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Frontostriatal and Mediotemporal Lobe Contributions to Implicit Higher-Order Spatial Sequence Learning Declines in Aging and Parkinson's Disease

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Abstract

Sequence learning depends on the striatal system, but recent findings also implicate the mediotemporal lobe (MTL) system. Schendan, Searl, Melrose, & Stern (2003) found higher-order associative, learning-related activation in the striatum, dorsolateral prefrontal cortex, and the MTL during the early acquisition phase of both implicit and explicit variants of a serial response time task. This functional magnetic resonance imaging (fMRI) study capitalized on this task to determine how changes in MTL function observed in aging and compromised frontostriatal function characteristic of Parkinson's disease (PD) patients impacts sequence learning and memory under implicit instructions. Brain activity was compared between "Sequence" and "Random" conditions in 12 non-demented PD patients and education and gender matched healthy control participants of whom 12 were age matched (MC) and 14 were younger (YC). Behaviorally, sequence-specific learning of higher-order associations was reduced with aging and changed further with PD and resulted primarily in implicit knowledge in the older participants. FMRI revealed reduced intensity and extent of sequence learning-related activation in older relative to younger people in frontostriatal circuits and the MTL. This was because signal was greater for the Sequence than Random condition in younger people, whereas older people, especially those with PD, showed the opposite pattern. Both older groups also showed increased

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activation to the task itself relative to baseline fixation. In addition, right MTL showed hypoactivation and left MTL hyperactivation in PD relative to the MC group. The results suggest changes in frontostriatal and MTL activity occur during aging that affect task-related activity and the initial acquisition phase of implicit higher-order sequence learning. In addition, the results suggest that Parkinson's disease adversely affects processes in the MTL including sequence learning and memory.

Keywords

Parkinson's disease; aging; implicit learning; medial temporal lobe; striatum; prefrontal cortex

Implicit sequence learning refers to the gradual acquisition of stimulus-response associations without awareness, whereas in explicit sequence learning, the learned associations are consciously accessible. Evidence from brain imaging studies of healthy young people has reliably implicated cortical and subcortical components of frontostriatal circuitry, including the dorsolateral prefrontal cortex (DLPFC), caudate, putamen, ventral striatum, and anterior cingulate, in implicit sequence learning (Berns, Cohen, & Mintun, 1997; Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Grafton, Hazeltine, & Ivry, 1995; Hazeltine, Grafton, & Ivry, 1997; Packard & Knowlton, 2002, for review; Peigneux et al., 2000; Rauch *et al.*, 1997; Rauch et al., 1995; Willingham, Salidis, & Gabrieli, 2002; Robertson, 2007; Wymbs & Grafton, 2009; Wymbs, et al., 2012). In addition, studies that required learning of a higher-order sequence, in which predictions must be based on more than pairwise associations of adjacent stimuli, found evidence that the medial temporal lobe (MTL) is necessary to encode and consolidate the sequential order of events (Albouy et al., 2008; Curran, 1997; Schendan, Searl, Melrose, & Stern, 2003). Altogether, these findings provide evidence for a complex interplay between traditionally explicit (MTL) and implicit (striatum) memory systems during higher-order implicit learning (Poldrack *et al.*, 2001). Both of these memory systems undergo structural and functional changes in normal aging as well as neurological disease states, as in Parkinson's disease (PD).

The present study aimed to compare fMRI response patterns in MTL and basal ganglia during the early acquisition phase of implicit sequence learning in PD and to determine how these patterns differ between PD, age-matched healthy control participants (MC), and a subset of younger control (YC) participants previously reported by Schendan, Searl, Melrose, and Stern (2003). The Serial Response Time task (SRT), developed originally by Nissen and Bullemer (1987), has been used in many studies of human implicit sequence learning. In the spatial SRT task, people see a sequence of cued locations (e.g., represented by squares) that is either presented pseudo-randomly or in a repeating sequence. The version of the SRT task in this study was originally implemented in Schendan, Searl, Melrose, and Stern (2003) and implicated MTL activity in the initial formation of higher-order associations among sequences of spatial locations during both explicit and implicit learning, regardless of conscious awareness of this newly acquired knowledge. In addition, different regions of the DLPFC and the striatum showed learning-related activation during explicit and implicit learning. By reliably activating both MTL memory structures and the basal ganglia during sequence learning, this SRT version is ideally suited to determine the

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interaction of different components of implicit and explicit learning and memory systems and how these change with neurological disorders and normal aging, which was the goal of the present study.

PD is an aging-related neurodegenerative disorder that disrupts the dopaminergic innervation of the striatum, affecting information processing in frontostriatal circuitry. The hallmark symptoms of PD are most recognized as motor disturbances (i.e., bradykinesia, rigidity, tremor, postural instability, and gait disturbance). However, a growing body of neuropsychological and neuroimaging evidence suggests that PD patients also suffer from cognitive difficulties, including executive and visuospatial dysfunction, as well as learning and memory deficits (Amick, Schendan, Ganis, & Cronin-Golomb, 2006; Cronin-Golomb & Amick, 2001; Dubois & Pillon, 1997; Schendan, Amick, & Cronin-Golomb, 2009; Davidsdottir, Wagenaar, Young, & Cronin-Golomb, 2008). Implicit learning in PD is one aspect of cognitive functioning that has received considerable attention (Jackson, Jackson, Harrison, Henderson, & Kennard, 1995; Smith, Siegert, McDowall, & Abernethy, 2001), as this addresses the role of the basal ganglia in learning and memory, an area of intense interest (Packard & Knowlton, 2002).

Functional neuroimaging studies of various implicit learning tasks (e.g., Weather Prediction, Tower of London) showed preserved performance but increased activation in the MTL and decreased activation in the striatum in PD patients compared to controls (Beauchamp, Dagher, Panisset, & Doyon, 2008; Dagher, Owen, Boecker, & Brooks, 2001; Moody, Bookheimer, Vanek, & Knowlton, 2004). Implicit sequence learning studies using the SRT task in PD patients have found impaired implicit sequence learning in many studies (Jackson, Jackson, Harrison, Henderson, & Kennard, 1995; Smith & McDowall, 2004; Werheid, Zysset, Muller, Reuter, & von Cramon, 2003; Westwater, McDowall, Siegert, Mossman, & Abernethy, 1998) but not all (e.g., Smith, Siegert, McDowall, & Abernethy, 2001). Inconsistencies across studies may be attributable to complexity of material to be learned, stage of learning examined, and heterogeneity of population.

