)	−39	−30	−48	−39	−16	−7
R FuG	AB	246	(30, −40, −6)	26	35	−43	−34	−12	−3
	BD	591	(32, −38, −11)	26	35	−43	−34	−15	−6
	DS	211	(27, −35, −18)	24	32	−39	−32	−20	−15
	EB	319	(26, −57, −9)	22	31	−59	−50	−12	−6
	HM	733	(36, −37, −15)	32	40	−41	−32	−19	−10
	KJ	655	(33, −41, −17)	28	37	−45	−36	−22	-13
	MM	105	(31, −39, −16)	26	32	-43	-34	-20	-12
	PM	382	(26, −45, −15)	21	30	-49	-40	-18	-11
	SS	341	(41, −40, −8)	37	45	-44	-35	-11	-6
	TB	946	(26, −50, −8)	22	31	-55	-46	-13	-4
	TM	761	(33, −38, −12)	29	38	-43	-34	-17	-8

All selected seed regions were significant at $p_{vox} < 0.05$, uncorrected.
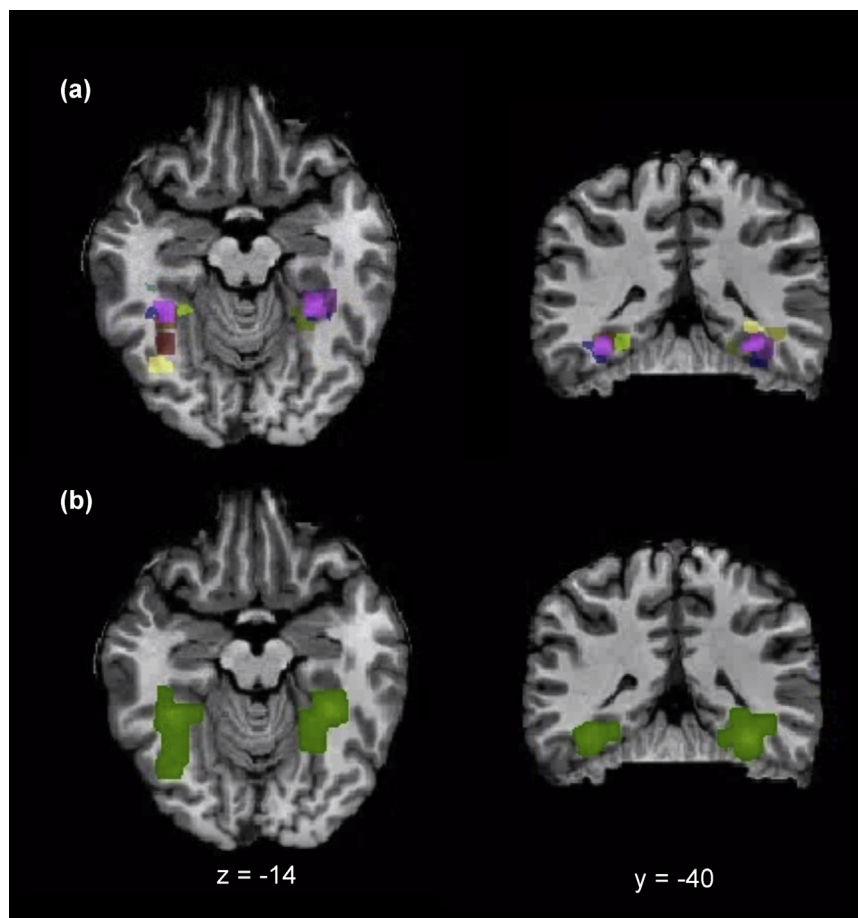
Fig. 2. Localization of individual seed regions for gPPI analysis. (a) Individual seeds localized to the left and right fusiform gyri by contrasting all trained letter conditions to a fixation baseline. Each subject is represented by a different color. (b) Probability map showing the spatial consistency of the seed regions across individual subjects on a template brain.

to contrast the different types of visual-motor learning within letters, we compared the training conditions to one another. Resulting statistical maps were subjected to cluster thresholding at the $p_{voxel} < 0.05$, $p_{cluster} < 0.05$ significance levels through Monte Carlo simulations iterated 1000 times [51]. These cluster corrected statistical maps were used as component maps in the conjunction analysis (Table 2).

Table 2
Specifications of regions functionally connected to the L FuG and R FuG by type of contrast.

