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**Behavioral and neural correlates of speech motor sequence learning in
stuttering and neurotypical speakers: an fMRI investigation**

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Abstract

Stuttering is a neurodevelopmental disorder characterized by impaired production of coordinated articulatory movements needed for fluent speech. It is currently unknown whether these abnormal production characteristics reflect disruptions to brain mechanisms underlying the acquisition and/or execution of speech motor sequences. To dissociate learning and control processes, we used a motor sequence learning paradigm to examine the behavioral and neural correlates of learning to produce novel phoneme sequences in adults who stutter (AWS) and neurotypical controls. Participants intensively practiced producing pseudowords containing non-native consonant clusters (e.g., “gvasf”) over two days. The behavioral results indicated that although the two experimental groups showed comparable learning trajectories, AWS performed significantly worse on the task prior to and after speech motor practice. Using functional magnetic resonance imaging (fMRI), the authors compared brain activity during articulation of the practiced words and a set of novel pseudowords (matched in phonetic complexity). FMRI analyses revealed no differences between AWS and controls in cortical or subcortical regions; both groups showed comparable increases in activation in left-lateralized brain areas implicated in phonological working memory and speech motor planning during production of the novel sequences compared to the practiced sequences. Moreover, activation in left-lateralized basal ganglia sites was negatively correlated with in-scanner mean disfluency in AWS. Collectively, these findings demonstrate that AWS exhibit no deficit in constructing new speech motor sequences but do show impaired execution of these sequences before and after they have been acquired and consolidated.

NEURAL CORRELATES OF SPEECH MOTOR SEQUENCE LEARNING

22 *Keywords:* speech motor control; motor sequence learning; stuttering; GODIVA model; fMRI
23

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1. Introduction

Stuttering is a neurodevelopmental disorder affecting children into adulthood with devastating social effects that impede speech communication. The presenting clinical signs of the disorder include involuntary repetitions and prolongations of phonemes, syllables, or words as well as involuntary silent pauses (Bloodstein & Ratner, 2008). Despite considerable research, researchers still have a relatively poor understanding of the neural deficits underlying the disorder (see Craig-McQuaide, Akram, Zrinzo, & Tripoliti, 2014, for a review). Neurobiological accounts of stuttering, such as Alm (2004), Max, Guenther, Gracco, Ghosh, and Wallace (2004), and recently Connally, Ward, Pliatsikas, Finnegan, Jenkinson, Boyles, and Watkins (2018) and Chang and Guenther (2020), propose that stuttering disfluencies arise from an underlying defect in the (left-lateralized) cortico-basal-ganglia-thalamocortical (cortico-BG) loops (Alexander, DeLong, & Strick, 1986), which are hypothesized to be responsible for selecting and triggering articulatory motor programs, while suppressing others, at the appropriate time during speech sequencing.

There is an extensive body of evidence (recently reviewed by Chang and Guenther [2020]) in favor of the idea that stuttering involves an inability to initiate, sustain, and/or terminate speech motor programs due to anomalous basal ganglia function. For example, numerous acoustic investigations have provided evidence that stuttering involves an impaired ability to transition from the articulatory gestures affiliated with an initial phoneme of a syllable to subsequent gestures, as indicated by protracted voice onset times and formant transition rates (e.g., Yaruss & Conture, 1993; Boustien, 1995; Robb & Blomgren, 1997; Robb, Blomgren & Chen, 1998; Chang, Ohde, & Conture, 2002). As for the underlying neural circuitry, abnormal functional activity in the basal ganglia has frequently been implicated in stuttering severity (Ingham, Grafton, Bothe, &

Ingham, 2012; Giraud, Neumann, Bachoud-Levi, von Gudenberg, Euler, Lanfermann, & Preibisch, 2008; Toyomura, Fujii, Kuriki, 2015; Connally *et al.*, 2018). In addition, stuttering-like disfluencies have been reported to emerge in neurodegenerative diseases that impair the function of the basal ganglia, such as Parkinson’s disease (Niethammer, Feigin, & Eidelber, 2012), and deep brain stimulation applied to either the subthalamic nucleus or the globus pallidus pars interna of the basal ganglia can relieve or exacerbate stuttering disfluencies (see Skodda, 2012, and Sapir, 2014, for reviews).

Moreover, and of particular relevance to the current research, some existing theoretical models attribute a role in speech *motor sequence learning* to the cortico-BG loops (e.g., Kotz & Schwartz, 2010; Guenther, 2016, chapters 8 & 10; see also Fee & Goldberg, 2011, for a similar account in songbird vocal learning). For example, in the *Gradient Order Directions Into Velocities of the Articulators* (GODIVA) model of speech sequencing and initiation (Bohland, Bullock, & Guenther, 2010; Civer, Bullock, Max, & Guenther, 2013; Guenther, 2016, chapter 8; Segawa, Masapollo, Tong, Smith & Guenther, 2019), it is proposed that continual speech motor practice leads to the formation of optimized motor programs for frequently occurring kinematic sequences, such as the successive gestures making up a word or syllable. These learned movement “chunks” or “templates” are then encoded and sequentially activated (or “read out”) by the cortico-BG loops during production. The fundamental idea is that the cortico-BG loops perform two distinct, but related, cognitive operations during the speech production process; namely, forming regularized motor “chunks” and initiating/activating motor programs affiliated with those “chunks.”

Results from a functional magnetic resonance imaging (fMRI) study of speech motor sequence learning with neurotypical adults (Segawa, Tourville, Beal, & Guenther, 2015) provided empirical support for this view. In the initial training phase of the study, participants performed a

71 cued motor sequence learning task (outside the scanner) in which they intensively practiced
72 producing pseudowords (monosyllabic CCVCC[C], where C = consonant, V = vowel) consisting
73 of consonant clusters that were either phonotactically legal in their native language of English
74 (e.g., “blerk”) or illegal in English but legal in other natural human languages (e.g., “gvasf”). The
75 results indicated that, behaviorally, motor practice led to measurable performance gains (i.e.,
76 reduced error rates and shorter utterance durations), presumably because the sequences of
77 independent speech movements had become concatenated into larger cohesive “chunks” (see
78 Segawa *et al.*, 2019, for supporting evidence).

79 In the subsequent test phase (inside the scanner), the authors measured the blood-oxygen-
80 level-dependent (BOLD) response during production of the practiced native and practiced non-
81 native words as well as a novel set of non-native words (matched in phonetic complexity) using
82 sparse sampling fMRI (see, e.g., Gracco, Pike, & Tremblay, 2005). In one set of imaging analyses,
83 BOLD activity associated with production of the novel non-native words was contrasted with that
84 of the practiced non-native words. The results demonstrated increased activation in (left-
85 lateralized) cortical (i.e., pre-supplementary motor area [pre-SMA], anterior insula [aINS], inferior
86 frontal sulcus [IFS], inferior parietal sulcus [IPS], ventral premotor cortex [vPMC]) and
87 subcortical basal ganglia (i.e., globus pallidus [GP]) regions involved in the cortico-BG loops
88 (Alexander *et al.*, 1986). It was further demonstrated that the degree of motor performance
89 improvements between training and test was correlated positively with activity in left aINS. The
90 cortical regions that were found to be recruited in this task overlap with activation foci that have
91 been identified in meta-analyses of working memory neuroimaging studies (see, e.g., Rottschy *et*
92 *al.*, 2012). Thus, these findings are in keeping with the hypothesis that the cortico-BG loops play
93 an important role in learning and sequencing speech movement “chunks” since a higher number

of “chunks” will have to be concatenated in a working memory repository (or phonological output store) and then subsequently “read out” during the production of novel sequences compared to practiced ones.

In another set of analyses contrasting BOLD activity associated with production of the practiced non-native and practiced native words, the authors found increased activation in a similar network of brain areas, although notably no increased activation was found in left GP for this contrast. They did, however, report increased activation in right cerebellum (lobule VI) for this contrast, suggesting that other subcortical structures that extend beyond the cortico-BG may also play a role in speech motor sequencing learning and motor control (a point we will return to in the general discussion).

Nevertheless, if the cortico-BG loops are involved in successively encoding and activating speech motor “chunks” during sequence learning and production, then that raises the possibility that the neural deficits hypothesized to underlie stuttering are related not only to problems with *motor execution*, but also to impaired *motor learning* mechanisms. Consistent with this view are experimental findings, in the behavioral literature, that adults who stutter (AWS) and adults with normal speech (ANS) often show differences in their ability to learn a variety of novel speech and non-speech (i.e., finger-tapping) movement sequences (e.g., Ludlow, Siren, & Zikria, 1997; Smits-Bandstra, De Nil, & Saint-Cyr, 2006; Namasivayam & van Lieshout, 2008). Qualitatively similar findings have also been found in patients with Parkinson’s disease (Ferraro, Balota, & Connor, 1993; Jackson et al., 1995; Helmuth, Mayr, & Daum, 2000; Vakil et al., 2000; Smits-Bandstra & Gracco, 2013; Mollaei, Shiller, & Gracco, 2013).

In the current research, we performed a functional brain-imaging study to distinguish between motor execution and motor learning impairments in AWS at both the behavioral and

neural levels, by replicating and extending Segawa *et al.* (2015) with a cohort of adult speakers with persistent developmental stuttering. Towards this end, we trained AWS and ANS to produce pseudowords containing non-native consonant clusters. Participants were first trained over several days (outside the scanner) to produce two sets of novel CCVCC pseudowords: (1) syllables that involved native consonant clusters (*practiced native*) and (2) syllables comprised of non-native consonant clusters that are phonotactically illegal in English (*practiced non-native*). Based on previous results (Segawa *et al.*, 2015; see also Segawa *et al.*, 2019), we expected to observe significantly larger performance gains throughout the course of training for the *practiced non-native* words than for the *practiced native* words (for which performance is already expected to be near ceiling at the beginning of training). During a subsequent testing phase (inside the scanner), we then contrasted BOLD activity associated with production of the *practiced non-native* and *practiced native* words, and the *practiced non-native* and *practiced native* sequences.

We hypothesized that, if motor sequence learning mechanisms *per se* are impaired in stuttering, then: 1) At the behavioral level, AWS should show a slower rate of learning over the course of training and/or poorer learning outcomes compared to ANS; and 2) At the neural level, contrasting the BOLD signal for the *novel non-native - practiced non-native* and/or *practiced non-native - practiced native* conditions should yield less activity in the regions of the cortico-BG circuit previously identified in Segawa *et al.* (2015) in AWS, especially left aINS, since they will not have fully formed cohesive motor “chunks” for the trained speech sequences.

If, however, the only core deficit underlying stuttering is related to motor implementation rather than to motor learning mechanisms, then: 1) At the behavioral level, AWS should show comparable performance gains over time but will display poorer (and/or slower) overall motor performance compared to ANS; and 2) At the neural level, AWS should show a similar reduction

in activations in the aforementioned regions of the cortico-BG circuit implicated in motor sequences learning and working memory (left aINS, pre-SMA, and IFS) as ANS, but should still show differences in primary motor (and possibly premotor) cortex; and 3) the size of the BOLD activity difference in (at least some of) these brain areas will covary with stuttering severity (and/or in-scanner disfluency) since mechanisms involved in selecting and initiating successive speech motor programs will be more impaired in AWS with more severe stuttering.

2. Material and Methods

2.1 Participants

Sixteen adults who stutter (AWS; 14 male, age range: 18-42, median age: 26) and fifteen adults who do not stutter (ANS; 13 male, age range: 18-40, median age: 25) were paid for participating in three testing sessions on three separate days. Participants reported normal (or-corrected-to-normal) vision and no history of hearing, speech, language or neurological deficits (apart from stuttering in the AWS group). All were right-handed (Oldfield, 1971; mean score = 80.46) and native speakers of American English with no previous experience with any of the languages used in stimulus creation (see following text). Individuals were excluded from taking part in the study if they were currently on medications that may have substantial effects on neural activity, or had claustrophobia preventing them from completing the MRI protocol. All participants underwent a magnetic resonance safety screening.