To understand the cognitive implications of PD neuropathology, both disease- and age-related neural changes must be defined and considered. The literature on healthy aging suggests cognitive changes occur across multiple domains, including explicit episodic memory that supports recall and recognition of items as familiar, for which the underlying mechanism is theorized to include a neuromodulatory deficit (Braver et al., 2001; Braver, Satpute, Rush, Racine, & Barch, 2005; Hasselmo & Eichenbaum, 2005). In comparison with younger adults, older adults show changes in the ability to learn to bind complex pieces of information associatively into memory, for which MTL dysfunction has been strongly implicated (Chalfonte & Johnson, 1996; Cabeza, Anderson, Houle, Mangels, & Nyberg, 2000; Mitchell, Johnson, Raye, & D'Esposito, 2000; Mitchell, Johnson, Raye, Mather, & D'Esposito, 2000). For example, aging related changes on explicit episodic recognition have been proposed to reflect problems with binding parts of an experience into memory and retrieving such bound representations (Bender, Naveh-Benjamin, & Raz, 2010; Naveh-Benjamin, Guez, & Shulman, 2004). However, there is also evidence that older individuals might be implicitly "hyperbinding" familiar but irrelevant associations reflecting an inability to suppress distractions (Campbell, Hasher, & Thomas, 2010). In contrast, implicit learning

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in older adults is relatively preserved, but the neural substrates serving this function differ from those in younger adults. During implicit learning, younger adults recruit the striatum and disengage the MTL as learning proceeds, whereas older adults have been found to recruit the MTL in a sustained manner in addition to the striatum (Dennis, & Cabeza, 2011; Rieckmann, Fischer, & Bäckman, 2010). Given the comparable behavioral performance of older adults, the additional MTL recruitment has been interpreted as compensation.

To investigate aging-related and PD-specific changes in behavior and fMRI activation patterns, we also examined younger adults and compared them to healthy adults who were matched in age and other demographic factors to the PD group. We predicted that, relative to the younger group, aging-related changes within frontostriatal circuits and the MTL that are common to the older groups would lead to a similar set of behavioral and fMRI activation changes in both older groups (e.g., MC and PD would show reduced implicit sequence learning and over-recruitment of the MTL), and the added pathology in the PD group would produce disease-specific activity patterns in frontostriatal circuits (e.g., under-recruitment).

METHOD

Participants

Table 1 summarizes group demographics. Of 12 participants with idiopathic PD, 8 had the body side of motor symptom onset on the left (LPD; mean age: 55.6 ± 7.5 years [range 44.4–62.9], mean education: 17 ± 3.3 years [range 12–21], 3 female), and 4 had the body side of motor symptom onset on the right (RPD; mean age: 59 ± 8.2 years [range 47–65.6], mean education: 15 ± 2 years [range 12–16], all female). Twelve healthy adults participated as matched control individuals (MC) and were chosen to match to LPD and RPD subgroups in age, education, and gender. Of the 12 MCs, 7 matched the LPD subgroup (mean age: 58.7 ± 6.5 years [range 49.6–61.5], mean education: 16.3 ± 2.2 years [range 13–19], 3 female), and 5 matched the RPD subgroup (mean age: 58.8 ± 8.9 years [range 42.7–67], mean education: 16.6 ± 1.67 years [range 14–18], all female). All participated with informed consent and approval of Massachusetts General Hospital and Boston University. Diagnoses were made by staff neurologists in the outpatient clinic of the Parkinson's Disease Center in the Department of Neurology, Boston Medical Center. The PD and MC groups were recruited through the Vision & Cognition Laboratory in the Department of Psychology at Boston University. Some of the MCs were also recruited through the Harvard Cooperative Program on Aging. Data for 14 younger control participants (YC; mean age 30 ± 6.9 years [range 19–42]) were chosen from our prior study (Schendan *et al.*, 2003) to match the PD and MC groups overall on education (mean 17 ± 1.4 years [range 16–20]) and gender (8 female).

Exclusion criteria for all participants included neurological disease (aside from PD for the PD group) or medical disorders that impair central nervous system function, head trauma with more than 30 seconds loss of consciousness or other complications, learning disability, psychiatric conditions, including schizophrenia, bipolar disorder, personality disorder, but not anxiety and depression because these conditions are often comorbid with PD, history of substance (drug, alcohol) dependence, intravenous drug use, history of electro-shock

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treatment, English as non-native language, and failure of screening in regard to specific MRI safety considerations.

All PD patients had unilateral disease onset and asymmetrical disease course in the "ON" state of dopaminergic medication. In the 8 LPD and 4 RPD subjects, the side of the dominant hand was also the more affected side. The average duration of disease was 4.58 ± 2.8 years. All patients were responsive to either levodopa-carbidopa or dopamine receptor agonists. Ten patients were on a combination of up to 3 medications including levodopa-carbidopa, dopamine receptor agonists (pramipexole, ropinirole, pergolide), catechol-O-methyl-transferase (COMT) inhibitors (entacapone, tolcapone), monoamine oxidase B (MAO-B) inhibitors (selegiline), amantadine, and anticholinergics (trihexyphenidyl), and 2 were on dopamine receptor agonists only. 4 patients were on antidepressants, 2 on antianxiety medications as needed, and 1 was taking wakefulness-promoting drugs (modafinil).

Scanning started within 2 ± 1.5 hours after the first dose of dopaminergic medication for the day. Before scanning, patients underwent a neurological assessment while on dopaminergic medication, including Hoehn and Yahr (HY) staging (Hoehn & Yahr, 1967) and the Unified Parkinson's Disease Rating Scale (UPDRS; Fahn & Elton, 1987). The mean UPDRS score (28.6 ± 9.01) included the mentation, behavior, mood and activities of daily living components rated by interview, motor examination, and therapy-related complications (e.g., dyskinesia, dystonia, clinical fluctuations, anorexia/nausea/vomiting, sleep disturbances, symptomatic orthostasis). In addition to tremor, all patients had at least two more cardinal motor symptoms: bradykinesia, rigidity, or postural instability (UPDRS motor score M = 25.89 ± 1.66). Ten PD patients had a HY score of 2, one LPD had 2.5, and one LPD had 3 (M = 2.1 ± .30). A score of 2 indicates mild bilateral involvement without impaired balance, and 3 indicates mild to moderate bilateral involvement with some postural instability (Hoehn & Yahr, 1967).

Clinical Neuropsychological Tests

To characterize the cognitive and behavioral profiles, PD and MC participants were tested on standard clinical neuropsychological tests: Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) and the Dementia Rating Scale (DRS) (Mattis, 1988) assessed current cognitive function; the American National Adult Reading Test (ANART) (Grober & Sliwinski, 1991) assessed premorbid intellectual functioning; Digit Symbol and Symbol Search subtests of the third version of the Wechsler Adult Intelligence Scale (WAIS-III) (Wechsler, 1997) assessed psychomotor speed; Trail Making A and B tests (Reitan & Wolfson, 1993) assessed complex attention and executive function. Emotional status was assessed using the Beck Depression Inventory-II (BDI-II) (Beck, 1997) and Spielberger State Trait Anxiety Inventory (STAI) (Spielberger, Gorusch, & Lushene, 1983).