Seed	Contrast	Cluster size (voxels)	Peak T	Peak (x,y,z)	Min x	Max x	Min y	Max y	Min z	Max z
L FuG	(dLet+dSh+trLet+trSh+tyLet+tySh) > 3(cLet+cSh)	10,988	3.38	(32, −41, 15)	-10	65	-54	-25	1	67
	(dLet+trLet+tyLet) > 3cLet	15,226	4.24	(−43,−75,−23)	-56	11	-88	-40	-40	-17
		3985	3.59	(32,−38,9)	13	37	-44	-20	-12	30
		8647	3.37	(41,−35,36)	23	69	-50	-22	13	58
	((dLet+trLet+tyLet) > (dSh+trSh+tySh)) > (3(cLet>cSh))	7529	3.58	(−43,−74,−24)	-53	17	-87	-55	-40	-15
	dLet > cLet	6717	5.81	(17,−23,42)	-35	20	-47	-5	15	47
		6104	4.44	(66,−29,30)	23	66	-59	-19	24	51
	(dLet > cLet) > (dSh > cSh)	–	–	–	–	–	–	–	–	–
	trLet > cLet	3756	5.00	(47, 29,39)	39	66	-37	-17	23	49
	(trLet > cLet) > (trSh > cSh)	6828	4.05	(47,−23,51)	32	66	-44	-17	21	57
	tyLet > cLet	5144	4.86	(47, 44,51)	29	66	-66	-27	29	57
	(tyLet > cLet) > (tySh > cSh)	2906	4.21	(59,−35,35)	40	66	-42	-22	29	52
	dLet > dSh	4797	5.31	(−52,16,9)	-59	-31	4	27	30	-3
	dLet > trLet	–	–	–	–	–	–	–	–	–
	dLet > tyLet	4206	4.36	(−13,−35,48)	-41	-7	-51	-14	29	67
	trLet > tyLet	–	–	–	–	–	–	–	–	–
	(dLet > tyLet) > (dSh > tySh)	–	–	–	–	–	–	–	–	–
	(dLet > cLet)∏(trLet > cLet)∏(tyLet > cLet)	902	–	–	39	66	-36	-24	25	48
R FuG	(dLet+dSh+trLet+trSh+tyLet+tySh) > 3(cLet+cSh)	–	–	–	–	–	–	–	–	–
	(dLet+trLet+tyLet) > 3cLet	–	–	–	–	–	–	–	–	–
	((dLet+trLet+tyLet) > (dSh+trSh+tySh)) > (3(cLet>cSh))	–	–	–	–	–	–	–	–	–
	dLet > cLet	–	–	–	–	–	–	–	–	–
	(dLet > cLet) > (dSh > cSh)	–	–	–	–	–	–	–	–	–
	trLet > cLet	2791	3.92	(32, −47, 48)	13	63	-56	-26	36	57
	(trLet > cLet) > (trSh > cSh)	–	–	–	–	–	–	–	–	–
	tyLet > cLet	–	–	–	–	–	–	–	–	–
	(tyLet > cLet) > (tySh > cSh)	–	–	–	–	–	–	–	–	–
	dLet > dSh	–	–	–	–	–	–	–	–	–
	dLet > trLet	–								
	dLet > tyLet	–	–	–	–	–	–	–	–	–
	trLet > tyLet	–								
	(dLet > tyLet) > (dSh > tySh)	–	–	–	–	–	–	–	–	–

All results are reported at $p_{vox} < 0.05$, $p_{cluster} < 0.05$.

2.3.2. Motion analysis

Functional connectivity studies may be especially susceptible to artifacts produced by head motion, even after standard motion correction techniques are applied. Although functional connectivity estimates from gPPI analyses are considered more robust to motion-related artifacts than standard functional connectivity estimates, for the reasons stated in the methods section, we followed the functional connectivity analysis with a motion analysis to document the amount of motion present in our sample and to ensure that motion had not driven the results of the functional connectivity analysis. We based our motion analysis on the framewise displacement (FD) statistic, which is a measure of the absolute displacement of the head between sequential images [41].

Different variants of the equation used to calculate the FD statistic exist. Yan et al. [45] directly compared each of these equations and determined that the variant proposed by Jenkinson et al. [52] is most consistent, with the variant proposed by Power et al. [41] following closely behind. However, the Jenkinson et al. [52] model requires the assumption that the average distance between the center of the brain and cortex is 80 mm. Given that our sample consists of 5 year old child brains, images of which were normalized to an atlas of an elderly woman [37], we turned to the variant proposed in Power et al. [41]. The Power et al. [41] equation for the FD statistic overestimates the degree of motion, and is not considered to be a conservative estimate of motion [45]. Therefore, we chose to calculate the FD statistic as described in Power et al. [41], because its assumptions are more plausible for our sample and would ensure that our motion analysis would not underestimate the amount of motion present in the sample. Thus,

FD time courses were constructed by summing the absolute value of the difference between time n and time $n-1$ for each of the rigid body motion parameters [41,42]. Summary FD scores were obtained for each subject by averaging FD scores within individual subjects across time to summarize subject-specific motion and for conditions by averaging FD scores within task conditions across time to summarize condition-specific motion.

2.3.2.1. Subject-specific summary FD. Subject-specific summary FD (sFD) scores allowed us to select a sub-sample of individuals with the lowest sFD that included only individuals below the median sFD. We reran the gPPI analysis on these individuals and compared these results to the results obtained in the full sample. If the results held with the low-sFD subsample, then our results were not likely to have been driven by motion artifacts due to the inclusion of high-sFD. Additionally, an ANCOVA was performed to directly compare results from the low-sFD group to those of the high-sFD group for the contrasts of interest. This analysis directly assessed the degree to which the results from the low-sFD group differed from those of the high-sFD group. A difference between low- and high-sFD groups would be evidence that high-sFD subjects drove the effects in the functional connectivity analysis.

2.3.2.2. Condition-specific summary FD. Condition-specific summary FD (cFD) scores were subjected to a One-Way ANOVA to determine if motion was significantly different in one condition compared to another across subjects. A difference between conditions in cFD would be evidence that task-correlated motion drove the effects in the functional connectivity analysis. We had no a priori reason to believe motion would differ across conditions,

because every task required subjects to remain still while passively viewing visual stimuli. However, we wanted to be sure that children were not moving more, by chance during a particular condition than during others.