An experimenter interviewed all participants to confirm the diagnosis of persistent developmental stuttering in AWS and to confirm normal speech production in ANS. None of the AWS were enrolled in a fluency-shaping program at the time of participation. The stuttering severity of each AWS was assessed using the Stuttering Severity Instrument, Edition 4 (SSI-4; Riley, 2008). As part of this assessment, each AWS was video recorded while reading aloud,

conversing with an experimenter, and speaking on the telephone. A certified speech-language pathologist then rated the frequency and durations of the stuttering events and the presence of physical concomitants that accompanied the moments of disfluency (e.g., eye-blinking). Stuttering severity in the AWS group ranged from 13 to 48, with a median of 27 and an interquartile range of 17–34. Five of the sixteen AWS were categorized as “very mild” (SSI-4 score <17), 1 as “mild” (18–24), 5 as “moderate” (25–31), 3 as “severe” (32–36), and 2 as “very severe” (37–46).

2.2 Stimuli

The speech stimuli consisted of several sets of monosyllabic pseudoword sequences (15 words/set). As shown in Table 1, all items contained either native or non-native syllable-initial (onset) and syllable-final (coda) consonant clusters. In the native sequences (e.g., BLERK, THRIMF, TRALF), the onset and coda clusters are phonotactically legal in English; in the non-native sequences (e.g., FPESHCH, GVASF, TPIPF), the clusters are phonotactically illegal in English, but do occur in some other natural human language. The non-native clusters were taken from a variety of languages and language families including Hebrew, Leti, Taba, Romani, Polish, Lithuanian, Romanian, Georgian, Tepehua, Hungarian, and Pima; participants reported no prior experience with any languages in which these consonant clusters readily occur. None of the stimuli were an orthographic or a phonological word according to the MRC Psycholinguistic Database (Coltheart, 1981). All items containing non-native clusters had a neighborhood size of 0, and no real English words could be created by adding, deleting, or substituting a single phoneme in any subsequence. All of the clusters – both native and non-native alike – were bi- or tri-consonantal and used in either onset position or coda position, but not both. Each cluster was used in only one word (i.e., no two words contained the same consonant cluster). The number of phonemes per word was counter-balanced across experimental conditions (see below).

-- [Insert Table 1 about here] --

To create the prompts for the elicited production task, a female native speaker of American English was recorded producing the words. The model speaker was phonetically trained and had previously practiced producing the sequences until each stimulus could be executed fluently (i.e., without vocoid epenthesis or phoneme omissions, swaps, or substitutions). All recordings took place in a sound-attenuated booth. The speech was recorded directly to a computer using Audacity® software (Version 2.0.3, Audacity Team) via a microphone (Samson C01U studio condenser) connected to a pre-amplifier (44.1-kHz sampling rate, 32-bit quantization). The speaker recorded multiple randomized repetitions of each token. From these repetitions, one instance of each token was selected on the basis of clarity and acoustic similarity in voice pitch (f_0) to the other stimuli in the set. Using Praat software (Boersma & Weenink, 2020), all recorded tokens were digitally edited to be matched for peak intensity and duration (i.e., 480 ms) without changing f_0 .

2.3 Procedure and Design

Participants completed a cued sequence production task (see, e.g., Segawa *et al.*, 2015, 2019). The experiment consisted of a training phase, during which participants learned to produce 15 words containing native clusters and 15 words containing non-native clusters, followed by a test phase, during which participants were tested on their ability to produce all 30 of the previously learned words and 15 novel words containing non-native consonant clusters. fMRI data were only collected during the test phase (see below). For both phases, participants were asked to repeat aloud each of the target words individually, which were presented both auditorily over noise-cancelling headphones and visually using text stimuli (as shown in Table 1). The training phase consisted of eight blocks of trials over two consecutive days (four on day one and four on day

two). Each training block contained eight repetitions of each word, for a total of 240 trials per block. The test phase consisted of eight blocks of trials performed on day three (inside the MRI scanner) after completing the training phase (outside the scanner on days one and two). Each test block contained five to six repetitions of each word, for an average of 40 trials per block, from three stimulus categories (conditions): (1) *practiced native* words (i.e., words comprising native clusters that were previously encountered in the training phase), (2) *practiced non-native* words (i.e., words comprising non-native clusters that were previously encountered in the training phase), and (3) *novel non-native* words (i.e., novel words with novel clusters that were not encountered in the training phase).

Participants were divided into four groups, each of which practiced producing a different subset of the native and non-native sequences during training. The non-native words that were not practiced during training were used as novel non-native words during the imaging session. Assignment of non-native words to the *practiced non-native* and *novel non-native* categories was counterbalanced across participants. The training phase occurred one to two days before the test phase to allow for sleep-mediated memory consolidation of the newly acquired motor traces (see, e.g., Fenn, Nusbaum, & Margoliash, 2003; Doyon, Bellec, Amsel, Penhune, Monchi, Carrier, Lehericy & Benali, 2009; Doyon, Albouy, Vahdat, & King, 2015; Vahdat, Fogel, Benali, & Doyon, 2017; cf. Brawn, Fenn, Nusbaum, & Margoliash, 2010; Pan & Rickard, 2015).

During training, participants were seated in a chair in front of a laptop (IBM Lenovo ThinkPad X61s) computer screen in a sound-treated laboratory room that was dimly lit. The auditory speech stimuli were presented over headphones (Sennheiser, HD 280 Pro) at a comfortable listening level and utterances produced by the participants were recorded with a Samson (Hauppauge, NY) C01U USB studio condenser microphone connected to the computer

via a MOTU microbook audio interface. Utterances were recorded using MATLAB (MathWorks Inc., Natick, MA) at 44.1 kHz. During test, participants laid supine in an MRI scanner. Instructions and visual stimuli were projected onto a screen viewed from within the scanner via a mirror attached to the head coil. The auditory stimuli were played over Sensimetrics model S-14 MRI-compatible earphones. Participants' productions were transduced by a Fibersound model FOM1-MR-30m fiber-optic microphone, sent to a Lenovo ThinkPad X61s, and recorded using MATLAB at 44.1 kHz.

The trial structure was identical during training and test. First, the orthographic display of a given syllable was centrally presented in tandem with its corresponding auditory prompt. Participants only heard each prompt once on each trial. Then, after the offset of the auditory presentation, a tone was presented for 50 ms. The time between stimulus offset and tone onset was randomly jittered between 500 and 1,000 ms. This tone served as a "GO" signal that prompted the participant to go ahead and repeat the token as clearly and accurately as possible. For all phases, participants produced the target syllables in a pseudorandom order. The combination of the auditory and orthographic presentations was necessary because prior studies have shown that listeners tend to perceive non-native consonant clusters as epenthesized disyllabic sequences (e.g., Pitt, 1998; Dupoux, Kakehi, Pallier, Hirose, & Mehler, 1999; Berent, Steriade, Lennertz, & Vaknin, 2007; Dupoux, Parlato, Frota, Hirose, & Peperkamp, 2011; Davidson & Shaw, 2012). Moreover, in another study that directly examined the effects of stimulus input modality (audio only vs. audio and text) on speakers' ability to produce non-native consonant clusters (Davidson, 2010), it was found that the presence of text led to an improvement in overall task performance.

For both phases, participants were instructed to repeat the target syllable as clearly and accurately as possible as soon as they heard the tone. Several familiarization trials with

experimenter feedback were included at the start of the experiment to confirm that participants understood the task instructions and were able to perform the task. The sequences used during these initial practice trials were not used at any point in the rest of the study. Feedback was not provided during the actual training or test phases.

2.4 Behavioral Data Analysis

We conducted several analyses to provide evidence, at the behavioral level, of incremental motor learning throughout the two days of speech motor practice (outside the MRI scanner), and that such learning was retained at test (inside the scanner). In the first analysis, we analyzed three temporally sensitive measures of learning across the eight training blocks (on days 1 and 2): (1) articulatory sequencing error rate, (2) utterance duration (interval from utterance onset to offset), and (3) reaction time (RT) (interval from the offset of the go-signal to utterance onset). These measures are generally believed to quantify the ease or difficulty with which speakers retrieve and execute speech sequences (Sternberg, Monsell, Knoll, & Wright, 1978) and are commonly used in both the motor sequence learning and second language learning literatures as measures of learning extent (see, e.g., Rauschecker et al., 2008; Nakamura, Sakai, & Hikosaka, 1998; Brawn *et al.*, 2010; Doyon *et al.*, 2015; Buchwald *et al.*, 2019; Segawa *et al.*, 2019). Sequencing errors were defined as phoneme additions (including schwa insertions), deletions, and substitutions, and utterance repetitions, omissions, and restarts. Based on prior findings reported with neurotypical speakers (Segawa *et al.*, 2015, 2019), we expected to observe greater learning (at least in the ANS group) for the non-native sequences because those sequences included both novel words and novel onset and coda clusters, whereas the native sequences included novel words with familiar onset and coda clusters.

In the second analysis, to provide evidence of the *retention* of learning at test, we examined the same three performance measures for each experimental group (ANS vs. AWS) and condition (*practiced native* vs. *practiced non-native* vs. *novel non-native*) during the test phase (on day 3). For each word production, each measure was calculated following the removal of noise associated with the scanner bore echo and peripheral equipment using a Wiener filter (Wiener, 1949). The coder was blind to the condition (*practiced* vs. *novel*) of the non-native syllables. We hypothesized that if participants formed regularized motor “chunks” for the previously trained speech sequences, then they should perform more accurately (and/or faster) at generating the *practiced native* and *non-native* sequences compared to the *novel non-native* sequences.

In a final analysis, we examined whether, and if so, how, speech motor practice influenced fluency in the AWS group. Toward this end, we compared the mean number of trials perceptually coded as containing at least one stuttering-like disfluency during both the training and test phases (for the AWS group only) for each stimulus condition (*practiced native* vs. *practiced non-native* vs. *novel non-native*). Disfluencies were determined by a certified speech-language pathologist.

For each of these analyses, custom MATLAB software (Natick, MA) was used to perceptually rate and acoustically measure onsets and offsets of syllables by viewing the waveform and spectrogram and listening to the audio files. Listeners first marked, blinded to experimental condition, whether the target phoneme sequence was produced fluently. Disfluent productions were further categorized into four possible disfluency types: (1) repetition (i.e., a phoneme or syllable was repeated); (2) prolongation (i.e., a phoneme was prolonged); (3) audible or silent blocking (involuntary filled or unfilled pauses in speaking), and (4) clustered (i.e., more than one stutter type was produced for a given sequence).

Each utterance was then marked for four possible sequencing error subtypes: (1) approximation of the target (i.e., phoneme deletion/omission, insertion, substitution, vowel epenthesis); (2) unrecognizable from target (i.e., an entirely different sequence was produced); (3) unfinished word (i.e., the sequence was produced by was not complete before the end of the recording window); and (4) silence (i.e., no sequence was produced at the time of recording). Since we were concerned with the learning of non-native phonotactics rather than sub-phonemic allophonic details, productions from either the model speaker or the participants were not judged on how natural they sounded in the language from which they were derived.

In addition, it is important to note that it was impossible to reliably distinguish involuntary “silent” pauses or blocks associated with stuttering from intentional speech onset delays with the audio-only recordings that we obtained in the scanner (although there were constraints on what the delay could be since the length of the recording window was fairly short). Thus, we suspect that trials with stuttering pauses were categorized as sequencing error subtype 4 (i.e., no sequence produced).¹ This is a limitation inherent in the current study and all studies of this kind in stuttering (unless video recordings of articulatory behavior inside the scanner are obtained).