Materials

Response cues were shown in 12-location, second order conditional (SOC) sequences. All SOC sequences have 3 occurrence of each of the 4 spatial locations (denoted here using numerals 1 [left-most] through 4 [right-most]) and 1 occurrence of each of the 12 possible

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transitions between two consecutive locations (i.e., “transition pairs”: 1–2, 1–3, 1–4, 2–1, etc.). In the Sequence condition, 1 SOC sequence was shown repeatedly (1-2-1-4-2-3-4-1-3-2-4-3); in the Random condition, pseudo-randomly generated (Emerson & Tobias, 1995) SOC sequences were shown once each. Consequently, higher-order associations among three or more consecutive locations in the Sequence must be encoded in order to demonstrate learning.

Neuroimaging of Implicit Learning

Neuroimaging methods were identical to the prior study with the YC group (Schendan *et al.*, 2003), except as noted. Functional scans were acquired in 4 implicit runs; each began and ended with fixation of a centered dot. Before the first run, participants were instructed and practiced the SRT task with one random SOC sequence of 12 locations. On each trial, one of four squares in a horizontal array turned from black to white, thereby “lighting up” that spatial location, for 1000 ms followed by the blank array for 250 ms. Note, in order to have the subjects learn the task implicitly, they were simply told to press the button corresponding to the white square, and were not informed about the repeating sequence. In all runs, Sequence and Random blocks alternated. Each Sequence block began at a unique random point of the sequence to ensure further that learning would be implicit. The Sequence repeated 4 times in each of 3 blocks per run. All runs had 3 30-s Sequence blocks (S). These were separated by 2 short 15-s Random blocks (r) comprised of 2 novel random sequences each because pilot work in earlier studies had concluded that longer intervening segments of random sequences impaired learning of the repeating sequence (Rauch *et al.*, 1997). In addition, two long 30-s Random blocks (R), comprised of 4 novel Random sequences each, started and/or ended all runs in order to equate total number of trials in both Sequence and Random conditions. For counterbalancing purposes, the exact position of these long Random blocks varied between three types of block orders: In runs with block order A, both long Random blocks preceded all Sequence blocks (i.e., R-R-S-r-S-r-S); with order B, one long Random came before and the other came after all Sequence blocks (i.e., R-S-r-S-r-S-R); with order C, both long Random blocks followed all Sequence blocks (i.e., S-r-S-r-S-R-R). In the original fMRI study with the YC group (Schendan *et al.*, 2003), the activation paradigm involved presenting the following set of four runs from first to last block order type: ABBC. Half of each MC and PD group received the same ABBC activation paradigm as used in our prior fMRI study with the YC (Schendan *et al.*, 2003). The other half of each MC and PD group received a slightly modified activation paradigm with the four runs having identical block orders: BBBB. Block order B was chosen for all runs in order to use the most balanced block order throughout all runs because Sequence and Random blocks alternate with each other throughout order B. Nonetheless, to ensure a valid comparison with the YC, as noted, the original ABBC paradigm used for all YC was used for half of each MC and PD group. A fixation rest (4 s) preceded some Random blocks in the ABBC paradigm (Schendan *et al.*, 2003) so, in the BBBB paradigm, the locations of these rests changed to counterbalance these locations between the two paradigms. Activation paradigm types (ABBC, BBBB) were kept the same between each PD and their matched control participant. Results were compared between ABBC and BBBB activation paradigms, and no systematic differences between these two paradigms were observed in any group that could explain the pattern of results and so data from both paradigms were considered altogether;

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nonetheless, to be cautious, we emphasize differences between the PD and MC groups over differences between the two older (PD, MC) and the younger (YC) groups.

Direct Memory Tests

The sequence learning task was administered implicitly: Participants were not instructed or otherwise informed that there was a repeating sequence. In our prior study with young subjects (Schendan et al., 2003), we found that MTL activity associated with the early acquisition of higher-order sequences occurred in both implicit and explicit instruction conditions, and, even under implicit instructions, could not be attributed to participants developing sequence knowledge during learning. In our prior work and in this study, explicit memory tests were given to assess conscious knowledge of sequence structure. These tests were administered only after all sequence learning runs had been completed. The most sensitive tests used for the YC group from our prior fMRI study (Schendan *et al*., 2003) were used to assess explicit awareness of the learned sequence in the PD and MC groups. Methods for these tests were otherwise identical to our previous study, except where noted. Tests vary in sensitivity to conscious awareness, as well as potential for nonconscious (implicit) memory influences (Shanks & Johnstone, 1999), and are described next in the order of administration.

Awareness—Immediately following all implicit runs, participants pressed a key to respond “yes (1), probably (2), unlikely (3), or no (4)” to four questions: “In the task you just performed, did you notice that: (i) the boxes lit up at random locations the entire time; (ii) some boxes lit up more often than other boxes; (iii) the task was easier at times and harder at other times; (iv) there was a repeating pattern of locations some of the time?”. The fourth question is the first information provided to subjects that introduces the idea of a repeating sequence and is the most basic test of sequence awareness and provides the least cues to the subject for retrieving memory.

Free Generation—The unfilled 4-square array was continuously shown. Participants pressed response keys in the order of the repeating sequence until cued to stop. This free recall test provides minimal cues to memory and places high demands on conscious memory but may be less sensitive for detecting conscious memory (Shanks & Johnstone, 1999).

Sequence Recognition—Full-sequence recognition provides the maximal set of cues possible for memory and was the most sensitive conscious memory test. Participants did the SRT task with the entire implicit Sequence and 4 new 12-item SOC sequences. After each sequence, subjects rated on a scale from 0 (least) – 10 (most), if they had experienced it in the implicit SRT task learning phase (Reber & Squire, 1998).

Magnetic Resonance Imaging (MRI)

High-resolution T1-weighted scans (MP-RAGE; 192×256) for anatomical localization and 4 T2*-weighted functional BOLD scans were acquired at 3 T (Siemens Allegra using a gradient-echo, echo-planar imaging (EPI) pulse sequence, 21 AC-PC slices were acquired (5 mm thick; 1 mm skip; TR=2 s, TE=30 ms; flip angle=90°, 64×64). After the first anatomical scan, a T1-weighted EPI image was acquired before the first functional scan for later co-

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registration of anatomical with functional images. If time allowed, a second MP-RAGE scan was acquired during the post-learning, explicit memory tests.

General Analysis

The YC data were taken from our prior study (Schendan, *et al.*, 2003). To compare LPD and RPD groups directly, ANOVAs included between-subject factors of 2 group (PD, MC) and 2 side of motor symptom onset subgroup (left, right). However, as no analysis revealed effects of side, perhaps due at least in part to the small sizes of these subgroups, this report focuses on analyses of the entire PD group, which was collapsed across left and right subgroups and included a between-subjects factor of group (PD, MC, YC) in the ANOVAs. All statistical tests were performed using SPSS 15 for Windows.