3. Results

3.1. Functional connectivity analysis

1. **Effects of training (trained vs. untrained stimuli).** First, to ensure that our training had an effect and to quantify that effect, we compared all trained symbols, including letters and shapes, to symbols with which they received no training, also including letters and shapes $(dLet + dSh + trLet + trSh + tyLet + tySh) > (3(cLet + cSh))$ (Fig. 3). Significant functional connectivity between the L FuG and right hemisphere superior parietal lobule (SPL), inferior parietal lobule (IPL), inferior parietal sulcus (IPS) and the posterior cingulate cortex (PCC) was observed to be greater for trained vs. untrained stimuli. There were no areas of greater functional connectivity for this contrast with the R FuG. Therefore, a widespread right parietal system was functionally connected with the L FuG during perception as a result of any type of training, indicating that functional connections may occur after relatively brief visual-motor training.
2. **Stimulus-specific training (trained letters vs. trained shapes).** Second, to assess the effects of training that may have been stimulus specific, we compared connectivity patterns that resulted from comparing trained vs. untrained letters to trained vs. untrained shapes $(((dLet + trLet + tyLet) > (dSh + trSh + tySh)) > (3(cLet > cSh)))$. This analysis showed no difference in connectivity with the seed regions for trained letters vs. trained shapes, indicating that the training effect seen in our first analysis was not specific to letters.
3. **Training effects within letters (each trained letter condition vs. untrained letters).** To better understand the training effects within letters, we then focused on the type of visual-motor training by comparing each of the three methods of visual-motor training to untrained letters (Fig. 4). Contrasting letters

trained through handwriting to untrained letters $(dLet > cLet)$, letters trained through tracing to untrained letters $(trLet > cLet)$, and letters trained through typing to untrained letters $(tyLet > cLet)$, all revealed an increase in functional connections between the L FuG seed and R IPS/SPL with the cluster being nearly exactly the same for each contrast (Fig. 4a). No areas were more functionally connected with the R FuG seed for letters trained through handwriting or through typing compared to untrained letters; however, contrasting letters trained through tracing to untrained letters revealed an increase in functional connections between the R FuG seed and R IPS/SPL and L MFG (Fig. 4b).

We investigated these results further by performing a conjunction analysis on the cluster corrected statistical maps produced by comparing functional connections with the L FuG during the perception of letters trained through handwriting, letters trained through tracing, and letters trained through typing to untrained letters. This analysis revealed that drawing letters, tracing letters, and typing letters all contributed to an increase in functional connections between the L FuG and a relatively restricted region of the anterior R IPS/IPL (Fig. 5).

4. **Stimulus-specificity for handwritten production (handwriting letters vs. drawing shapes).** To compare the effects of self-production as a function of stimulus type, we then directly compared the effects of handwriting letters to drawing shapes $(dLet > dSh)$ (Fig. 5). This contrast revealed heightened functional connectivity between the L FuG seed and a left premotor area, the L IFG, for letters trained through handwriting compared to shapes trained through drawing. No areas were functionally connected with the R FuG seed for this contrast.
5. **Effects of the type of visual-motor training with letters: handwriting vs. tracing and typing.** Our fifth hypothesis was that positive effects in visual-motor functional connectivity from handwriting practice with letters would display some specificity to the visual-motor training method Fig. 6. To test this hypothesis, we compared the three conditions of visual-motor training to one another within letters. First, we compared letters trained through handwriting to letters trained through tracing $(dLet > trLet)$. Then, we compared letters trained through tracing to letters trained through typing $(trLet > tyLet)$.
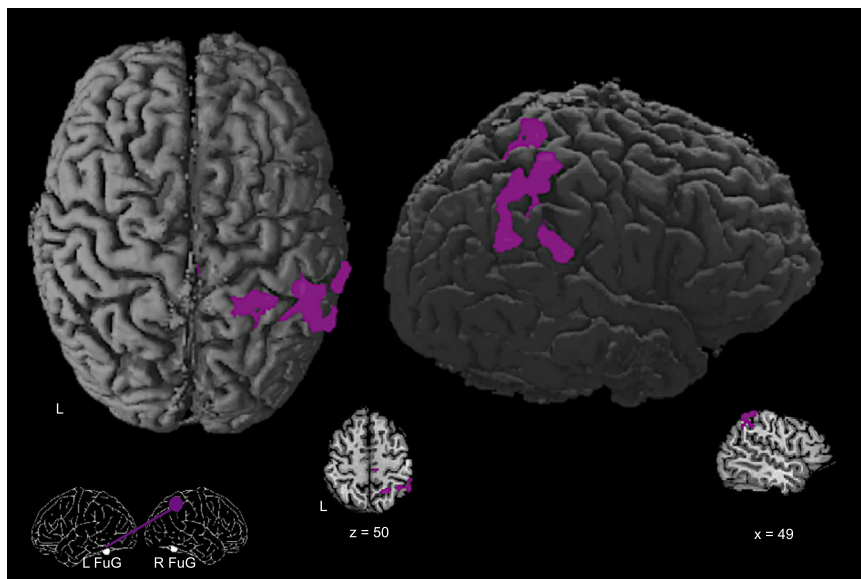