Mean disfluency rates for each subject were calculated as the percentage of trials that contained one or more disfluency error sub-types, and mean error rates for each subject were calculated as the percentage of trials that contained one or more error subtypes. For each production containing no sequencing or disfluency errors, utterance onset and offset were automatically labeled based on sound pressure level thresholds, then hand-checked. Note that, in

¹ In order to explore the possibility that some trials containing “silent” stuttering blocks were included in the neuroimaging analyses, we plotted histograms showing the distribution of RT scores for each stuttering speaker in the test phase (inside the scanner). Critically, these plots did not reveal a bimodal distribution with one peak having frequency values clustered to the right. This finding suggests that any potential blocks or pauses were likely categorized as silent errors (sequencing subtype 4 as described above) and were excluded from the imaging analyses, in which case, the BOLD responses would not have been contaminated by potential delays in RT. See *Supplementary Materials* for further details.

the neuroimaging component of the study (described below), only trials in which participants produced the target sequence accurately and fluently were analyzed.

2.5 fMRI Paradigm

In addition to the three speaking conditions (*practiced native* vs. *practiced non-native* vs. *novel non-native*), a silent baseline condition was intermixed during imaging in which participants viewed a series of asterisks on the screen instead of the orthographic stimulus and rested quietly instead of uttering a word. FMRI data were acquired using a sparse sampling protocol (see, Belin, Zatorre, Hoge, Evans, & Pike, 1999; Gracco *et al.*, 2005; Perrachione & Ghosh, 2013) that allowed participants to produce the target syllables during *silent* intervals between volume acquisitions. A single volume was acquired approximately 4 sec after speech onset on each trial which aligns with the 4-6 second delay in peak BOLD response onset (Belin *et al.*, 1999). By scanning between speech productions, this protocol avoids the influence of scanner noise on speaker performance and brain activity responses and image artifacts resulting from speech-induced motion of the head.

The cued sequence paradigm was identical to that used during training (outside of the scanner) except with an additional pause after the production of each syllable to temporally align the image acquisition to the expected peak of the hemodynamic response. As previously described, the test phase consisted of eight blocks of trials which corresponded to eight functional runs. A single volume was recorded on each trial and the delay between volumes was approximately 10 seconds. Each functional run contained five or six productions of each stimulus item, for a total of 40 trials per run (each run lasted approximately 6-7 minutes). This resulted in a total of 320 test trials (80 trials per condition, including baseline). Conditions were shuffled and pseudorandomly distributed across the eight functional runs with at least eight instances of each condition appearing in each run.

2.6 Image Acquisition

All neuro-imaging data were acquired using a 3-Tesla Siemens TIM Trio scanner, equipped with a 32-channel head coil. T2*-weighted gradient echo echo-planar fMRI images were collected to assess blood-oxygen-level-dependent (BOLD) responses during the test phase. 41 horizontal slices were collected in each functional volume (in-plane resolution = 3.1 mm², slice thickness = 3 mm, gap = 25%, acquisition time = 2.5 sec, TE = 20 msec); volumes were automatically aligned to the AC–PC line. Prior to collecting functional data, a gradient-echo field map sequence was collected; the resulting magnitude and phase images was enabled offline correction of magnetic field distortions in functional images during data preprocessing (see below; Jezzard & Balaban, 1995). Structural images were collected using a T1-weighted multi-echo MPRAGE pulse sequence (MEMPRAGE, voxel size = 1 mm³, 176 axial slices, 256 X 256 FOV, repetition time [TR] = 2530 msec, echo time [TE] = 3.44 ms, flip angle = 7°).

2.6 fMRI Data Analysis

Image preprocessing. Functional data were processed using tools from the following software packages that were integrated into a processing stream within SPM12 (Statistical Parametric Mapping, v12; www.fil.ion.ucl.ac.uk/spm/): FreeSurfer (Fischl et al., 2002; Dale, Fischl, & Sereno, 1999; Fischl, Sereno, & Dale, 1999; www.freesurfer.net), Artifact Detection Tools (ART; www.nitrc.org/projects/artifact_detect/), and the CONN toolbox (Whitfield-Gabrieli & Nieto-Castañón, 2012). Freesurfer was used to remove non-brain components of the T1 structural volumes, segment the brain into gray matter, white matter, and cerebral spinal fluid components, generate a reconstruction of the cortical surfaces of each hemisphere and to identify cortical and subcortical regions of interest (ROIs) (see below). Functional data were preprocessed through two pipelines: a surface/vertex-based pipeline for analysis of cortical responses and a

volume/voxel-based pipeline for analysis of subcortical basal ganglia and cerebellar responses. Prior to pre-processing, the first volume of each functional series was removed because it served only as a trigger for the initial experimental trial.

A surface/vertex-based analysis pipeline was used to assess BOLD response differences for each experimental group and for each contrast in the cerebral cortex. Functional images from each subject were simultaneously realigned to the mean subject image and unwarped (motion-by-inhomogeneity interactions) using the SPM12 realign and unwarp procedure (Andersson, Hutton, Ashburner, Turner, & Friston, 2001). Outlier scans were detected using ART based on framewise displacement (scan-to-scan motion threshold of 0.9 mm) and mean signal change (scan-to-scan signal change threshold of 5 standard deviations above the mean (see Nieto-Castañón, 2020 for details²). Functional volumes from each subject were then co-registered with their high-resolution T1 structural images and resliced using SPM12's inter-modal registration procedure with a normalized mutual information objective function. The functional data were then resampled at the location of the FreeSurfer *fsaverage* level-8 tessellation (163,842 vertices and 327,680 faces) projected on each subject-specific cortical surface, averaged across 10 intervals along the normal between the white matter and pial surfaces, and smoothed using iterative diffusion smoothing with a series of 40 discrete steps, approximately equivalent to a 8 mm full-width half-maximum (FWHM) two-dimensional Gaussian smoothing kernel (Hagler *et al.* 2006, Nieto-Castañón, 2020).

A volume/voxel-based analysis pipeline was used to identify differences in subcortical BOLD responses. Following the realignment, unwarping steps described above, functional volumes, and the original T1 structural volumes, were simultaneously segmented and normalized

² Framewise displacement is computed at each timepoint by considering a 140x180x115mm bounding box around the brain and estimating the largest displacement among six control points placed at the center of this bounding-box faces. Global BOLD signal change is computed at each timepoint as the change in average BOLD signal within SPM's global-mean mask scaled to standard deviation units.

directly to Montreal Neurological Institute (MNI) space using SPM12's combined normalization and segmentation procedure (Ashburner & Friston, 2005). Prior to MNI-normalization, both the functional and anatomical volume origins were centered to coordinates $[0, 0, 0]$ in order to improve the quality of the iterative procedure initial starting estimates. Functional volumes were then spatially smoothed using an 8 mm FWHM Gaussian kernel in order to increase BOLD signal-to-noise ratio and reduce the influence of residual variability in functional and gyral anatomy across subjects (Nieto-Castañón, 2020).

Subject-level BOLD contrast analyses. After preprocessing, BOLD responses were estimated using a general linear model (GLM) in SPM 12. Because functional volumes were acquired in a sparse sequence, the hemodynamic response function for each stimulus event was modeled as a finite impulse response. The model included four condition-specific variables (practiced native, practiced non-native, novel non-native, and baseline). Trials with productions that were perceptually rated as containing either a sequencing error (e.g., phoneme deletions, insertions or substitutions) or a stuttering-like disfluency (e.g., phoneme/syllable repetitions, prolongations, or blocks), or were found to be outliers by ART were modeled as separate conditions (one individual regressor per trial) thereby removing variability resulting from these trials from the effects- and contrast-of-interest estimates. For each individual run, regressors were added to the model to remove linear effects of time (e.g. signal drift, adaptation) and the six motion covariates (taken from the realignment step) and a constant term.

The model was estimated at each vertex (surface-based analysis) or voxel (volume-based) analysis for each participant, resulting in two surface maps (one for each hemisphere) and a volume map of the model regressor coefficients for each condition. These condition estimates were then contrasted to yield effect-size maps of the following contrasts of interest: differences in the

response during *novel non-native* and *practiced non-native* conditions (novel non-native – practiced non-native), and differences in the response during the *practiced non-native* and *practiced native* conditions (practiced non-native – practiced native).

Group-level analyses. Group-level *t*-statistics were calculated separately for each contrast map. Cluster-level inferences were based on Threshold Free Cluster Enhancement (TFCE; Smith and Nichols, 2009) with default parameters $E=0.5$ and $H=2$. This method assigns TFCE scores to each voxel characterizing the amount of cluster-like local spatial support at each location. These scores are then compared to their expected distribution under the null-hypothesis, estimated using 1000 randomization/permutation iterations and results are thresholded at Family-wise Error false positive probability (p_{FWE}) < 0.025 . This threshold was used as a conservative means to ensure a cluster-level $p_{FWE} < 0.05$ across the cortical surface and subcortical volume analyses.

2.7 Region-of-interest-based analyses

To increase statistical power and sensitivity, we supplemented the surface/vertex-based and volume/voxel-based analyses with region-of-interest (ROI) analyses (Nieto-Castañón, Ghosh, Tourville, & Guenther, 2003) based on *a priori* hypotheses derived from the results of Segawa *et al.* (2015). Specifically, we used anatomically-defined ROIs in the speech production network that overlapped areas of significant BOLD activity in Segawa *et al.* (2015) for each contrast of interest. The predefined cortical and subcortical (basal ganglia and cerebellar) ROIs are listed in Table 2; a detailed description of the anatomical landmarks used for cortical parcellation are provided in Tourville and Guenther (2003). For the *novel non-native – practiced non-native contrast*, we tested whether brain activity was significantly different in globus pallidus internal (GPi) and external (GPe) segment ROIs; these ROIs were derived from the probabilistic atlas of the basal ganglia described by Keuken and colleagues (2014). For the *practiced non-native – practiced native*

contrast, we tested whether brain activity was significantly different in right cerebellum lobule VI, which was derived from the SUI probabilistic atlas of the cerebellum (Diedrichsen *et al.*, 2009). Affirmation of differences in these ROIs would replicate the Segawa *et al.* (2015) findings with neurotypical speakers.

2.8 Brain-behavior correlation analyses

Two types of hypothesis-driven analyses were conducted to identify potential relationships between behavioral measures and brain activity. In the first type of analysis, we conducted multiple correlation tests to identify relationships between behavioral measures of motor sequence learning success and BOLD activity. Specifically, we tested for correlations between (i) the mean BOLD response in the cortical and subcortical ROIs that showed task-activated clusters in Segawa *et al.* (2015; Table 2) in both the *novel non-native – practice non-native* and the *practiced non-native – practiced native* contrasts and (ii) each of the three motor learning indices (error rate, utterance duration, and RT). No correction was applied for the number of ROIs in this ROI list. FreeSurfer was used to define the ROIs on each individual cortical surface using the labeling system described in Cai, Tourville, Beal, Perkell, Guenther, and Ghosh (2014). These ROIs included left-lateralized aINS, vPMC, anterior frontal operculum [aFO], preSMA, IFS, posterior superior temporal sulcus [pSTs], and planum temporale [PT], as well as left GPi and GPe. For each participant, we computed each motor learning index by subtracting the mean performance difference between the *novel non-native* and *practiced non-native* productions. We normalized for differences between participants by dividing these differences by the *practiced non-native* syllable measure, on a per-participant basis. For example, the utterance duration learning measure was the mean duration difference between the *novel non-native* and *practiced non-native* productions divided by mean

duration of the *practiced non-native* productions. Each motor learning index was then correlated with the mean beta coefficient within each significant cluster from each contrast.