Response Time (RT) Analyses—RT analyses were the same as those in Schendan, *et al.* (2003); note, RT data of 6 subjects (1 YC, 2 MC, 3PD) were missing due to technical problems. First, median RTs (maximum cut-off of 1250 ms, which is the total trial time) were calculated within-group for all Sequence and Random blocks by run. The 2 short Random blocks were analyzed together as if they were 1 long block so that the same number (4) of trials comprised all Random RT blocks (i.e., 2 short Random each had 2 trials for a total of 4 trials altogether, and 2 long Random each had 4 trials). As in Schendan, *et al.*, (2003), we conducted the analysis of higher-order associative learning between 3 or more consecutive locations based on methods described by Curran (1997). To do so, RTs to each pair of consecutive locations ("transition pairs") were compared between Sequence and Random conditions. For all 12 transition pairs, the median RT to the second location of each pair was determined, separately, for Sequence and for Random blocks in each run. For example, for the transition pair 2–*4*, the RT to *location 4* is taken only if it is preceded by location 2). The median RT for each of the 12 transition pairs was then averaged across all Sequence and Random blocks, separately, in each run. Next, a difference score for each transition pair was calculated by subtracting the resultant mean RT for the Sequence condition from that for the Random condition in each run, and planned contrasts (1-sample t-tests) defined pairs differing reliably from zero.

MRI Analyses—Functional MRI (fMRI) data were preprocessed using statistical parametric mapping software (SPM5; Wellcome Dept. of Cognitive Neurology) running on *Matlab* software (*Mathworks*, Natick, MA). Functional imaging runs were realigned for motion correction and normalized to the standard EPI template and resliced into 3×3×3 mm resolution in Montreal Neurological Institute (MNI) space). The registration of the EPI data to the template was checked for each individual subject. The fMRI data were then smoothed with a Gaussian kernel of 8-mm^3. Statistical analyses used the general linear model. High-pass filtering was applied, but global signal scaling was not used to avoid spurious deactivations. Design matrices were modeled using a boxcar function convolved with a canonical hemodynamic response function.

As in Schendan, *et al.*, (2003), learning-related activation was defined as the linear contrast of Sequence relative to Random blocks. As we used SOC sequences, this contrast demonstrates sequence-specific activation or associative learning processes. In addition to

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the learning-related contrasts, the Random blocks were also compared to the baseline fixation condition in order to assess baseline SRT task activation independent of learning. Detailed statistical comparison of results for short and long random revealed no systematic differences, and so results were collapsed across these random block lengths for analysis. Contrast images for each subject were used in second-level analyses treating subjects as a random effect. Detailed statistical comparison of results for ABBC and BBBB sets of runs revealed no systematic differences, and so results were collapsed across these run set types for analysis. Within- and between-group analyses were performed using one- and two-sample t-tests for contrasts of Sequence versus Random. To compare with published results (Schendan *et al.*, 2003), for each YC, MC, and PD group, separately, the group averaged, statistical parametric maps (SPMs) for the average of all runs, as well as each run individually, were examined initially using a whole-brain voxel-wise comparison at uncorrected *p*-value (p_u)=.0167 (alpha .05 divided by 3 for the regions of interest [ROIs] of MTL, basal ganglia, and DLPFC; with the exception of those runs noted at p_u =.05 in Figure 3a). The coordinates for the ROIs were also taken from the same study. For the ROI analyses, each region was defined using a 5 mm diameter sphere centered at the coordinate of each learning-related activation maximum in order to facilitate the extraction of percent signal change estimates for each condition. These values, which were later entered into the general linear model, were extracted using the *Marsbar* extraction utility (Brett, Johnsrude, & Owen, 2002). ANOVAs included a between-subject factor of group and within-subject factor of run, and planned pairwise contrasts between groups and levels of factors determined the pattern of effects on signal change. Analyses on performance and signal change data were conducted with and without covariates of Age, Gender, and Paradigm version (ABBC, BBBB); note, results remained the same for both analyses so we report results with covariates, as these were typically more powerful, except where noted.

RESULTS

Clinical Neuropsychological Tests

A within-subjects factor of test was added to evaluate differences between tests. Statistical threshold was set at $p < 0.05$. Huynh-Feldt correction for violations of the sphericity assumption was applied, as needed. Independent-sample *t*-tests were performed to detect subtle group differences. ANOVAs did not include the YC level of group because no neuropsychological data had been collected for the YC group. Results of analyses comparing between LPD and RPD and their corresponding MCs suggested no effect of group (PD *vs*. MC, $F[1,10] = 0.90$ $p = .771$) or match (left-type *vs*. right-type, $F[1,10] = 0.029$ $p = .869$). Results comparing the entire PD and MC groups suggested no significant differences between them (PD *vs*. MC, $F[1,12] = 1.01$ $p = .336$). Table 1 details results of the clinical tests. In sum, the PD and MC groups were well-matched cognitively, as well as on age, education, and gender. Any implicit associative learning-related differences between groups cannot be attributed to these demographic factors and cognitive and emotional functions (i.e., general intelligence, psychomotor speed, complex attention, executive function, and emotion).

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Performance

Sequence *versus* Random—Previously for the YC group (Schendan *et al.*, 2003), RTs differed between conditions, blocks, and runs. Here, median RT data were entered into an omnibus mixed-factor repeated measures ANOVA with a group factor (YC, MC, PD) and within-subject factors of condition (Sequence, Random), block (1–3), and run (1–4) and covariates of Age, Gender, and Paradigm version. Overall, RT results showed a main effect of condition (F[1,26] = 4.77, p = 0.038) and interactions of condition by block by run (F[6,156] = 2.92, p = 0.026, Greenhouse-Geisser *epsilon* for sphericity correction (ε) = .647) and condition by block by group (F[4,52] = 3.21, p = .0199, Huynh-Feldt *epsilon* correction for sphericity (ε) = 1). Thus RTs differed between conditions, demonstrating sequence-specific learning, and this learning varied across blocks and runs. Further, critically, how sequence specific learning varied across blocks differed between groups. Pairwise contrasts between groups determined which groups differed. Comparing YC and MC groups showed a significant main effect of condition (F[1,18] = 5.84, p = 0.026) and interactions of condition by block by run (F[6,108] = 2.85, p = 0.041, GGε = .540) and condition by block by run by group (F[6,108] = 3.86, p = 0.012, GG ε = .540), demonstrating that how sequence-specific learning varied across trials (i.e., blocks and runs) differed between younger and older healthy groups. Comparing YC and PD groups showed no effects. Comparing MC and PD groups showed significant interactions of condition by block by run (F[6,84] = 3.42, p = 0.023, GGε = .533) and condition by block by group (F[2,28] =3.98, p = 0.046, GG ε = .730), demonstrating that sequence-specific learning varied across blocks and runs in the older groups and how this learning varied across trials differed between the MC and PD groups. To assess whether each group learned, within-group ANOVAs were done. The YC group showed main effects of condition (F[1,12] = 50.98, p = 0.00001; note, with covariates but also significant without) and block (F[2,24] = 3.68, p = 0.04; note, no covariates). The MC group showed a significant interaction of condition by run by block (F[6,36] = 3.69, p = 0.006). The PD group showed no effects. Thus both healthy YC and MC groups, but not the PD group, demonstrated sequence-specific learning, and this learning differed across trials in the older MC group.