Fig. 3. Effects of sensorimotor training on symbols. Functional connections between the L FuG and a right parietal region (purple), including the R SPL, R IPS, and R IPL, for the perception of trained letters and shapes compared to untrained letters and shapes. Talairach coordinates are provided. Sagittal view is of the right hemisphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
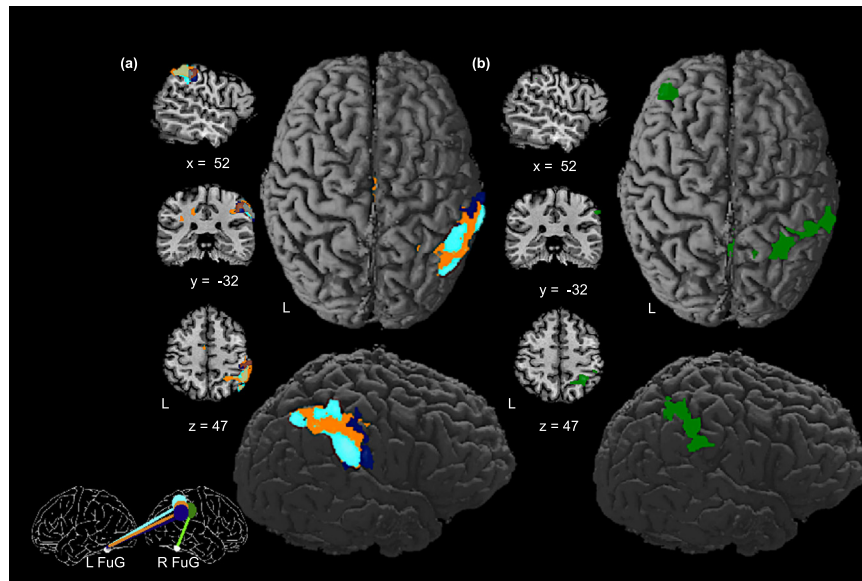
Fig. 4. Effects of sensorimotor training with letters. (a) Functional connections between the L FuG and a right parietal region, including the R SPL, R IPS, and R IPL, for the perception of letters trained through handwriting (orange), tracing (dark blue), and typing (cyan) compared to untrained letters. (b) Functional connections between the R FuG and a right parietal region and L MFG for the perception of letters trained through tracing (green) compared to untrained letters. Talairach coordinates are provided. Sagittal view is of the right hemisphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)


Fig. 5. Conjunction analysis of contrasts presented in Fig. 4a. Functional connections between the L FuG and anterior R IPS/IPL (turquoise) were stronger during the perception of letters trained through drawing and letters trained through tracing and letters trained through typing than untrained letters. Talairach coordinates are provided. Sagittal view is of the right hemisphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
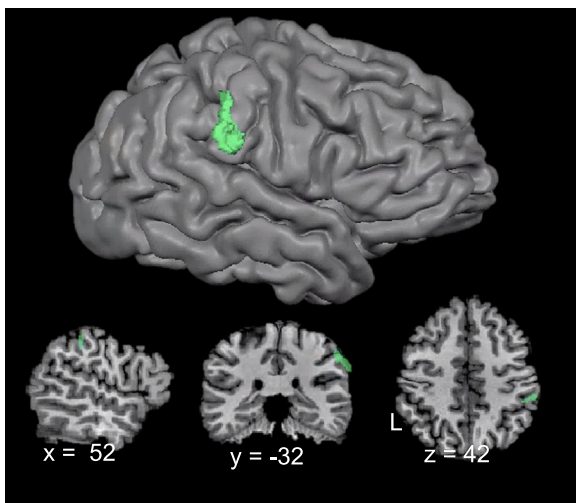
Finding that no areas were more functionally connected with either the L FuG or the R FuG for either contrast, we then compared letters trained through handwriting to letters trained through typing (dLet > tyLet). This contrast revealed an increase in functional connections for letters trained through handwriting between the L FuG seed and a left dorsal sensorimotor area, encompassing L dPrG, L dPoG, and the area of the central sulcus between them. No areas were more functionally connected with the R FuG seed for these contrasts. No significant functional connections remained after controlling this contrast for drawing practice with shapes ((dLet > tyLet) > (dSh > tySh)) for either the L FuG or R FuG seeds. Therefore, the effects seen above for

the comparison of handwriting vs. typing may not be letter specific; rather, they appear to be most associated with handwriting experience.

3.2. Motion analysis

The median sFD was 0.1977 mm and scores ranged from 0.0601 mm to 1.3905 mm. Subjects were grouped based on a median split of sFD. After separating the data into low- and high-FD subsamples, we first ran the full gPPI analysis low-FD participants. Compared with the analysis on the full sample, analysis on the low-FD subsample produced very similar results. With the exception of one cluster, the same set of significant clusters were found with both analyses using the same correction for multiple testing. This was despite the reduced sample size (N=4) of the low-FD analysis. Therefore, our results are not likely to have been driven by the inclusion of high-FD subjects introducing motion artifacts into the group results. Secondly, we added a sFD group variable to the gPPI design matrix to directly compare the strength of the interactions between low- and high-FD groups of participants. The results of this analysis were the same as the original gPPI and the interaction of the groups with the contrasts of interest did not reach significance even at $p_{vox} < 0.001$, uncorrected. This was further evidence that movement artifacts present in the high-FD participants did not artificially induce the experience-based effects in functional connectivity observed in the full sample. Finally, differences in the cFD did not reach significance (F(7, 136)=1.15, p=0.34), demonstrating that differing amounts of movement during specific conditions could not explain differences in functional connectivity between conditions fig. 7.