In the second type of analysis, SSI measures (Riley, 2008) of stuttering frequency and overall severity (described above) were used, in addition to the number of disfluencies produced during the test phase (inside the scanner), to identify potential relations between stuttering-related behaviors and brain activity. Specifically, we tested for correlations in the AWS group only with the mean composite SSI scores and mean disfluency rates during test (inside the scanner) and the mean BOLD responses in 8 hypothesis-based ROIs within the cortico-BG loops (listed in Table 2) in the *novel non-native – practice non-native* contrast (based on fluent trials only). These ROIs included left premotor and primary motor areas (ventral and mid premotor cortices [vPMC, midPMC] + ventral and mid motor cortices [vMC, midMC]), left medial prefrontal areas (left supplementary motor area [SMA] + preSMA), as well as several left-lateralized subcortical basal ganglia sites (GPi, GPe, caudate, putamen, ventral anterior nucleus [VA], ventral lateral nucleus [VL]).

3. Results

3.1 Behavioral Measures of Speech Motor Sequence Learning

3.1.1. Training Phase. Table 3 shows a summary of the behavioral results for each phase of the experiment and for each group (ANS vs. AWS). Our first set of analyses examined, at the behavioral level, whether participants showed evidence of incremental speech motor sequence learning over the course of the training phase by examining the time course of improvement in each of the three performance measures – mean error rates, utterance durations, and RTs – over the two days of speech motor practice. Each performance measure was averaged within each group, training block, condition, and participant. Again, duration and RT measures were only

analyzed for utterances that were perceptually coded as having been properly executed (see above) on each day. To visualize the running estimate of the learning trajectories across participants, Figure 1 plots the time course of improvement of each performance measure as a function of group, training block, and condition. Separate analyses of variance (ANOVAs) were performed on the mean sequencing error rates, durations, and RTs with training block (1-8) and condition (native vs. non-native) as within-subjects factors and group (ANS vs. AWS) as a between-subjects factor. In these and all subsequent ANOVAs, Greenhouse-Geisser corrections were applied when appropriate and partial eta-squared effect sizes were calculated for all main effects and interactions. Post-hoc pairwise comparisons were reported as significant at the .05 level.

-- [Insert Table 3 about here] --

The ANOVA performed on mean error rates (shown in Figure 2A) revealed a significant main effect of group [$F(1,29) = 5.036, p = .033, \eta^2_p = .148$], such that AWS [mean (M) = 32.7; standard deviation (SD) = 13.6] produced more sequencing errors than ANS [$M = 23.0; SD = 10.3$] in general. There were also highly significant main effects of condition [$F(1,29) = 208.822, p < .001, \eta^2_p = .878$] and block [$F(7,203) = 15.985, p < .001, \eta^2_p = .355$]. There was one significant interaction, the stimulus condition $\times$ block interaction [$F(7,203) = 8.863, p < .001, \eta^2_p = .234$]. All other interactions were not significant [$p > .05$, in all cases]. Simple effects tests on the condition $\times$ block interaction revealed that there was a significant effect of block on both the native sequences [$F(7,210) = 2.958, p = 0.045, \eta^2_p = 0.090$] and the non-native sequences [$F(7,210) = 20.495, p < 0.001, \eta^2_p = 0.406$]. However, the effect size was considerably larger in the non-native condition, indicating that sequence learning was greater for novel words with unfamiliar consonant clusters than novel words with familiar consonant clusters during training. This is in keeping with the

behavioral results reported by Segawa and colleagues (2015, 2019) which found that relatively less learning occurred for native compared to nonnative sequences.

The ANOVA performed on mean utterance duration (shown in Figure 1B) revealed a main effect of group [$F(1,29)=8.963, p=.006, \eta^2_p=.236$], such that ANS produced shorter utterances [$M=.59; SD=.05$] than AWS [$M=.69; SD=.10$]. There was also a significant effect of stimulus condition [$F(1,7)=34.318, p<.001, \eta^2_p=.542$], such that the native sequences [$M=.61; SD=.09$] were uttered faster than the non-native sequences [$M=.67; SD=.10$], as well as a condition X block interaction [$F(7,203)=2.066, p=.049, \eta^2_p=.066$]. All other interactions were not significant [$p>.05$, in all cases]. Consistent with our expectations based on our prior studies, simple effects tests on the condition X block interaction revealed that there was a significant effect of block on the non-native sequences [$F(7,210)=3.340, p=0.002, \eta^2_p=0.100$] but not on the native sequences [$F(7,210)=1.038, p=0.382, \eta^2_p=0.033$]. Thus, participants only got reliably faster at executing novel words during training if they contained unfamiliar consonant clusters, likely because they were near ceiling performance for novel words using native clusters.

The ANOVA performed on mean RT scores (shown in Figure 1C) revealed a highly significant effect for block [$F(7, 203) = 16.742, p < .001, \eta^2_p = .366$], such that participants got faster at initiating their utterances during the training sessions. The effect of group, condition, and all other interactions were not significant [$p > .05$ in all cases].

In an additional analysis, we examined the mean disfluency rates for the AWS group only. These are shown in Figure 2 averaged across each sequence type (native vs. non-native) and block (1-8). An ANOVA on these scores – sequence type X block – showed no significant main effects or interaction [$p > .05$ in all cases], indicating that stuttering speakers were equally disfluent for both sequence types throughout training.

3.1.2. Test Phase.

Our second set of analyses examined the same set of behavioral measures of sequence generation while participants were inside the MRI scanner during the test phase. Each performance measure was averaged within each group, testing block, condition, and participant. Duration and RT measures were again only analyzed for utterances coded as properly sequenced productions (see above) on each day. Table 4 shows the mean error rates during the test phase as a function of error subtype and sequence type. Figure 3 shows the mean error rates, utterance duration, and RT scores as a function of group, test block, and condition. Separate analyses of variance (ANOVAs) were performed on the mean error rates, utterance durations, and RTs with group (AWS vs. ANS) as a between-subjects factor, and test block (1-8) and experimental condition (*practiced native* vs. *practiced non-native* vs. *novel non-native*) as within-subjects factors.

-- [Insert Table 4 about here] --

The ANOVA performed on mean error rates (shown in Figure 3A) revealed significant main effects of block [$F(7, 196) = 5.291, p < .001, \eta^2 = .159$] and condition [$F(2, 56) = 91.164, p < .001, \eta^2 = .765$]. There was no significant main effect of group [$F(1, 28) = 1.371, p = .251, \eta^2 = .092$] or interaction effects [$p > .05$ in all cases]. Post-hoc *t*-test comparisons performed on the main effect of condition indicated that, regardless of group, the mean error rates for the *practiced native* syllables [$M = 4.0; SD = 7.2$] were significantly lower than the *practiced non-native* syllables [$M = 34.8; SD = 20.2; t(30) = -9.357, p < .001, \text{Cohen's } d = 2.03$] and *novel non-native* syllables [$M = 44.7; SD = 21.0; t(30) = -10.978, p < .001, d = 2.59$], and the mean error rates for the *practiced non-native* syllables were lower than the *novel non-native* syllables [$t(30) = -4.653, p < .001, d = .48$].

Measures of mean utterance duration (shown in Figure 3B) patterned similarly. The ANOVA performed on mean utterance durations indicated that there also significant main effects of block [$F(7, 203) = 2.638, p = .033, \eta^2 = .083$] and condition [$F(2, 58) = 40.733, p < .001, \eta^2 = .584$]. Again, there was no significant main effect of group [$F(1, 29) = .112, p = .740, \eta^2 = .004$] or interaction effects [$p > .05$ in all cases]. Post-hoc *t*-test comparisons performed on the main effect of condition indicated that, regardless of group, participants were faster at executing the *practiced native* syllables [$M = .67; SD = .15$] than the *practiced non-native* syllables [$M = .74; SD = .16; t(30) = -6.797, p < .001, d = .45$] and *novel non-native* syllables [$M = .77; SD = .19; t(30) = -7.454, p < .001, d = .58$]. In addition, participants were faster at executing the *practiced non-native* syllables than the *novel non-native* syllables [$t(30) = -3.044, p = .005, d = .17$].

The ANOVA performed on mean RT scores (shown in Figure 3C) showed no main effect of condition [$F(2, 58) = 1.688, p = .200, \eta^2_p = .054$]. There were, however, significant main effects of block [$F(7, 203) = 3.114, p = .039, \eta^2_p = .097$] and group [$F(1, 29) = 5.926, p = .021, \eta^2_p = .170$], such that AWS took longer to initiate their utterances [$M = .71; SD = .36$] than ANS [$M = .48; SD = .16$]. The condition X group interaction effect also reached statistical significance [$F(2, 58) = 4.973, p = .012, \eta^2_p = .146$]. Simple effects tests on the condition X group interaction indicated that the effect of condition approached significance in AWS [$F(2, 30) = 3.555, p = .053, \eta^2_p = .369$], but not in ANS [$F(2, 28) = .540, p = .589, \eta^2_p = .037$]. All other interactions were not significant [$p > .05$ in all cases].

Finally, we again examined mean disfluency rates for the AWS group while in the scanner. These scores are shown in Figure 4. The ANOVA performed on these scores – sequence type X block – revealed a highly significant main effect of condition [$F(2, 30) = 9.959, p < .001, \eta^2_p = .399$]. There was no significant main effect of block or interaction effect [$p > .05$ in both cases].

Post-hoc *t*-test comparisons performed on the main effect of condition indicated that AWS were less disfluent when executing the *practiced native* syllables [$M = 4.6$; $SD = 11.8$] compared to the *practiced non-native* syllables [$M = 7.5$; $SD = 15.6$; $t(15) = -2.442$, $p = .027$, $d = .32$] and *novel non-native* syllables [$M = 9.8$; $SD = 19.4$; $t(15) = -3.476$, $p = .003$, $d = .49$]. As well, AWS were less disfluent when producing the *practiced non-native* than the *novel non-native* syllables [$t(15) = -2.307$, $p = .036$, $d = .19$].

In summary, we obtained similar results as in our prior studies on speech motor sequence learning with neurotypical speakers (Segawa *et al.*, 2015, 2019): Participants – ANS and AWS alike – showed incremental improvements in performance speed and accuracy, especially for the non-native ones, with repetition and practice during training and these gains were maintained overnight in-between experimental sessions. Moreover, the data indicated that, after two days of extended speech motor practice, speakers from both groups showed further performance gains in accuracy (reduced error rate) for the *practiced non-native* sequences throughout the test phase (Figure 3A). Although both groups showed comparable gains, the AWS produced the sequences slower and/or less accurately prior to and after training. Overall, these findings provide evidence at the behavioral level that AWS do not show deficits in the acquisition or retention of new speech motor sequences. Finally, RT scores were notably higher during test (inside the scanner) than during training (outside of the scanner), especially for the AWS group. Given that the experimental paradigm during the test phase was identical to that used during the training phase, this difference in initiation speed likely reflects differences in performance anxiety outside versus inside the scanner.

3.2. Neural correlates of speech motor sequence learning

3.2.1 FMRI analysis

Before performing direct group comparisons, we first assessed differences between the condition-specific brain activations using the pooled results (ANS and AWS combined). Figure 5A and Table 5 show the brain regions that were significantly more active during the execution of *novel non-native* than *practiced non-native* syllables (TFCE cluster-level $p_{FWE} < .05$)³ aggregated across both experimental groups. The surface/vertex-based analyses revealed that the production of *novel non-native* syllables resulted in greater BOLD response in pre-SMA, aINS, aFO, pFO, and IPS bilaterally. In the left hemisphere, additional cortical clusters were noted with peaks in IFS, vPMC, pIFG, midPMC, vMC, midMC, STG, and inferior temporal occipital (ITO) region. The volume/voxel-based analysis found no statistically significant differences in subcortical activity. We supplemented this voxel-based analysis with a hypothesis-based subcortical ROI analysis based on the results reported in Segawa *et al.* (2015) for this contrast. Specifically, we tested for differences in activation in left globus pallidus internal (GPi) and external (GPe). Results from this analysis demonstrated that left GPi was more activate during production of the *novel non-native* than the *practiced non-native* words [$t(30) = 2.22, p\text{-unc} = .034, r = .375$], in effect replicating the finding of Segawa *et al.* (2015) for the same contrast. No significant groups effects (ANS vs. AWS) were found for any cortical or subcortical region in either hemisphere for the *novel non-native* - *practiced non-native* contrast; nevertheless, we present the results (albeit non-significant) for the two groups separately in Figures 5B.