Higher-Order Associative Learning—As in Schendan, *et al.*, (2003), results of a finer-grained analysis are reported that pinpoint higher-order associative learning across three or more consecutive locations for each location in the sequence and for each group. The fundamental unit for this analysis was a transition pair (i.e., two consecutive locations) because each SOC sequence in both the Sequence and Random conditions had exactly the same set of all 12 possible transition pairs, and these pairs occurred with the same frequency in all SOC sequences. Thus the transition pairs and their frequencies do not distinguish between the two conditions; in contrast, triplets and longer sequences differed between the two conditions. Consequently, the only way learning can be demonstrated is if a higher-order association beyond the transition pairs has been acquired (i.e., between three or more consecutive locations). A significant difference score (Sequence - Random) for a transition pair means that a higher-order association has been learned. Each transition pair can thus show a difference between Sequence and Random conditions only if learning of the Sequence results in association of this pair of locations (i.e., transition pair) with one or more preceding locations (i.e., a higher-order sequence of three or more locations) in the

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repeating Sequence. In other words, a transition pair will show a difference only if the *triplet* (i.e., the higher-order association) or a longer sequence, of which the transition pair is a part, had been learned. Consequently, if such longer sequences had been learned, the RT difference between Sequence and Random will be greater than zero, but if not, then the difference will not differ from zero. Thus a significant transition pair demonstrates that a triplet or longer sequence ending in that transition pair had been learned. Figure 1b plots the RT difference of Random minus Sequence at each transition pair for each group and the results of *t*-tests that determine whether each difference differs significantly from zero in order to indicate which higher-order sequences each group learned. These results showed that all groups demonstrated higher-order associative learning at multiple locations in the sequence. However, the YC group learned more and longer sequence transitions in each run than both MC and PD.

To assess whether difference scores varied between locations, runs, and groups, these scores were analyzed in a mixed ANOVA with within-subject factors of transition (pair 1–12) and run and between-subject factor of group. As found previously for the YC group (Schendan *et al.*, 2003), results over all groups confirmed that higher-order associative learning differed across runs overall ($F[3,143] = 4.46$, $p = 0.004$), as the score was higher for later than earlier runs. Further, learning differed between transitions ($F[11,143] = 24.90$, $p < 0.0001$), as some transitions showed higher learning scores (e.g., 3–2) than others (e.g., 1–4). Run interacted with transition ($F[33,143] = 1.65$, $p < 0.012$), as how much the score increased with run differed between transitions. More important, how scores varied across transitions differed between groups, as transition by group was significant ($F[22,143] = 1.79$, $p = 0.014$), and results of planned contrasts of pairs of groups indicated that this was due to differences between the YC and MC groups who showed a significant transition by group interaction ($F[11,96] = 2.52$, $p = 0.004$). In addition, omnibus results also showed that groups differed in associative learning across runs, as run by group was significant ($F[6,143] = 2.50$, $p < 0.021$), and results of planned contrasts indicated that group was significant in runs 2 ($F[2,36]=3.22$) and 4 ($F= 3.88$), ps < .05, because the PD group showed smaller learning scores than the YC group in these runs 2 ($F[1,24] = 6.75$, $p = 0.01$) and 4 ($F = 8.06$, $p = 0.005$), as well as run 1 ($F= 4.13$, $p = 0.043$). This result reflected the observation that PD patients showed a reduced higher-order associative learning score in these runs compared with the YC group. When age, gender, and paradigm version covariates were added to the omnibus ANOVA, no effects were significant, but pairwise comparisons indicated that 5 transitions and runs 1 and 4 differed significantly (ps < .03). To focus on the clearest learning effects, the ANOVA with covariates was conducted on the 4 transitions showing significant positive learning differences in all runs in the YC group (Fig. 1b): 1–2, 4–1, 3–2, and 2–4. Results showed a significant interaction of transition by run by group ($F[2,28]=3.96$, $p=.030$, with a quadratic trend for run and linear trend for transition), suggesting that how higher-order associative learning varied across transitions and runs differed between groups. Pairwise contrasts between groups suggested that transition by run was significant for MC and PD groups (F [1,16]=6.175, p =.024, with a linear trend for transition and quadratic trend for run), though transition by run by group was only marginal (F [1,16]=4.36, p =.053, with a linear trend for transition and quadratic trend for run). Overall, this provides further evidence that higher-order associative learning differed in PD

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relative to healthy controls, even for associations acquired most rapidly (i.e., within Run 1) and present across all runs.

Subsequent Direct Memory Tests—Results of direct tests of explicit episodic memory assessed how much awareness about the repeating sequence each group had acquired. These explicit memory tests are critical for demonstrating that MTL activity is related to implicit sequence learning and not explicit awareness. Our previous work in younger participants demonstrated MTL activity during learning in both implicit and explicit variants of the SRT task, regardless of subject's awareness of the repeating sequence (Schendan et al. 2003).

Awareness: Results of univariate ANOVAs with a between-subjects factor of Group showed that explicit knowledge questions following the implicit SRT task revealed only that the YC group endorsed the idea that "some boxes lit up more often than other boxes" (question ii) more than both older groups (both *p*s<.02; *M*(YC) = 3.15, *M*(MC) = 1.89, *M*(PD) = 1.8; *F*[2,31]=5.06, *p*=.013). However, endorsement of this incorrect statement (in fact, all locations were equiprobable) does not indicate explicit sequence knowledge. Groups did not differ on question i (*M*(YC) = 1.53, *M*(MC) = 1.22, *M*(PD) = 1.5), question iii (*M*(YC) = 2.15 and *M*(PD) =1.30, *p* = 0.020); *M*(MC) = 2.00), or the critical, repeating sequence, question iv (*M*(YC) = 1.62, *M*(MC) = 1.67, *M*(PD) = 1.7). However, no group effect was significant when age, gender, and paradigm version covariates were included in the ANOVAs (*F*s<3, *p*s>.07), suggesting no group differences in sequence awareness.

Free Generation: The YC group recalled a significantly longer consecutive string of spatial locations from the repeated sequence than the MC group (*p* = 0.026; Figure 2a). Mean generation span was above chance (3.71 [Rauch, et al., 1997]) only for the YC group (*M* = 5.6 *t* = 2.795, *p* = 0.016; MC: *M* = 3.3, *t* = −0.647, *p* = 0.534; PD: *M* = 4.2, *t* = 0.16, *p* = 0.356). Thus the YC, on average overall as a group (but see Schendan, et al., 2003 for aware *vs*. unaware subgroups), were somewhat aware and significantly more so than the MC group, and these results provided no clear evidence that MC and PD groups had conscious knowledge about the repeating sequence. However, group was not significant when age, gender, and paradigm version covariates were included in the ANOVA (*F*[2,29]=1.04, *p*=.37), suggesting no overall group differences in recall.