Thus, we have demonstrated that our data are suitable for the gPPI functional connectivity analysis and have taken several steps to account for motion. Additionally, we have demonstrated that a reanalysis with the lowest motion subjects produced the same results as with the full sample, which strongly indicates that these results were not driven by the presence of high-motion subjects. Furthermore, we have demonstrated that the motion between conditions was not significantly different, which strongly indicates that these results were not driven by the presence of task-correlated motion (Fig. 8).
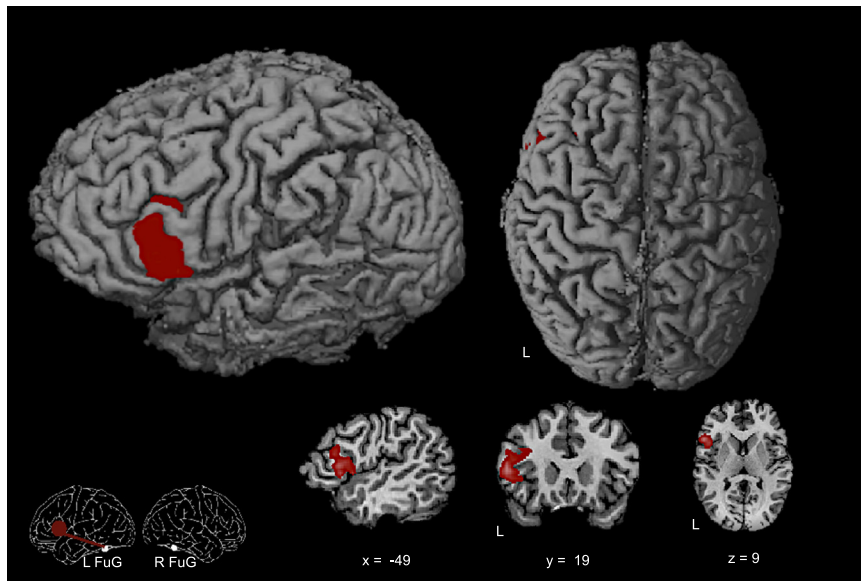
Fig. 6. Effects of handwriting experience as a function of stimulus type. Functional connections between the L FuG and L IFG (cayenne) for the perception of letters trained through handwriting compared to shapes trained through drawing. Talairach coordinates are provided. Sagittal view is of the right hemisphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
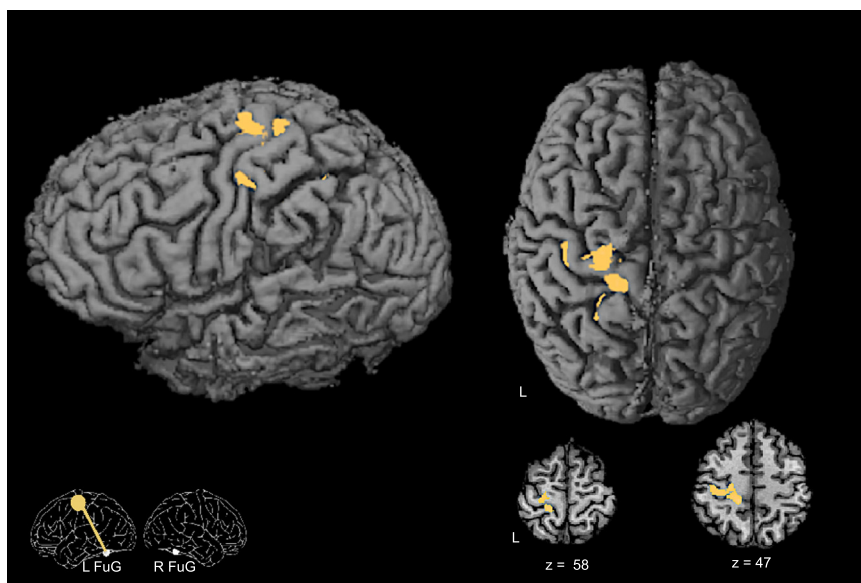

Fig. 7. Effects of handwriting vs. typing experience of letters. Functional connections between the L FuG and dorsal sensorimotor area (yellow), including the L dPrG and the L dPoG, for the perception of letters trained through handwriting compared to letters trained through typing. Talairach coordinates are provided. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The natural assumption from previous neuroimaging work is that the reason visual and motor regions co-activate is from stronger neural connections as a result of handwriting, but until now this had not been confirmed empirically. The findings here show unequivocally that this network of regions becomes more strongly functionally connected after handwriting experience.