Figure 6A and Table 6 show the brain regions that were significantly more active during the execution of *practiced non-native* than *practiced native* syllables (TFCE cluster-level $p_{FWE} < .05$). The surface/vertex-based analyses revealed that the production of *practiced non-native* words resulted in greater BOLD response in pSTG and OC bilaterally. The increased activity in OC likely

³ For each of the two speech contrast conditions, we report the results from one-sided (positive only) tests.

reflects greater attention and/or depth of processing while reading the non-native text stimuli, rather than differences in speech production. In the left hemisphere, additional cortical clusters were noted with peaks in pre-SMA, aINS, FO, pIFG, vPMC, midPMC, aSTG, vSC, and IPS. The volume/voxel-based analysis found increased activation in the right cerebellum (lobule XIII) during the *practiced non-native* than the *practiced native* condition. A hypothesis-based subcortical ROI analysis based on the results reported in Segawa *et al.* (2015) for this contrast was then performed. Specifically, we tested for differences in activation in right cerebellum (lobule VI). Results from this analysis demonstrated that this region was more activate during production of the *practiced non-native* than the *practiced native* words [$t(30) = 2.61, p\text{-FDR} = .014, r = .430$], again replicating findings from Segawa *et al.* (2015). As in the other contrast, no region in either hemisphere was found to be significantly more active for this contrast in either group (ANS vs. AWS) at the cortical or subcortical levels, further bolstering the view that speech motor learning mechanisms are unimpaired in stuttering; again, we present the results (albeit non-significant) for the two groups separately in Figures 6B.

Although no significant groups effects were found, several interesting trends emerged that are worth noting and speculating about. First, brain areas linked to speech premotor planning (vPMC, mid-PMC, FO, aINS) showed a non-significant trend toward greater activation in ANS during novel sequence production, suggesting that neurotypical speakers may show greater learning of the motor “chunks” for the trained sequences than AWS. Second, brain areas involved in auditory processing (pSTG) showed a non-significant trend toward greater activation in AWS during novel sequence production, suggesting that, following motor practice, stuttering speakers may still be relying more on auditory feedback to guide them toward the intended production

targets compared to ANS. Critically, however, there were no brain areas activated in AWS that fell outside the network of areas identified in ANS and in the previous study (Segawa *et al.*, 2015).

3.3 Brain-behavior correlation analysis

The correlation tests between behavioral measures of learning success and mean activation in the 12 hypothesis-based ROIs from Segawa *et al.* (2015) (listed in Table 2) for the *novel non-native* – *practiced non-native* contrast revealed no group differences at either the cortical or subcortical levels. In the analyses performed on the pooled data (ANS and AWS combined), we found that learning success, as measured by the participant-normalized difference in mean error rates between the *novel non-native* and *practiced non-native* words, was positively correlated with the mean activation in both the left [$t(29) = 2.22, p = .034, r = .381$] and right aINS [$t(29) = 2.38, p = .024, r = .404$], such that processing in these areas increased most with learning in participants who showed the greatest performance improvements. These results provide support for the hypothesis that these premotor areas are involved in speech motor sequence learning. No significant correlations emerged between the participant-normalized differences in mean utterance duration (between the *novel non-native* and *practiced non-native* words) and the mean activation in any of the ROIs (in all cases, $p_{unc} > .05$).

-- [Insert Figure 7 about here] --

Finally, within the AWS group, we assessed whether stuttering severity (as indexed by SSI scores and mean disfluency rates inside the scanner during the test phase) was correlated with activation in 8 hypothesis-based ROIs within the cortico-BG loops (listed in Table 2) for the *novel non-native* – *practiced non-native* contrast. Correlation tests revealed that mean disfluency rates inside the scanner were negatively correlated with mean activation in left GPe [$t(14) = -2.88, p\text{-FDR} = .047, r = -0.609$] and left caudate [$t(14) = -2.74, p\text{-FDR} = .047, r = -.591$] (i.e., the more

disfluent participants were during the test phase, the less BOLD activity change was observed in these basal ganglia sites for this contrast). Although this result is correlational and therefore a causal relationship cannot be firmly established, it is nonetheless compatible with the idea that speakers are more likely to stutter when the left GPe and left caudate are more hypoactive. In contrast, no statistically significant correlations were found between SSI scores and activation in any of the 8 ROIs. The latter result is perhaps unsurprising given that the SSI does not measure disfluency during *non-native* sequence production and its affiliated scores were not based on speech uttered inside the scanner.

-- [Insert Figure 8 about here] --

In summary, we found that, across both ANS and AWS, greater activity was observed during the production of novel sequences in brain regions previously associated with learning and maintaining speech motor programs, including lateral premotor cortex, FO, aINS, posterior superior temporal cortex, and right cerebellum (Segawa *et al.*, 2015; Guenther, 2016). Measures of learning success correlated positively with activity in the left and right aINS, suggesting that this brain area plays an important role in speech motor learning. In AWS, measures of in-scanner disfluency rates were negatively correlated with activity in left-lateralized basal ganglia structures. Overall, these findings provide evidence that the neurobiological correlates of speech motor sequence learning do not differ across ANS and AWS.

4. Discussion

The goal of the current study was to investigate the behavioral and neural correlates of speech motor sequence learning in both stuttering and neurotypical adult speakers. Using a cued sequence production task, we examined changes in the performance of pseudowords containing non-native consonant clusters over time as a function of speech motor practice. At the behavioral level, we

found that, although AWS were less accurate and/or slower compared to ANS at producing words involving non-native consonant clusters regardless of whether the words were practiced, they showed statistically comparable improvements in these measures with practice to those seen in ANS. At the neural level, we found that, across both groups and both speech contrasts (*novel non-native* – *practiced non-native* and *practiced non-native* – *practiced native*), practice producing words with non-native clusters led to significant decreases in BOLD activity in brain areas implicated in verbal working memory and speech motor planning (including left preSMA, FO, aINS, and bilateral IPS), suggesting that processing load and articulatory effort decreases as the nervous system forms regularized motor programs (or “chunks”) for coordinated sequences of vocal tract gestures. Critically, there were no reliable group differences in either contrast map in any areas involved in the cortico-BG loops (or otherwise). Furthermore, the degree of motor performance gains in accuracy were correlated positively with activity in the left and right aINS. Collectively, these findings suggest that cortico-BG-mediated mechanisms involved in *learning* novel phoneme sequences are not impaired in AWS; instead, the impairment is primarily in the *motor execution* of speech sequences, novel and learned alike.

As in our prior study (Segawa *et al.*, 2015), we also observed pre- to post-training BOLD activation changes in cortical areas that extend beyond those implicated in verbal working memory and speech motor planning processes. Specifically, we found greater activity in areas related to auditory processes (left PT, aSTG, and pSTG) during production of the *novel non-native* sequences relative to the *practiced non-native* sequences (Figure 5). These auditory regions are thought to be involved in guiding speech movements based on self-generated auditory feedback (Hickok, 2012; Guenther, 2016). During speech production, activity in this area has been reported to be greater when there is a mismatch between predicted and actual auditory feedback (Tourville, Reilly, &

Guenther, 2008). Several existing theoretical models of speech production (e.g., Hickok, 2012; Guenther, 2016) propose that error signals arising from these regions are used to fine-tune speech motor programs over the course of repeated production attempts. Thus, learning is thought to rely on the transmission of these auditory error signals to frontal regions involved in motor planning and execution.

We also noted greater activity in cortical areas related to orthographic processing (left ITO) during production of the *novel non-native* sequences relative to the *practiced non-native* sequences (Figure 5). Prior neuroimaging studies suggest that ITO is a higher-level visual processing area involved in identifying letters and words from lower-level shape images (see, e.g., Pugh, Mencl, Jenner, Katz, Frost, Lee, *et al.*, 2001) and is therefore highly likely to be related to viewing and decoding the pseudowords that our participants were instructed to produce, rather than speech motor control. In contrast, production of the *practiced non-native* sequences did not produce significantly greater activity compared with the *practiced native* sequences in ITO (Figure 6). This difference between the condition-specific brain activations suggests that pseudowords containing unfamiliar phonotactic sequences are more difficult to process prior to reading and repetition practice (cf. Pugh, Frost, Sandak, Landi, Rueckl, Constable, Seidenberg, Fulbright, Katz, & Mencl, 2008).

The finding that the right cerebellum (which is structurally and functionally connected with left cortical areas) was recruited to a greater extent across both groups during the production of the *practiced non-native* than the *practiced native* sequences (shown in Figure 6) was also observed by Segawa *et al.* (2015) in ANS, and corroborates other reports with neurotypical participants that the cerebellum plays an important role in motor sequence learning and fine motor control (e.g., Doyon, Song, Karni, Lalonde, Adama, & Ungerleider, 2002; Ackermann, 2008; Guenther, 2016,

chapter 2). For example, Bohland and Guenther (2006) found that different regions of the cerebellum showed differential sensitivity to syllable complexity (e.g., “stra vs. ta”) and serial complexity (e.g., “da-da-da” vs. “da-ru-ti”) in ANS, suggesting involvement in speech sequencing. That said, it is somewhat surprising that we did not find significant differences in cerebellar activity in the *novel non-native* – *practiced non-native* contrast. However, this comparison possessed less statistical power because of more removed error trials.

An alternative, but not mutually exclusive, interpretation to the cortical activity differences we found for both speech contrasts is that these differences result from the fact that the *novel non-native* and *practiced non-native* sequences are more difficult to *vocally imitate* than the *practiced native* sequences. Two sources of evidence support this conjecture. First, prior neuroimaging studies (e.g., Irwin, Frost, Mencl, Chen & Fowler, 2011) have reported that imitation of auditory speech produces additional significant activations in left IFG and aINS when compared to a passive listening condition. Second, current computational models, such as the *Directions Into Velocities of the Articulators* (DIVA) model (Guenther, 2016), propose that speech motor learning is driven by initial mismatches between newly acquired sound targets and one’s own production attempts as represented in the auditory cortex. Auditory error signals are then transformed into corrective motor commands, and these corrective commands alter the feedforward commands for the next production attempt. As the feedforward commands improve, fewer error signals are generated and thus the contribution of the feedback control system gradually diminishes. The DIVA model thus predicts decreases in BOLD activation in both motor planning and auditory cortical areas as a consequence of imitation-based learning. In this view, the nervous system begins to form speech motor programs via an imitation-based learning mechanism, which may account for the increased activity in motor and auditory areas for the *novel non-native* compared with the

749 *practiced non-native* sequences, and also for the *practiced non-native* compared with the *practiced*
750 *native* sequences.

751 A number of other neuroimaging studies, compiled and discussed in a meta-analysis by
752 Belyk and colleagues (Belyk, Kraft, & Brown, 2015), have reported that AWS tend to display
753 higher activity in a number of right-hemisphere regions during fluently produced native speech
754 when compared to ANS. Some researchers have proposed that this right-hemisphere cortical
755 hyperactivity arises from impaired left-hemisphere function (see, e.g., Fox *et al.*, 1996; Belyk *et*
756 *al.*, 2015; Guenther, 2016; Neef, Anwender, Butfering, Schmidt-Samoa, Friederici, Paulus, &
757 Sommer, 2017; cf. Connalley *et al.*, 2018). In view of these results, it is perhaps surprising that no
758 clusters emerged in the right hemisphere for AWS in the current study. However, it is possible that
759 right-hemisphere hyperactivity in AWS may not occur in all speaking situations. In particular, the
760 current speaking task likely required more attention and articulatory effort than those in prior
761 studies since the participants were required to produce non-native sequences of segments, which
762 is known to be difficult (e.g., Davidson, 2006, 2010). In addition, there may be methodological
763 issues that concern the difference between the experimental procedures we used to test for a
764 functional activation difference between ANS and AWS and the ones used by other research
765 groups as the existing literature on stuttering encompasses a diverse set of sampling protocols and
766 motor tasks.