Full Recognition: As in Reber and Squire (1998) on which this test was based, a recognition score was computed for each subject (1 Sequence rating – mean of 4 Random distractor ratings) and entered into an ANOVA with a group factor. The main effect of group was not reliable (*F*[2,34] = 1.02, *p* = 0.371). To determine whether subjects discriminated the Sequence from the Random distractor sequences, the full-sequence recognition ratings (Figure 2b) were also assessed in an ANOVA with within-subject factor of Condition (sequence, distractors) and between-subject factor of Group. The main effect of Condition was significant (*F*[1,32] = 9.54, *p* = 0.004), and no group effects were found. No effects were significant when age, gender, and paradigm version covariates were included in this ANOVA (*F*s<1.1, *p*s>.36), suggesting no recognition nor group differences overall.

Awareness: Finally, if we define aware subjects as those with a free generation score of 6 or higher and full sequence recognition score (sequence minus all distractors) above 1.8 (i.e.,

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above that achieved by amnesic patients) (Reber & Squire, 1998) and sequence rated higher (more familiar) than the average of the distractors, then no MC or PD individual met this criterion; in contrast, 7 YC were aware, as reported previously (Schendan, et al., 2003). Notably, only 1 MC and 2 PD individuals achieved a free generation score of 6 (and no higher score was attained by anyone in these older groups). As 7 YC but no older participant (MC or PD) met the criterion for awareness of sequence knowledge, learning was primarily implicit for all older individuals and, as reported previously (Schendan, et al., 2003), for a subset of 8 out of 15 total YC individuals; however, we note that 1 MC and 2 PD individuals might have had some explicit sequence knowledge, based on achieving a free generation score of 6. Thus brain activation, on average, reflects the development of some explicit sequence knowledge for a subset of the younger group but no one in the two older (MC, PD) groups, for whom learning is primarily implicit.

In contrast, on the RT measures of implicit higher-order associative learning (Figures 1ab), the MC and PD groups differed in how sequence-specific learning varied across trials. Further, relative to the YC group, both older groups differed in aspects of higher-order associative learning: The MC group learned different transitions, while the PD group showed less higher-order associative learning across trials, and both groups learned fewer and shorter higher-order associations. This suggests a dissociation between implicit and explicit knowledge such that, while both MC and PD groups showed overall less or different kinds of implicit and explicit knowledge relative to the YC group, the MC and PD groups differed from each other primarily in implicit knowledge.

Functional MRI (fMRI)

Learning-Related Activation (Sequence > Random)

Each Run of Each Group: The contrast of sequence relative to random defines brain activity that is due to learning higher-order associations among 3 or more consecutive locations in the repeating sequence. First, the question of whether each group showed learning-related activation in each run, as found previously for the young group (Schendan *et al.*, 2003), was assessed. Indeed, all groups demonstrated significant learning-related activation (Sequence > Random, Figure 3a) in MTL, striatal, and DLPFC regions of interest (ROIs). However, the YC group demonstrated bilateral activation of both MTL and striatal regions (Figure 3a, Tables 2–3), while the corresponding activated regions in MC and PD groups were often only unilateral.

All Runs between Pairs of Groups: To define group differences directly and increase the power to do so, statistical parametric maps (SPMs) from each pair of groups (two-sample *t*-tests) were compared with data collapsed across runs (Figure 4). Results suggested that learning-related activation (Sequence > Random) in bilateral MTL and basal ganglia and right DLPFC was greater in the YC group than both MC and PD groups, and left DLPFC was also greater in the YC than MC group. No area showed greater activation for older (MC, PD) than younger (YC) groups. This suggests that implicit learning-related activation in the MTL and frontostriatal circuits may decline with normal aging. In addition, results comparing MC and PD groups directly revealed lateralized differences in MTL activation: In PD (relative to MC), right MTL showed hypoactivation, whereas left MTL showed

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hyperactivation. In other words, the direction of MTL alteration in PD differed between hemispheres: While right MTL showed an exacerbation of the decrease with normal aging, the left MTL showed an amelioration of such a decrease in PD relative to the MC group. This suggests that learning-related MTL activation decreases with normal aging, and the disease process in PD further alters the normal aging pattern. In sum, results suggested that, while all groups showed learning-related activation, younger people showed more learning-related activity than older people, and the PD group showed additional abnormal hypoactivation and hyperactivation in the right and left MTL, respectively. Figure 3b further pinpoints the aging related hypoactivation in all ROIs and PD-related changes in the MTL.

Percent Signal Change in Aging-Related ROIs—To define more precisely the group differences in learning-related activation, percent signal changes were extracted for the Sequence and Random conditions, separately, in a subset of the ROIs (taken for this analysis from Table 1 of Schendan, *et al.*, 2003) that showed aging-related group differences in the above results for learning-related activation (YC > MC in Fig. 4): right MTL [27 −21 −18], left MTL [−30 −21 −9], right putamen [30 0 −3], left putamen [−27 6 −1], right DLPFC [36 27 39], and left DLPFC [−36 27 39]; note, the MTL ROIs correspond to the hippocampus head and subiculum ROIs of Schendan, *et al.*, 2003. These data were entered into ANOVAs that also included covariates of age, gender, and paradigm version.

Learning-Related Activation (Difference of Sequence > Random): Omnibus Run by ROI and Group: Percent signal changes for learning-related activation (difference of Sequence minus Random conditions) were extracted for each run and group separately (Figure 4) and entered into an omnibus ANOVA with within-subject factors of Run and ROI and a between-subject factor of Group, and covariates of age, gender, and paradigm. Results revealed a significant main effect of Group (F[2,32] = 7.17, p = 0.003), as learning-related activation was higher for the YC group (M = 0.202) than both older groups (MC M = −0.065; PD M = −0.081; ps < .002). This further confirms overall sequence-specific, learning-related hypoactivation with aging in all frontostriatal and MTL ROIs.

Condition Effects (Sequence vs. Random): Omnibus Condition by Run, ROI and Group: To characterize condition effects more precisely and sensitively, percent signal changes for Sequence versus Random conditions were separately extracted for each run and entered into an ANOVA with within-subject factors of condition, run, ROI and a between-subject factor of group, and covariates of age, gender, and paradigm. Results revealed a significant main effect of condition (F[1,32] = 7.08, p = 0.012), demonstrating signal changes differed overall between Sequence and Random conditions. The condition by group interaction was significant (F[2,32] = 7.17, p = 0.003), demonstrating the condition effect differed overall between groups. To interpret the condition by group interaction, planned contrasts used the same ANOVA on each pair of groups, separately. Results revealed that Sequence and Random conditions differed between YC and MC groups, as Condition by Group was significant (F[1,21] =6.41, p = .019). The two conditions also differed between the YC and PD groups, as the main effect of Condition (F[1,21] =7.99, p = .010) and Condition by Group interaction were significant (F[1,21] =12.74, p = .002). Between MC and PD groups, the main effect of Condition was significant (F[1,19] =4.66, p = .044), but

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Condition by Group was not (F[1,19] =0.24, p = .629), suggesting that condition effects were present in the older people but were indistinguishable between the two older groups. This provides further evidence that sequence-specific, learning-related activation differed between the younger (YC) and both older groups (MC, PD). However, as Figure 5a shows, this was because signal was higher overall for Sequence than Random in the YC group but not the MC and PD groups who showed instead lower signal overall for Sequence than Random. Planned contrasts using the same ANOVA on each group, separately, suggested that the YC group showed significant main effects of Condition (F[1,13] =7.95, p = .014) and ROI (F[5,65] =7.07, p = .001), the MC group showed no reliable effects (Condition, F=. 72, p = .421), and the PD group showed a significant main effect of Condition (F[1,8] =6.44, p = .035); note, for the YC group, effects were found only without covariates, which have little or no relevance for this group anyway. Overall, this suggests that the YC group showed greater signal for Sequence than Random, whereas the PD group showed the opposite (i.e., less signal for Sequence than Random), and the MC group showed a nonsignificant minimal difference between conditions in the same direction as the PD group. Notably, Run was not significant in these percent signal change results, supporting the validity of collapsing across runs for more power in the above comparisons of learning-related SPM activation between pairs of groups (Figure 3b).