Naturalistic handwriting is a complex task involving many brain systems dynamically interacting with one another to produce the desired outcome. As children learn to write, they must integrate visual, proprioceptive, haptic, working memory, and motor information together to produce a symbol by hand. Here, we take one small aspect of handwriting, producing an individual letter from a model, and test whether this kind of experience changes functional connectivity patterns in the brain relative to other forms of visual-motor experience with symbols. We therefore compared functional connectivity patterns in the whole brain when children viewed letters and simple shapes after various forms of training in the laboratory. We were particularly interested in connectivity with the anterior FuG that might emerge after different types of visual-motor experiences with letters. Our hypothesis was that functional connectivity among visual (FuG) and motor (specifically parietal and frontal) regions would be stronger for letters learned through handwriting than through other visual-motor methods. We addressed this hypothesis by demonstrating greater functional connectivity among FuG and motor systems as a function of (a) training (trained vs. untrained letters), (b) stimulus
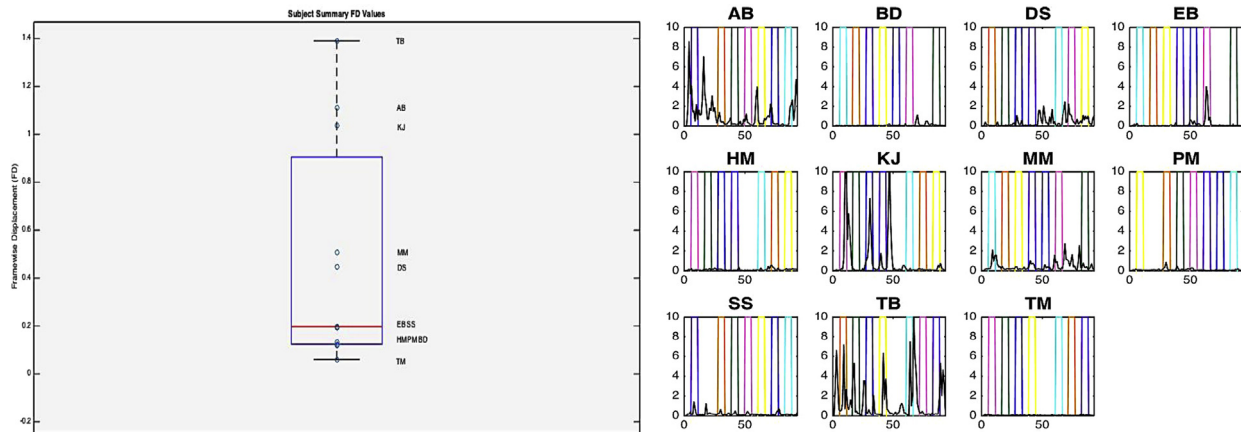
Fig. 8. Motion analysis results for subject-specific FD scores. Subjects below the median were selected as a low-motion sub-sample. Subject initials are reported, because this is the reporting style used in James and Engelhardt [14] and, thus, allows the reader to reference the behavioral scores reported therein.

(letters trained through handwriting vs. shapes trained through drawing), and (c) method of visual-motor training (letters learned through handwriting vs. typing). The primary results supported a specific version of our hypothesis, showing that the strength and pattern of functional connectivity was reflective of the training itself, regardless of stimulus, but importantly, was also affected by the type of visual-motor experience (handwriting) during symbol learning. During visual perception of individual letters, visual and motor brain regions exhibited coordinated activation that was strongest for letters after handwriting practice. In summary, the results suggest that one mechanism by which handwriting practice leads to adult-like patterns of BOLD signal change in pre-literate children is through augmentation of visual-motor communication pathways that reflect different aspects of the handwriting experience.

Remarkably, these training-based differences were present after a considerably brief amount of training, indicating that functional connections can be strengthened after a rather short amount of handwriting practice. Similar exposure times have been shown to correspond to changes in the magnitude of the BOLD signal during letter perception [13–15,22] and to behavioral changes in letter knowledge [6]. Although it is possible that this functional network was present prior to our training manipulation, the fact that the training conditions differentially affected subsequent functional connections indicates that the effects found among training conditions could not have been solely driven by pre-existing functional connections.

On the other hand, it is possible that changes in functional connectivity due to training may correlate with the strength of the pre-existing functional connection, such that the presence of pre-existing functional connections indirectly influenced our results. That is, it is possible that the strengthening of a weaker pre-existing functional connection may be more readily detected than the strengthening of a strong connection, or that strong pre-existing connections are more readily strengthened than weak connections. The fact that handwriting, tracing, and typing are equally common methods of letter learning in preschool makes this interpretation unlikely for the training effects, but it could be a plausible interpretation for the stimulus effects. We found only one effect that was specific to the stimulus: functional connectivity between the L FuG and the L IFG was stronger for letters trained through handwriting than shapes trained through drawing. This functional connection may have been more readily strengthened for letters than for shapes, because children are likely more experienced with writing letters than drawing the particular shapes chosen in this study. Similarly, they may already have some phonological and semantic associations with letters. Indeed, their

behavioral scores indicate that they did have more experience with letters than the chosen shapes and that they did have some phonological associations with letters. However, we find this interpretation unsatisfactory, because we did not find any effects specific to the stimulus for tracing or typing, which would be expected under this interpretation, because shapes are rarely traced and never typed whereas these are common activities for letters.

In the following, we first consider the broad visual-motor network that results from visual-motor training in general and then, more specifically, we consider how different types of training with letters affected functional connectivity patterns. We then discuss results that suggest that components of this network have different functions for supporting visual letter processing. Namely, functional connections between the visual and parietal regions appear to be related to visual-motor training in general, connectivity between visual regions and ventral frontal regions increased as a result of writing letters compared to shapes, and functional connections between visual and dorsal frontal regions increased as a result of handwriting letters compared to other visual-motor training methods.

4.1. Fusiform gyri – right parietal cortex: training network

The functional connectivity found between the L FuG and the R parietal cortex, specifically the R SPL, R IPS, and R IPL, was revealed in a variety of contrasts that targeted the effects of training. Our overall training contrast, comparing the perception of trained symbols vs. untrained symbols, resulted in functional connectivity between the L FuG visual region and right parietal cortex, as did our contrasts between the specific training conditions with letters and untrained letters. This right parietal region also displayed functional connections with the R FuG for the perception of letters learned through tracing vs. untrained letters, which was the only contrast showing significant functional connections with the R FuG. Furthermore, a conjunction analysis confirmed that handwriting, tracing, and typing training all increased functional connections between the L FuG and an anterior portion of the R IPS/ IPL during subsequent letter perception. We therefore regard this pathway as reflecting visual-motor training, in general.