767 In the current study, it was further demonstrated, in AWS, that in-scanner mean disfluency
768 covaried with BOLD responses in left-lateralized basal ganglia sites (GPe and left caudate), such
769 that the more disfluent participants were during the test phase, the less BOLD activity change was
770 observed in these basal ganglia sites for the *novel non-native* – *practiced non-native* contrast. This
771 negative correlation does not establish a causal relationship; rather it establishes an associative

link. Although this relationship is broadly consistent with the long-standing view that stuttering reflects a malfunction within the cortico-BG loops (e.g., Fox *et al.*, 1996; Alm, 2004; Giraud *et al.*, 2007; Watkins *et al.*, 2008; Kell, Neumann, von Kriegstein, Posenenske, von Gudenberg, Euler, & Giraud, 2009; Lu, Chen, Ning, Ding, Guo, Peng, Yang, Li, & Lin, 2010; Connally *et al.*, 2018; Chang & Guenther, 2020), it would appear to be at odds with other neuroimaging studies (albeit that did not use a sparse-sampling method) which have reported *positive* relationships between basal ganglia activity and stuttering severity (e.g., Giraud *et al.*, 2008; see also, Metzger *et al.*, 2018, for a similar finding based on manual motor responses in AWS). Differences between the findings of the current study and these prior studies suggest that the brain mechanisms underlying *general stuttering traits* and *transient disfluent states* are dissociable (for further discussion, see Connally *et al.*, 2018).

At first glance, the lack of a robust learning difference between the ANS and AWS groups may appear to be at odds with other behavioral studies reporting motor learning deficits in AWS (Ludlow, Siren, & Zikria, 1997; Smits-Bandstra, De Nil, & Saint-Cyr, 2006; Namasivayam & van Lieshout, 2008). Several important differences between those studies and ours might account for the divergent results. First, our study measured sequence and cluster learning using perception-based segmental transcription and simple acoustic measures. However, this approach required transcribers to make categorical decisions regarding the segments that speakers produce, and thus did not permit quantitative analysis of the presence or magnitude of various gestures, or how speakers continually updated their implementation of novel gestural scores. In contrast, other motor learning studies (e.g., Namasivayam & van Lieshout, 2008) used kinematic measures to compare more subtle aspects of speech articulation between ANS and AWS. Thus, subtle differences in the articulatory correlates of speech motor learning may exist between ANS and

795 AWS. Ongoing studies based on the current design that use electromagnetic articulography will
796 determine whether AWS and ANS differ in their ability to learn to execute and coordinate inter-
797 articulator movements for novel speech sequences.

798 Second, our study focused on the learning of new phoneme sequences within a single
799 syllable, with which the participants had no prior experience since they violated English
800 phonotactic constraints. This task required learning of new articulatory sequences at a motoric
801 level. In contrast, other studies in the literature on speech motor sequence learning in AWS have
802 involved production of more extended, multi-syllabic sequences that conform to English
803 phonotactics (e.g., Namasivayam & van Lieshout, 2008). However, there is evidence that multi-
804 syllabic sequences places higher demands on phonological working memory mechanisms than
805 motor programming mechanisms. For example, in another fMRI study, McGettigan, Warren,
806 Eisner, Marshall, Shanmugalingam, and Scott (2010) found that activity in planum temporale, a
807 region implicated in auditory working memory, increased as the number of syllables in either a
808 non-word repetition or passive listening task increased, whereas activity in left SMA, a region
809 implicated in speech motor programming, increased as the number of consonant clusters increased.
810 In this light, the increased error rates reported in other studies with AWS might be interpreted
811 instead as reflecting differences in phonological working memory mechanisms, rather than motor
812 learning or implementation mechanisms (see also, Yang, Jia, Fox, Siok, & Tan, 2019). Yet others
813 have reported some differences between ANS and AWS in their degree of *motor adaptation*
814 following an experimental perturbation to their online sensory feedback (e.g., Daliri, Wieland, Cai,
815 Guenther, & Chang, 2018), suggesting that mechanisms involved in *updating* existing motor
816 programs may still be impaired in stuttering.

It is also important to note that the present fMRI measures focused on the *outcome* of speech motor sequence learning, not the *online process* of learning. However, previous research, spanning a wide range of learning tasks and paradigms, has shown that participants may have similar learning outcomes despite very different learning trajectories (see Karuza, Emberson, & Aslin, 2014, for a review). Thus, despite showing comparable BOLD activity patterns at test, ANS and AWS may not show evidence of the same, qualitative neural changes during the course of speech motor sequence training. It would therefore be of interest to conduct further fMRI studies, based on the current design, that perform BOLD contrast analyses throughout both the training and test phases.

Finally, the current study focused primarily on the mechanisms of *speech motor learning and control*, from the phonetic encoding stage down to the motor commands to the speech articulators. Specifically, we examined how ANS and AWS learn to transform discrete “phonological chunks” (speech sounds, which can be phonemes, syllables, or words) into a set of articulator movements that achieves the intended auditory “target.” We employed monosyllabic, pseudoword stimuli to provide a “purer” measure of the speech motor system, as this would limit the recruitment of higher-level cognitive and linguistic processing strategies to assist task performance (see also, McGettigan *et al.*, 2010). However, any viable theory of speech production learning will ultimately have to explicate how these phonological chunks are integrated with prosodic structures, as well as their relation to syntactic/semantic planning processes. Future studies will aim to develop predictions from the highly controlled laboratory experiments presented here and elsewhere (e.g., Segawa *et al.*, 2015, 2019) that may be tested when speakers are instructed to produce novel speech sequences in a more natural context.

References

- Ackermann, H. (2008). Cerebellar contributions to speech production and speech perception: psycholinguistic and neurobiological perspectives. *Trends in Neurosciences*, 31, 265–272.
- Alexander, G.E., DeLong, M.R., & Strick, P.L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience*, 9, 357-381.
- Alm, P. A. (2004). Stuttering and the basal ganglia circuits: a critical review of possible relations. *Journal of Communication Disorders*, 37, 325–369. doi: 10.1016/j.jcomdis.2004.03.001
- Anderson, J.L., Hutton, C., Ashburner, J., Turner, R., & Friston, K. (2001). Modeling geometric deformations in EPI time series. *NeuroImage*, 13(5), 903-919.
- Ashburner, J., & Friston, K.J. (2005). Unified segmentation. *NeuroImage*, 26(3), 839-851.
- Belin, P., Zatorre, R. J., Hoge, R., Evans, A. C., & Pike, B. (1999). Event-related fMRI of the auditory cortex. *NeuroImage*, 10, 417–429.
- Belyk, M., Kraft, S. J., & Brown, S. (2015). Stuttering as a trait or state—an ALE meta-analysis of neuroimaging studies. *European Journal of Neuroscience*, 41, 275–284.
- Berent, I., Steriade, D., Lennertz, T., & Vaknin, V. (2007). What we know about what we have never heard: Evidence from perceptual illusions. *Cognition*, 104, 591-630.
- Bloodstein, O., and Ratner, N. B. (2008). A handbook on stuttering. Clifton Park, NY: Thomson/Delmar Learning, 552.
- Bohland, J.W., Bullock, D., & Guenther, F.H. (2010). Neural representations and mechanisms for the performance of simple speech sequences. *Journal of Cognitive Neuroscience*, 22(7), pp. 1504-1529.
- Boersma, P., & Weenink, D. (2020). Praat: doing phonetics by computer [Computer

- 862 program]. Version 6.1.09, retrieved 26 January 2020 from <http://www.praat.org/>
- 863 Bohland, J. W., & Guenther, F. H. (2006). An fMRI investigation of syllable sequence production.
- 864 *NeuroImage*, 32, 821–841.
- 865 Boutsen, F. (1993). A comparative study of stress timing of stutterers and nonstutterers. *Journal*
- 866 *of Fluency Disorders*, 20, 145-155.
- 867 Brawn, T.P., Fenn, K.M., Nusbaum, H.C., & Margoliash, D. (2010). Consolidating the effects of
- 868 waking and sleep on motor-sequence learning. *Journal of Neuroscience*, 30(42), 13977-
- 869 13982.
- 870 Buchwald, A., Calhoun, H., Rimikis, S., Steinberg Lowe, M, Wellner, R., & Edwards, D. (2019).
- 871 Using tDCS to facilitate motor learning in speech production: The role of timing. *Cortex*,
- 872 274-285.
- 873 Byrd, D. (1996). Influences on articulatory timing in consonant sequences. *Journal of Phonetics*,
- 874 24(2), 209-244.
- 875 Cai, S., Tourville, J. A., Beal, D. S., Perkell, J. S., Guenther, F. H., & Ghosh, S. S. (2014). Diffusion
- 876 imaging of cerebral white matter in persons who stutter: evidence for network-level
- 877 canomalies. *Frontiers in Human Neuroscience*, 8, 18 pages.
- 878 Chang, S.-E., Ohde, R.N., & Conture, E.G. (2002). Coarticulation and formant transition rate in
- 879 young children who stutter. *Journal of Speech, Language, and Hearing Research*, 45, 676-
- 880 688.
- 881 Chang, S.-E., & Guenther, F.H. (2020). Involvement of the cortico-basal-ganglia-thalamocortical
- 882 loop in developmental stuttering. *Frontiers in Psychology*, 10, 1-15.
- 883 Cholin, J., Levelt, W.J.M., & Schiller, N.O. (2006). Effects of syllable frequency in speech
- 884 production. *Cognition*, 99, 205-235.

- 885 Civier, O., Bullock, D., Max, L., and Guenther, F. H. (2013). Computational modeling of stuttering
 886 caused by impairments in a basal ganglia thalamocortical circuit involved in syllable
 887 selection and initiation. *Brain and Language*. 126, 263–278.
- 888 Connally, E.L., Ward, D., Pliatsikas, C., Finnegan, S., Jenkinson, M., Boyles, R., Watkins, K.E.
 889 (2018). Separation of trait and state in stuttering. *Human Brain Mapping*, 39, 3109-3126.
- 890 Coltheart, M. (1981). The MRC Psycholinguistic Database. *The Quarterly Journal of*
 891 *Experimental Psychology Section A*. 33(4), 497-505.
- 892 Craig-McQuaide, A., Akram, H., Zrinzo, L., & Tripoliti, E. (2014). A review of brain circuitries
 893 involved in stuttering. *Frontiers in Human Neuroscience*, doi: 10.3389/fnhum.2014.00884
- 894 Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface- based analysis—I. Segmentation
 895 and surface reconstruction. *NeuroImage*, 9, 179–194.
- 896 Daliri, A., Wieland, E. A., Cai, S., Guenther, F. H., & Chang, S. E. (2018). Auditory-motor
 897 adaptation is reduced in adults who stutter but not in children who stutter. *Developmental*
 898 *Science*, 21:e12521.
- 899 Davidson, L. (2006). Phonology, phonetics, or frequency: Influences on the production of non-
 900 native sequences. *Journal of Phonetics*, 34, 104-137.
- 901 Davidson, L. (2010). Phonetic bases of similarities in cross-language production: Evidence from
 902 English and Catalan. *Journal of Phonetics*, 38, 2, 272-288.
- 903 Davidson, L., & Shaw, J. (2012). Sources of illusion in consonant cluster perception. *Journal of*
 904 *Phonetics*, 40(2), 234-248.
- 905 Diedrichsen, J., Balsters, J. H., Flavell, J., Cussans, E., & Ramnani, N. (2009). A probabilistic atlas
 906 of the human cerebellum. *Neuroimage*, 15, 39-46.
- 907 Doyon, J., Song, A. W., Karni, A., Lalonde, F., Adams, M. M., & Ungerleider, L. G. (2002).