Condition Effects (Sequence vs. Random): Omnibus Condition by ROI and Group: In order to increase power in this signal change analysis, as had been done for the SPM contrasts, another ANOVA was conducted on data collapsed over all runs with within-subject factors of condition, ROI and a between-subject factor of group, and covariates of age, gender, and paradigm. Results showed a significant interaction of Condition by Group (F[2,32] =4.39, p = 0.021). Planned contrasts using the same ANOVAs on pairs of groups indicated a significant main effect of Condition (F[1,21] = 5.74, p = 0.026) and a Condition by Group interaction between YC and PD groups (F[1,23] = 8.16, p = 0.009; YC *vs*. MC, F = 3.59, p = 0.072; MC *vs*. PD, F = .003, p = 0.955). As Figure 5 shows, this was because percent signal changes were higher for Sequence than Random in the younger YC group but not in the PD group which instead showed lower signal for Sequence than Random in all ROIs except left DLPFC where signal was comparable between conditions, and the MC group showed a similar pattern to the PD group overall.

Task-Related Activation (Random > Baseline)—Results so far suggest that the reduced learning-related activation with aging could be due to greater activity in the ROIs in response to the SRT task itself in older relative to younger people. To evaluate this observation more precisely, SPMs were produced of one-sample (p_u < 0.001) and two-sample t-tests (p_u < 0.0167 = alpha .05 ÷ 3 ROIs of basal ganglia, MTL, and DLPFC) of the Random condition greater than Fixation collapsed across runs. This defined activation related to the task itself in the absence of sequence-specific learning. Figure 3c shows the within-group fMRI results, showing higher and greater extent of activation in MTL, striatal, and DLPFC ROIs in both older (MC, PD) than younger (YC) groups. Accordingly, as Figure 7b shows, comparisons between groups showed that both older groups (MC and PD) relative to the younger (YC) group showed hyperactivation in the striatum, the MTL, and DLPFC during the SRT task. Specifically, for MC relative to YC groups, the Random

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condition hyperactivation was found bilaterally in the ROIs of striatum, MTL, and DLPFC. For PD relative to YC groups, hyperactivation was found in the ROIs of left caudate, left putamen, and left DLPFC. Between the two older groups (MC *vs*. PD), no differences reached the threshold; note, at the most liberal threshold ($p_u < 0.05$), for MC relative to PD, task activation was greater in MTL and striatum, hinting that PD may paradoxically reduce aging-related hyperactivation, shifting task-related activity overall toward the level in the younger group (as evident in Figure 5). In sum, performing the SRT task (with unique Random SOC sequences) activated the MTL, basal ganglia, and DLPFC more in older than younger people, and this hyperactivation with aging resulted in less sequence-specific learning related activation in these areas. Thus aging increases MTL and frontostriatal task-related activation in the direction (Sequence > Random) found in the young group.

DISCUSSION

These results indicate that Parkinson's disease affects the MTL and frontostriatal processes involved in sequence learning. In addition, by examining both younger and older control participant groups, the results also demonstrate changes in the MTL and frontostriatal processes related to aging. Importantly, the PD-related changes were obtained even though the PD group and their age, education, and gender-matched control group were indistinguishable on standard clinical neuropsychological tests of general intelligence, psychomotor speed, complex attention, executive function, and emotional status. We discuss the behavioral findings first and then how the neuroimaging findings relate to the performance findings.

Behaviorally, aging results in a decline in sequence-specific learning of higher-order associations, and PD exacerbates this. The basic RT results comparing the times in Sequence and Random conditions directly (Fig. 1a) show that older (PD and MC) groups are slower overall on the sequence learning task than the younger (YC) group, consistent with slowed processing speed with normal aging and basal ganglia disease (Schendan, Amick, and Cronin-Golomb, 2009; Grahn, Parkinson, & Owen, 2008; Salthouse, 1996). More important, RTs also reveal that sequence-specific learning varies across trials, and the pattern of this variation differs between the YC and MC groups, as well as between the MC and PD groups. These group differences reflect the finding of sequence-specific learning in both healthy groups, which varies across trials in the MC group. In contrast, the PD group shows no evidence for such learning effects. Thus an overall benefit of learning the sequence on speed of SRT task performance was found only in the healthy groups, not in PD patients, but this benefit for healthy people was reduced for older relative to younger groups. Further, older healthy people differ in how learning develops across trials from PD patients. Together, these basic RT findings indicate that implicit sequence-specific learning performance changes with aging and further with PD.

Additional detailed analyses of RTs yielded higher-order associative learning scores at each location in the sequence illustrate (Fig. 1b) that, compared to the younger group, both older groups learn to associate different segments of the repeating sequence, and, in each run, associated across a smaller number of higher-order location sequences and across shorter segments of the repeating sequence. More important, overall statistical results show that

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how associative learning scores vary across locations differs between the YC and MC groups, demonstrating that implicit higher-order associative sequence learning changes with aging. Such aging-related decreases are consistent with evidence that aging affects the initial phase of implicit sequence learning but not later learning phases (Daselaar, Rombouts, Veltman, Raaijmakers, & Jonker, 2003) and that aging impairs implicit learning of higher-order sequences of three or more locations (Howard & Howard, 1997), even in people between 34 and 52 years old (Feeney, Howard, & Howard, 2002). In the present study, patients with PD show a smaller degree of higher-order associative learning in most runs relative to the YC group. In addition, MC and PD groups differ regarding how higher-order associative learning scores vary across trials and among different locations (i.e., at least between the four sequences that were most rapidly learned and retained in the YC group: 3-1-2, 3-4-1, 1-3-2, and 3-2-4; Fig. 1b). Overall, the RT results suggest that PD exacerbates the implicit higher-order associative learning impairment that accompanies aging.