One interpretation is that this pathway is involved in the visual to motor transformation required to accomplish all writing, tracing, and typing tasks. Communication between visual and motor brain regions may use transformations accomplished through parietal cortex to effectively communicate between visual and motor brain regions. Such an interpretation would appear to receive support from the breadth of studies demonstrating the involvement of parietal cortex in visually guided actions [53].

However, the typical finding has been that activation in *left* parietal cortex is stronger during the perception of letters learned through handwriting [14] and during the act of handwriting itself. For instance, a recent study from Kadmon Harpaz, Flash, & Dinstein [54] found that the left IPS was routinely recruited while participants wrote individual letters and that the pattern of activation within this region was specific to the letter being written (e.g., e vs. a). On the other hand, there are indications that both left and right parietal cortex are recruited while writing individual letters, although activation in left parietal cortex is generally stronger than in right parietal [27]. Nonetheless, finding functional connections between the fusiform gyri and right parietal cortex was unexpected. We can only attribute this difference to the fact that prior studies did not look at functional connectivity. Rather, they looked at changes in the height of activation across tasks. Given that this is an unexpected result and the absence of prior studies looking at functional connections during letter perception and handwriting, this result is particularly difficult to interpret.

Perhaps the most parsimonious interpretation is one that involves attention and effort when perceiving newly learned forms. The right parietal lobe has been considered for many years to be important for visual attention [55]. Our findings would fit well with such an interpretation if we assume that processing recently learned stimuli recruits more attentional mechanisms than processing well-learned stimuli. Similarly, recent visual-motor experiences may make certain spatial features of the letterforms more salient and, thus, the fusiform and right parietal functional connections may be due to the orienting of spatial attention for the purpose of letter recognition. Such an interpretation would be supported by studies that have indicated a role for R IPS in the visual perception of newly learned forms [53]. However, without directly manipulating attention in the current study, such interpretations should be considered with caution.

4.2. Fusiform gyri – inferior frontal gyrus: written language pathway

The pathway between the left visual cortex and the L IFG has long been thought to underlie language processing. Numerous studies, dating back to Broca [56], have suggested a crucial role for the L IFG in language production. The L IFG has traditionally been associated with speech articulation; however, handwriting, being one form of language production, also recruits this region [21]. Here, we find that this region is functionally connected to the L FuG – a language region associated with the visual perception of orthography in the left visual cortex – during the perception of letters learned through handwriting when compared to shapes learned through drawing.

If we consider the L IFG as playing a crucial role in the sequencing of motor actions – whether in speech articulation or handwriting – we might consider that the L FuG – L IFG connection may be strengthened by sequential actions. For instance, practice reproducing written forms that require a specific motor sequence would increase the utility of this region during subsequent visual perception of the practiced form. A similar mechanism may be at work in phonological processing. Practice articulating a letter sound requires a specific motor sequence that may increase the utility of this region during subsequent auditory perception of the practiced sound. If the L IFG is concerned with the ordering of motor movements to produce speech and written symbols, then experiences that direct the attention of the observer to the relationship between the motor sequence and the features produced, auditory or visual, may effectively train this area to more readily realize the relationship between important features for the purpose of perception. The role of the L IFG in the sequencing of motor actions is supported by the earlier finding that the L IFG response is increased after handwriting and tracing practice with

letters compared to typing [14]. Here, we show that the L IFG response is more highly correlated with the L FuG response during the perception of letters learned through handwriting than shapes learned through drawing. We interpret this functional connection as a visual-motor connection that may be strengthened by the stroke-by-stroke creation of visual features through sequential motor actions.

Given that the L IFG is traditionally associated with the speech articulation, an obvious alternative interpretation of this finding is that the L FuG is being driven by the visual stimulus at the same time that the L IFG is being recruited for sub-vocal articulation and, thus, these two regions exhibit correlated activity. However, this is not likely for two reasons. First, the gPPI method explicitly accounts for the main activation associated with the task, and so, the functional connections observed between the L FuG and L IFG are task-based correlations that are present above and beyond any co-activation driven by independent aspects of the task (e.g., visual letter perception, sub-vocal articulation). Second, if this correlation were due to the simultaneous, yet independent, effects of visual letter perception and sub-vocal articulation, then it would be expected that the correlation strength would be greater in all letter conditions when compared to shape conditions. However, this was not the case.

4.3. Left fusiform gyrus – left precentral and postcentral gyri: specificity of handwriting practice

Perhaps most interestingly, when we compared handwriting experience to typing experience during subsequent letter perception, a functional pathway from the left visual regions to the left dorsal primary motor/somatosensory cortices was revealed. That is, activation patterns in the L FuG were more correlated with patterns in the L dPrG/dPoG when children perceived letters with which they had received handwriting rather than typing training. Handwriting's ability to strengthen functional connections between the L FuG – L dPrG/dPoG indicates that handwriting may be particularly effective at pairing motor and proprioceptive information with visual information for the purpose of visual perception. The L dPrG is a well-documented primary motor region and the L dPoG is a well- documented somatosensory region [57,58]. Both regions are known to function together during motor actions and their coordination is particularly important during fine motor movements, such as handwriting [59,60]. The integration of motor and somatosensory regions during writing may be more related to the necessity of using proprioceptive feedback from a writing implement to modulate fine motor movements, which is relevant during handwriting practice, but not during typing practice. Our results indicate that the coordination of motor and somatosensory brain regions is influential in guiding perceptual decisions about visually presented stimuli with which the observer has received stimulus-specific motor training (i.e., creating a form feature-by-feature). This interpretation is in line with behavioral results from James and Gauthier [63], in which motor execution and proprioceptive feedback elicited during handwriting interfered with visual letter recognition performance and with neuropsychological case studies indicating that one's ability to write letters is related to one's ability to recognize letters [61,62]. In light of these studies, our results indicate a functional role for motor and somatosensory systems in visual letter recognition by way of functional communication pathways between L FuG and L dPrG/dPoG.