- 908 Experience-dependent changes in cerebellar contributions to motor sequence learning.
 909 *Proceedings of the National Academy of Sciences*, U.S.A., 99, 1017–1022.
- 910 Doyon, J., Gabbitov, E., Vahdat, S., Lungu, O., & Boutin, A. (2018). Current issues related to motor
 911 sequence learning in humans. *Current Opinion in Behavioral Sciences*, 20, 89-97.
- 912 Dupoux, E., Kakehi, K., Pallier, Y., Hirose, C., & Mehler, J. (1999). Epenthetic vowels in
 913 Japanese: a perceptual illusion? *Journal of Experimental Psychology: Human Perception*
 914 *and Performance*, 25(6), 1568-1578.
- 915 Dupoux, E., Parlato, E., Frota, S., Hirose, Y., & Peperkamp, S. (2011). Where do illusory vowels
 916 comes from? *Journal of Memory and Language*, 64(3), 199-210.
- 917 Fee, M.S., & Goldberg, J.H. (2011). A hypothesis for basal ganglia dependent reinforcement
 918 learning in the songbird. *Journal of Neuroscience*, 198, 152-70.
- 919 Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2003). Consolidation during sleep of perceptual
 920 learning of spoken language. *Nature*, 425, 614–616.
- 921 Ferraro, F. R., Balota, D. A., & Connor, L. T. (1993). Implicit memory and the formation of new
 922 associations in nondemented Parkinson’s disease individuals and individuals with senile
 923 dementia of the Alzheimer type: a serial reaction time (SRT) investigation. *Brain and*
 924 *Cognition*, 21, 163–180.
- 925 Fischl, B., Sereno, M. I., & Dale, A. M. (1999). Cortical surface-based analysis—II: Inflation,
 926 flattening, and a surface-based coordinate system. *NeuroImage*, 9, 195–207.
- 927 Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole
 928 brain segmentation: Automated labeling of neuroanatomical structures in the human brain.
 929 *Neuron*, 33, 341–355.
- 930 Fox, P. T., Ingham, R. J., Ingham, J. C., Hirsch, T. B., Downs, J. H., Martin, C., Jerabek, P., Glass,

- 931 T., & Lancaster, J. L. (1996). A PET study of the neural systems of stuttering. *Nature*,
932 382(6587), 158–161.
- 933 Giraud, A.-L., Neumann, K., Bachoud-Levi, A.-C., von Gudenberg, A. W., Euler, H. A.,
934 Lanfermann, H., & Preibisch, C. (2008). Severity of dysfluency correlates with basal
935 ganglia activity in persistent developmental stuttering. *Brain and Language*, 104, 190-199.
- 936 Gracco, V.L., Tremblay, P., Pike, G.B. (2005). Imaging speech production using fMRI.
937 *NeuroImage*, 26, 294-301.
- 938 Guenther, F.H. (2016). *Neural Control of Speech*. Cambridge, MA: MIT Press.
- 939 Hagler Jr, D. J., Saygin, A. P., & Sereno, M. I. (2006). Smoothing and cluster thresholding for
940 cortical surface-based group analysis of fMRI data. *NeuroImage*, 33(4), 1093-1103.
- 941 Hayasaka, S., & Nichols, T. E. (2003). Validating cluster size inference: Random field and
942 permutation methods. *NeuroImage*, 20, 2343–2356.
- 943 Helmuth, L., Mayr, U., & Daum, I. (2000). Sequence learning in patients with Parkinson’s disease:
944 a comparison between spatial-attention and number-response sequences.
945 *Neuropsychologia*, 38, 1443–1451.
- 946 Hickok, G. (2012). Computational neuroanatomy of speech production. *Nature Reviews*
947 *Neuroscience*, 13, 135–145.
- 948 Ingham, R. J., Grafton, S. T., Bothe, A. K., & Ingham, J. C. (2012). Brain activity in adults who
949 stutter: Similarities across speaking tasks and correlations with stuttering frequency and
950 speaking rate. *Brain and Language*, 122(1), 11–24.
- 951 Irwin, J.R., Forst, S.J., Mencl, E.W., Chen, H., & Fowler, C.A. (2012). Functional activation for
952 imitation of seen and heard speech. *Journal of Neurolinguistics*, 24(6), 611-618.
- 953 Jackson, G. M., Jackson, S. R., Harrison, J., Henderson, L., & Kennard, C. (1995). Serial reaction

- 954 time learning in Parkinson's disease: evidence for a procedural learning deficit.
- 955 *Neuropsychologia*, 33, 577–593.
- 956 Jezzard, P., & Balaban, R.S. (1995). Correction for geometric distortion in echo planar images
- 957 from B0 field variations. *Magnetic Resonance in Medicine*, 34(1), 65-73.
- 958 Karuza, E.A., Emberson, L.L., & Aslin, R.N. (2014). Combining fMRI and behavioral measures
- 959 to examine the process of human learning. *Neurobiology of Learning and Memory*, 109,
- 960 193-206.
- 961 Kell, C. A., Neumann, K., von Kriegstein, K., Posenenske, C., von Guden- berg, A. W., Euler, H.,
- 962 & Giraud, A.-L.L. (2009). How the brain repairs stuttering. *Brain*, 132(10), 2747–2760.
- 963 Keuken, M. C., Bazin, P.-L., Crown, L., Hootsmans, J., Laufer, A., Müller-Axt, C., Sier, R., van
- 964 der Putten, E.J., Schafer, A., Turner, R., Forstmann, B. U. (2014). Quantifying inter-
- 965 individual anatomical variability in the subcortex using 7T structural MRI. *Neuroimage*,
- 966 94, 40–46. <https://doi.org/https://doi.org/10.1016/j.neuroimage.2014.03.032>
- 967 Kotz, S. A., & Schwartze, M. (2010). Cortical speech processing unplugged: a timely subcortico-
- 968 cortical framework. *Trends in Cognitive Science*, 14, 392–399.
- 969 Lu, C., Chen, C., Ning, N., Ding, G., Guo, T., Peng, D., Yang, Y., Li, K., & Lin, C. (2010). The
- 970 neural substrates for atypical planning and execution of word production in stuttering.
- 971 *Experimental Neurology*, 221(1), 146–156.
- 972 Ludlow, C. L., Siren, K., & Zikria, M. (1997). Speech production learning in adults with chronic
- 973 developmental stuttering. In W. Hujstijn, H. F. M. Peters, & P. H. H. M. Van Lieshout
- 974 (Eds.), *Speech production: Motor control, brain research and fluency disorders* (pp. 212–
- 975 229). Amsterdam: Elsevier Science Publishers.

- 976 Max, L, Guenther, F.H., Gracco, V.L., Ghosh, S.S., Wallace, M.E. (2004). Unstable or
 977 insufficiently activated internal models and feedback-biased motor control as sources of
 978 dysfluency: a theoretical model of stuttering. *Contemporary Issues in Communication*
 979 *Science and Disorders*, 31, 105–22.
- 980 Metzger, F.L., Auer, T., Helms, G., Paulus, W., Frahm, J., Sommer, M., & Neef, N.E. (2018).
 981 Shifted dynamic interactions between subcortical nuclei and inferior frontal gyri during
 982 response preparation in persistent developmental stuttering. *Brain Structure and Function*,
 983 223, 165-182.
- 984 McGettigan, C., Warren, J.E., Eisner, F., Marshall, C.R., Shanmugalingam, P., & Scott, S.K.
 985 (2011). Neural correlates of sublexical processing in phonological working memory.
 986 *Journal of Cognitive Neuroscience*, 23(4), 961-977.
- 987 Mollaei, F., Shiller, D.M., & Gracco, V.L. (2013). Sensorimotor adaptation of speech in
 988 Parkinson’s Disease. *Journal of Movement Disorders*, 28, 1668-1674.
- 989 Nakamura, K., Sakai, K., & Hikosaka, O. (1998). Neuronal activity in medial frontal cortex during
 990 learning of sequential procedures. *Journal of Neurophysiology*, 80, 2671–2687.
- 991 Namasivayam, A. K., & Van Lieshout, P. H. H. M. (2008). Bite-block perturbation in people who
 992 stutter: immediate compensatory and delayed adaptive processes. *Journal of*
 993 *Communication Disorders*, 41, 372-294.
- 994 Neef, N. E., Anwender, A., Butfering, C., Schmidt-Samoa, C., Friederici, A. D., Paulus, W., &
 995 Sommer, M. (2017). Structural connectivity of right frontal hyperactive areas scales with
 996 stuttering severity. *Brain*, <https://doi.org/10.1093/brain/awx316>
- 997 Niethammer, M., Feigin, A., & Eidelber, D. (2012). Functional neuroimaging in Parkinson’s
 998 disease. *Cold Spring Harbor Perspectives in Medicine*, 2, a009274.

- 999 Nieto-Castañón, A., Ghosh, S.S., Tourville, J.A., Guenther, F.H., 2003. Region of interest based
1000 analysis of functional imaging data. *Neuroimage* 19, 1303–1316.
- 1001 Nieto-Castañón, A. (2020). Handbook of functional connectivity magnetic resonance imaging
1002 methods in CONN. Boston, MA: Hilbert, Press.
- 1003 Pan, S.C., & Rickard, T.C. (2015). Sleep and motor learning: Is there room for consolidation?
1004 *Psychological Bulletin*, 141(4), 812-834.
- 1005 Perrachione, T.K., & Ghosh, S.S. (2013). Optimized design and analysis of sparse-sampling fMRI
1006 experiments. *Frontiers in Neuroscience*. 18(7), doi: 10.3389/fnins.2013.00055
- 1007 Pitt, M.A. (1998). Phonological processes and the perception of phonotactically illegal consonant
1008 clusters. *Perception & Psychophysics*, 60(6), 941-951.
- 1009 Pugh, K. R., Mencl, W. E., Jenner, A. R., Katz, L., Frost, S. J., Lee, J. R., et al. (2001).
1010 Neurobiological studies of reading and reading disability. *Journal of Communicable*
1011 *Diseases*, 34, 479–492.
- 1012 Pugh, K.R., Forst, S.F., Sandak, R., Landi, N., Rueckl, J.G., Constable, T.R., Seidenberg, M.,
1013 Fulbright, R., Katz, L., & Mencl, W.E. (2008). Effects of stimulus difficulty and repetition
1014 on printed word identification: an fMRI comparison of non-impaired and reading disabled
1015 adolescent cohorts. *Journal of Cognitive Neuroscience*, 20(7), 1146-1160.
- 1016 Rauschecker, A. M., Pringle, A., & Watkins, K. E. (2008). Changes in neural activity associated
1017 with learning to articulate novel auditory pseudowords by covert repetition. *Human Brain*
1018 *Mapping*, 29, 1231–1242.
- 1019 Riley, G.D. (2009). Stuttering severity instrument for children and adults (SSI-4) 4th ed. Pro-Ed,
1020 Inc; Austin, TX.
- 1021 Robb, M., & Blomgren, M. (1997). Analysis of F2 transitions in the speech of stutterers and

- 1022 nonstutterers. *Journal of Fluency Disorders*, 22, 1-16.
- 1023 Robb, M., Blomgren, M., & Chen, Y. (1998). Formant frequency fluctuation in stuttering and
- 1024 nonstuttering adults. *Journal of Fluency Disorders*, 23, 73-84.
- 1025 Rong, F., Isenberg, A.L., Sun, E., & Hickok, G. (2018). The neuroanatomy of speech sequencing
- 1026 at the syllable level. *PLoS ONE*, 13(10): e0196381.
- 1027 Rottschy, C., Langner, R., Dogan, I., Reetz, K., Laird, A. R., Schulz, J. B., & Eickhoff, S. B.
- 1028 (2012). Modelling neural correlates of working memory: A coordinate-based meta-
- 1029 analysis. *NeuroImage*, 60, 830–846.
- 1030 Sapis, S. (2014). Multiple factors are involved in the dysarthria associated with Parkinson’s
- 1031 disease: a review with implications for clinical practice and research. *Journal of Speech*
- 1032 *and Hearing Research*, 57, 1330–1343.
- 1033 Segawa, J., Tourville, J.A., Beal, D.S., & Guenther, F.H. (2015). The neural correlates of speech
- 1034 motor sequence learning. *Journal of Cognitive Neuroscience*, 27(4), 819-831.
- 1035 Segawa, J., Masapollo, M., Tong, M., Smith, D.J., & Guenther, F.H. (2019). Chunking of
- 1036 phonological units in speech sequencing. *Brain and Language*, 104636.
- 1037 Shima, K., & Tanji, J. (2000). Neuronal activity in the supplementary and presupplementary motor
- 1038 areas for temporal organization of multiple movements. *Journal of Neurophysiology*, 84,
- 1039 2148–2160.
- 1040 Skodda, S. (2012). Effect of deep brain stimulation on speech performance in Parkinson’s disease.
- 1041 *Parkinson’s Disease*, 2012, 850596.
- 1042 Smith, S. M., & Nichols, T. E. (2009). Threshold-free cluster enhancement: addressing problems
- 1043 of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*,
- 1044 44(1), 83-98.

- 1045 Smits-Bandstra, S., De Nil, L. F., & Rochon, E. (2006). The transition to increased automaticity
 1046 during finger sequence learning in adult males who stutter. *Journal of Fluency Disorders*,
 1047 *31*, 22–42.
- 1048 Smits-Bandstra, S., & Gracco, V. (2013). Verbal implicit sequence learning in persons who stutter
 1049 and persons with Parkinson’s disease. *Journal of Motor Behavior*, *45*, 381–393.
- 1050 Sternberg, S., Monsell, S., Knoll, R. L., & Wright, C. E. (1978). The latency and duration of rapid
 1051 movement sequences: Comparisons of speech and typing. In G. E. Stelmach (Ed.),
 1052 *Information processing in motor control and learning* (pp. 117–152). New York:
 1053 Academic Press.
- 1054 Tourville, J.A. & Guenther, F.H. (2003). A cortical and cerebellar parcellation system for speech
 1055 studies. Boston University Technical Report CAS/CNS-03-022. Boston, MA: Boston
 1056 University.
- 1064 Tourville, J. A., Reilly, K. J., & Guenther, F. H. (2008). Neural mechanisms underlying auditory
 1065 feedback control of speech. *Neuroimage*, *39*, 1429–1443.
- 1066 Toyomura, A., Fujii, T., Kuriki, S. (2015). Effect of an 8-week practice of externally triggered
 1067 speech on basal ganglia activity of stuttering and fluent speakers. *NeuroImage*, *109*, 458-
 1068 468.
- 1069 Vahdat, S., Fogel, S., Benali, H., & Doyon, J. (2017). Network-wide reorganization of procedural
 1070 memory during NREM sleep revealed by fMRI. *Elife*, *11*, e24987.
- 1071 Watkins, K.E., Smith, S.M., Davis, S., & Howell, P. (2008). Structural and functional
 1072 abnormalities of the motor system in developmental stuttering. *Brain*, *131*, 50-59.
- 1073 Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). CONN: a functional connectivity toolbox for
 1074 correlated and anticorrelated brain networks. *Brain connectivity*, *2*(3), 125-141

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- 1075 Wiener, N. (1949). Extrapolation, interpolation, and smoothing of stationary time series.
1076 Cambridge, MA: MIT Press.
- 1077 Yang, Y., Jia, F., Fox, P.T., Siok, W., & Tan, L.H. (2019). Abnormal neural response to
1078 phonological working memory demands in persistent developmental stuttering. *Human*
1079 *Brain Mapping*, 40(1), 214-225.
- 1080 Yaruss, J.S. & Conture, E.G. (1993). F2 transitions during sound/syllable repetitions of children
1081 who stutter and predictions of stuttering chronicity. *Journal of Speech, Language, and*
1082 *Hearing Research*, 36, 883-896.

1083 **Figure captions**

1084 **Figure 1:** Time course of improvement of behavioral performance measures during training
 1085 (outside the scanner) for the *practiced native* and *practiced non-native* sequences as a function of
 1086 training block (1-8) and group (ANS vs. AWS). (A) Mean percentage of errors of each sequence
 1087 type; (B) Mean durations of the properly executed utterances of each sequence type; (C) Mean
 1088 reaction times of the properly executed utterances of each sequence type. The shaded area
 1089 represents the standard error of the mean.

1090 **Figure 2:** Mean percentage of disfluency errors for the *practiced native* and *practiced non-native*
 1091 sequences during training (outside the scanner) as a function of training block (1-8) for AWS only.
 1092 The shaded area represents the standard error of the mean.

1093 **Figure 3:** Behavioral performance measures during test (inside the scanner) for the *practiced*
 1094 *native*, *practiced non-native*, and *novel non-native* sequences as a function of as a function of test
 1095 block (1-8) and group (ANS vs. AWS). (A) Mean percentage of errors of each sequence type; (B)
 1096 Mean durations of the properly executed utterances of each sequence type; (C) Mean reaction
 1097 times of the properly executed utterances of each sequence type. The shaded area represents the
 1098 standard error of the mean.

1099 **Figure 4:** Mean percentage of disfluency errors (inside the scanner) for the *practiced native*,
 1100 *practiced non-native*, and *novel non-native* sequences during test (inside the scanner) as a function
 1101 of test block (1-8) for AWS only. The shaded area represents the standard error of the mean.

1102 **Figure 5:** Brain areas showing greater BOLD activation for *novel non-native* than *practiced non-*
 1103 *native* trials. (A) Averaged across all 15 ANS and 16 AWS; (B) 15 ANS (left) and 16 AWS (right).
 1104 Activation is displayed on a canonical inflated cortical surface. Colors indicate the relative

1105 significance level at each voxel/vertex for the comparison of *novel non-native* and *practiced non-*
 1106 *native* trials.

1107 **Figure 6:** Brain areas showing greater BOLD activation for *practiced non-native* than *practiced*
 1108 *native* trials. (A) Averaged across all 15 ANS and 16 AWS; (B) 15 ANS (left) and 16 AWS (right).
 1109 Activation is displayed on a canonical inflated cortical surface. The significant subcortical cluster
 1110 is shown on a slice through the cerebellum at $z = -54$ in the MNI template (bottom right); left and
 1111 right hemispheres are indicated by L and R, respectively. Colors indicate the relative significance
 1112 level at each voxel/vertex for the comparison of *novel non-native* and *practiced non-native* trials.

1113 **Figure 7:** Significant correlation between participant-normalized difference in mean error rates
 1114 between the *novel non-native* and *practiced non-native* words and the mean activation in left aINS
 1115 [$r = .381, p = .034$] (left panel) and right aINS [$r = .404, p = .024$] (right panel).

1116 **Figure 8:** Significant correlation between mean disfluency rates during the test phase (inside the
 1117 scanner) and mean activation in left GPe [$r = -0.609, p\text{-FDR} = .047$] (left panel) and left caudate
 1118 [$r = -.591, p\text{-FDR} = .047$] (right panel).

1119

1120 **Table captions**

1121 **Table 1.** International phonetic alphabet (IPA) transcription and orthography for experimental
1122 stimuli used to elicit the *native* (left) and *non-native* (right) target onset and coda clusters
1123 (underlined).

1124 **Table 2.** List of the predefined cortical and subcortical regions-of-interest (ROIs) used in the
1125 current analyses.

1126 **Table 3.** Summary of the ANOVA results for the behavioral analyses. Shown are the F value, the
1127 degrees of freedom, p value, and partial-eta-squared value for each effect.

1128 **Table 4.** Mean error rates in the test blocks (inside the scanner) by error subtype and sequence
1129 type.

1130 **Table 5.** Summary of significant cortical and subcortical activation peaks for the *novel non-native*
1131 – *practiced non-native* contrast. From left to right, the columns show the anatomical region
1132 name(s), number of peaks, MNI stereotactic coordinates, cluster size, TFCE value, and p_{FWE} value.
1133 Cluster regions are listed in descending order of number of significant vertices.

1134 **Table 6.** Summary of significant cortical and subcortical activation peaks for the *practiced non-*
1135 *native – practiced native* syllable contrast. From left to right, the columns show the anatomical
1136 region name(s), number of peaks, MNI stereotactic coordinates, cluster size, TFCE value, and p_{FWE}
1137 value. Cluster regions are listed in descending order of number of significant vertices.

AWS

Mean Disfluency Rate (%)

25
20
15
10
5

1

2

3

4

5

6

7

8

Training Block

Sequence Type

- Practiced Native
- Practiced Nonnative

Left Lateral

Right Lateral

Left Medial

Right Medial

ANS

Left Lateral

Right Lateral

Left Medial

Right Medial

AWS

Left Lateral

Right Lateral

Left Medial

Right Medial

Left Lateral

Right Lateral

Left Medial

Subcortical Cerebellum

$z = -54$

ANS

Left Lateral

Right Lateral

Left Medial

Right Medial

AWS

Left Lateral

Right Lateral

Left Medial

Right Medial

Left Lateral

Left aINS: $r = .381$

Right Lateral

Right aINS: $r = .404$

Left GPe: $r = -.609$

Left Caudate: $r = -.591$

Supplementary Material for:

Neural correlates of speech motor sequence learning in stuttering vs. nonstuttering adults: an fMRI investigation

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S1. Given the differences in reaction time (RT) scores between the ANS and AWS groups, is the timing assumption of the sparse sampling protocol valid?

As shown in Figure 3C, the RT scores were approximately 200 ms longer in AWS than in ANS. These delayed responses were unlikely to have an effect on the obtained BOLD signal in the test phase given i.) the width of the delay of the hemodynamic response, and ii) that task performance is well under one second (as shown in Figure 3B). Nevertheless, in order to address the possibility that some trials containing “silent” stuttering blocks were included in the neuroimaging analyses, we here provide supplementary data showing the distribution of RT scores for the AWS group (Figure S2) as a whole and for each individual stuttering speaker (Figure S3) in the test phase. Critically, these plots did not reveal a bimodal distribution with one peak having frequency values clustered to the right. This finding suggests that any potential stuttering blocks or pauses were likely categorized as silent errors (sequencing subtype 4 as described; see main text for explanation) and were excluded from the imaging analyses, in which case, the BOLD responses would not have been contaminated by potential delays in RT.

S2. Figures for Supplementary Material

Figure S1: Histogram plots of reaction time (RT) measures (30 ms bins) for the properly sequenced productions (pooled across experimental conditions and AWS participants) during the test phase (inside the scanner). Note that the audio recording window was three seconds in duration (starting from the offset of the “go” signal).

Figure S2: Histograms plots of reaction time (RT) measures (30 ms bins) for the properly sequenced productions during the test phase (inside the scanner) for each participant in the AWS group. The counts are plotted as a function of experimental condition (*practiced native* vs. *practiced nonnative* vs. *novel nonnative*) and participant (n=16). P = patient.

Figure S3: Boxplots of mean percentage of errors during the test phase (inside the scanner) as a function of experimental group (ANS vs. AWS) and sequence type (*practiced native* vs. *practiced non-native* vs. *novel non-native*).

Figure S4: Boxplots of mean utterance durations (for properly sequenced productions only) during the test phase (inside the scanner) as a function of experimental group (ANS vs. AWS) and sequence type (*practiced native* vs. *practiced non-native* vs. *novel non-native*).

Figure S5: Boxplots of mean reaction times (for properly sequenced productions only) during the test phase (inside the scanner) as a function of experimental group (ANS vs. AWS) and sequence type (*practiced native* vs. *practiced non-native* vs. *novel non-native*).

Figure S6: Boxplots of mean percentage of disfluencies during the test phase (inside the scanner) as a function of sequence type (*practiced native* vs. *practiced non-native* vs. *novel non-native*) for the AWS group only.