However, and importantly, there was no difference in functional connectivity between handwriting and tracing training. This suggests that handwriting and tracing are not different in their functional connectivity patterns with the L FuG or the R FuG. Thus, the connectivity is due to a component that is shared between

handwriting and tracing, but not typing. The obvious similarities are that a form is created, feature by feature, through a movement pattern that is specific to the written product, and that both handwriting and tracing occur through the use of a writing implement. To our knowledge, only one fMRI study has compared free-hand writing experience to tracing experience [14]. In this study, a ROI analysis showed that activation in the L FuG was greater for letters trained through handwriting than through tracing, both of which were greater than for letters trained through typing [14]. Whole brain contrasts indicated a slightly different pattern of activation in the L PrG: greater activation for letters trained through handwriting than for letters trained through tracing or typing. Taken together with the present results, we now assume that the handwriting-specific L PrG activation seen in our previous study was not a due to greater handwriting-specific functional connections with visual regions. Rather, we assume that it was due to a strengthening of functional connections resulting from stimulus-specific motor movements. However, our previous study also indicated that the amplitude of signal after tracing was less than handwriting in the same areas shown in the current study to be functionally connected to the L FuG – implying that there are processing differences in this region for letters trained through handwriting and letters trained through tracing. Our interpretation is that handwriting and tracing both require a stimulus-specific motor movement using similar hand positions and similar visual guidance and, thus, result in a similar degree of visual-motor functional connectivity among these regions.

4.4. Implications for letter learning

Communication among sensory and motor brain regions during letter perception may constitute a flexible neural representation for letters that comes online early and remains foundational to robust recognition abilities in adulthood. This interpretation is in line with neuropsychological case studies that suggest a role for sensory and motor coordination in support of letter perception. Individuals who lack the ability to write also demonstrate impaired letter recognition abilities [61] and individuals with impaired letter imagery abilities are better able to manipulate mental images of letters if they are allowed to 'write' the letterform with their finger [62]. Additionally, there is evidence that as the amount of experience with a written symbol increases, sensory and motor systems increasingly interact with each other during letter processing. James and Gauthier [63] demonstrated that overlearned stimuli (e.g., letters) are particularly susceptible to perceptual identification errors when the observer is asked to simultaneously write a categorically different, but perceptually similar, letter, such as writing the uppercase letter G while perceiving the uppercase letter C. The motor action along with proprioceptive feedback while writing one letter was enough to cause errors in visual identification of a different letter when both occurred simultaneously – especially when the features of the two letters were similar. This behavioral work suggests that communication among somatosensory and visual regions with the motor system contributes to literate letter identification abilities and parallels the present neuroimaging work showing that letter perception in preliterate children is supported by visual-motor communication pathways that are strengthened through handwriting training. Further, the neuroimaging work of James and Gauthier [21] suggests that letter perception in literate adults is supported by a similar set of visual and motor brain regions, although further work is needed to determine if functional connections between these regions remain in the literate adult state.

In all, handwriting practice appears to be particularly effective at increasing functional connections between visual and motor brain regions. Although our study does not directly address whether or not this increase in functional connectivity is correlated with increases in letter recognition ability, other studies have indicated that practice handwriting results in greater gains in letter recognition than typing or visual-only practice [6]. Thus, we suggest that handwriting practice contributes to the strengthening of functional connections that come to play a functional role in letter recognition. Therefore, it will be important to make use of technological advances to ensure that handwriting practice remains a central activity in early letter learning, whether the handwriting occurs with paper and pencil or stylus and tablet.

Despite the suitability of the gPPI method for this study, it remains limited in that inferences regarding the direction of neural signals cannot be discerned, as it is a non-directed FC approach [34]. Thus, we make no inferences regarding the direction of influence between visual and motor brain regions. Future studies are needed to address the question concerning whether or not visual input feeds forward to the motor system, or motor actions inform the visual system, or whether the functional coordination between the visual and motor systems is truly bidirectional.

5. Conclusions

Previous research has shown that handwriting individual letters facilitates letter recognition ability and the emergence brain responses that are stronger for letters over other stimuli relative to other types of practice with letters. Regions displaying such response properties include visual and motor brain regions and, crucially, evidence suggests that these responses are sensitive to the type of motor experience that a child has received with a particular letter. Our results indicate that these experience-based neural responses to letters are supported by a functionally connected visual-motor network that reflects different aspects of the handwriting experience. Building on previous research, this study indicates that these visual-motor pathways may be instrumental in the development of letter recognition ability. This visual-motor network for letter perception is strengthened after relatively brief handwriting experience; however, the influence of handwriting practice with letters on long-term brain development remains an open question. Future work is necessary to determine the stability of this network over time.

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