Participants learned the SRT sequences implicitly, meaning they were not told there was a sequence at the start of testing. From our previous work (Schendan, *et al.*, 2003), we know that some subjects become explicitly aware of the sequence over the course of learning. In order to assess sequence awareness, a set of explicit episodic memory tests were administered after the learning session ended. The results of these subsequent explicit memory tests indicate that the initial acquisition phase of higher-order sequence learning assessed here produces explicit knowledge in only a subset of the younger people, as reported previously (Schendan, *et al.*, 2003), and in none of the older people. Only some of the younger people freely recall part of the sequence above chance levels (Schendan, *et al.*, 2003); neither the MC or PD group shows such evidence of explicit knowledge. Overall, no clear evidence was found for explicit knowledge in both older groups or for aging or PD related differences in such explicit knowledge. Most important, altogether, the implicit and explicit behavioral findings indicate that learning was implicit in both the MC and PD groups in this experiment, occurring without subsequent evidence of conscious awareness of sequence knowledge. The results indicate a dissociation between implicit and explicit knowledge such that the MC and PD groups differ from each other primarily in implicit knowledge. This behavioral data supports the idea that the learning-related brain activity differences between the MC and PD groups reflects differences in implicit higher-order associative learning.

The neuroimaging results implicated frontostriatal and MTL sources of these behavioral changes in implicit higher-order associative learning and memory in our MC group, and MTL sources of changes in this learning in our PD group. All groups demonstrated significant learning-related activation (Sequence > Random) in all ROIs, consistent with the behavioral evidence of sequence-specific implicit learning of higher-order associations in all groups. The re-analysis presented here in this subgroup of young participants, who are education and gender matched to the older groups, confirmed the previous findings that higher-order, implicit sequence learning activates the basal ganglia, MTL, and DLPFC bilaterally (Schendan, *et al.*, 2003). In contrast, relative to this younger group, both MC and PD groups show hypoactivation in these regions related to higher-order sequence learning.

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Critically, the hypoactivation related to implicit sequence-specific learning in both MC and PD groups was found to be driven by the relative hyperactivation in all ROIs while performing the SRT task itself. First, the learning-related hypoactivation with aging in the frontostriatal and MTL ROIs was due to greater activation for the Sequence than Random condition in younger YC group but not the older MC and PD groups who instead collectively show a reversal of this pattern (i.e., greater activation for the Random than Sequence condition). This reversal was significant in PD and evident in direction but not reliable in the MC group. Similar reversals of patterns between younger and older groups have been reported for memory encoding (Duverne *et al.*, 2009; S. L. Miller *et al.*, 2008), and for effects of episodic recollection of arbitrary associations in the default mode network (de Chastelaine *et al.*, 2011). Here, the reversal of pattern is significant in PD patients. Importantly, previous work has associated over-recruitment of brain regions with worse performance (e.g., de Chastelaine *et al.*, 2011). Here, over-recruitment of frontostriatal and MTL regions during the SRT task and concomitant reductions in learning related activation coincide with worse implicit learning performance with higher-order associative sequences with aging, which is exacerbated in PD.

Second, findings in the Random condition alone (relative to Baseline fixation) indicate that the older MC and PD participants hyperactivate frontostriatal circuits and the MTL relative to the younger group. The random condition requires participants to make simple visual-motor associations between the four visual locations and four response keys and/or simple stimulus-stimulus associations between two adjacent locations in this experiment. This finding may be consistent with evidence for “hyperbinding” of irrelevant associative information in aging (Campbell, Hasher, & Thomas, 2010) – in this case nonspecific visual-motor associations and/or simple stimulus-stimulus associations. One may speculate that frontostriatal circuits and the MTL are over-recruited for this irrelevant hyperbinding process in aging. The frontostriatal regions would also be recruited while encoding the simple associations in both older groups, but this would not result in sequence-specific learning because of the absence of higher-order sequence information transfer from the MTL to the striatum, which is critical in the early phases of learning assessed here (Curran, 1997; Schendan, *et al.*, 2003). Alternatively, these findings are also consistent with the hypothesis that hyperactivation represents a deficit regulating activity in a brain structure, which can result in a performance decline (Tinaz, Schendan, & Stern, 2008). This contrasts with most studies that instead interpret hyperactivation as due to compensatory processes that can result in more normal performance (e.g., Mallol *et al.*, 2007).

Prior work has focused on implicit learning of simple or first-order conditional sequences that differ in item and bi-item frequencies and do not require the acquisition of higher-order associations among three or more items. Prior studies have also examined later phases of sequence learning and overlearning. Results of these prior studies implicated frontostriatal circuits but not MTL structures (e.g., Grafton, Hazeltine, & Ivry, 1995; Nissen & Bullemer, 1987; Nissen, Knopman, & Schacter, 1987; Nissen *et al.*, 1989; Rauch *et al.*, 1995; Rauch *et al.*, 1997; Reber & Squire, 1994; Reber & Squire, 1998; Willingham, Salidis, & Gabrieli, 2002). In contrast, the present study used a higher-order sequence learning task on which patients with MTL amnesia are impaired (Curran, 1997). The results of our earlier study in

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younger participants found that the MTL is important in higher-order sequence learning and is recruited especially in the initial stages of learning, regardless of conscious awareness of sequence information (Schendan, *et al.*, 2003). The present work in older subjects and PD patients demonstrates aging-related hyperactivation, or over-recruitment, of the MTL and frontostriatal regions at a basic procedural level, revealed especially by examining the Random condition in which no learning of a specific sequence beyond simple stimulus-stimulus associations [i.e., 2 consecutive locations] can occur). Consequently, higher-order sequence learning-related activity in the MTL and striatal circuits and corresponding performance measures also change with aging. Altogether, these findings are consistent with evidence that different learning processes are involved in sequence learning in younger and older people (Weiermann and Meier, 2012).

These aging-related changes affect interpretation of disease related changes in implicit sequence learning in PD. PD patients show changes in the MTL beyond the changes in normal aging. Specifically, relative to their age matched control group, PD patients show right MTL hypoactivation (i.e., an exacerbation of the aging pattern) and left MTL hyperactivation (i.e., an amelioration of the aging pattern). The reasons for these MTL differences are unclear. However, one reason could be due to the focus of the present research on the early acquisition phase of implicit sequence learning. Our subjects had no exposure to the sequence before the first scanning run, whereas other studies scanned subjects after the sequence was learned, as detailed previously (Schendan, *et al.*, 2003). The early phase of learning examined here is especially sensitive to the role of the MTL in creating new higher-order associations and our results provide evidence for MTL changes in both aging and PD. In particular, the MTL has been implicated in relational learning (Cohen & Eichenbaum, 1993) or perception and memory of spatial locations and paths (Murray, Bussey, & Saksida, 2007). Previously in the young normal group, right more than left MTL was related to implicit higher-order associative learning (Schendan *et al.*, 2003). Right MTL hypoactivation in PD may thus reflect changes in relational learning or resolving ambiguity among sequence locations. Instead, left MTL hyperactivation might reflect a compensatory process due to a relatively intact dopaminergic system in the ventro-tegmental area, which links to the MTL (Lees and Smith, 1983; Torak and Morris, 1986; Taylor and Saint-Cyr, 1995). Finally, we note the caveat that the relatively small samples could explain the lack of evidence here for an effect of side of disease onset and differences between PD and MC groups in frontostriatal regions.

In conclusion, by comparing non-demented PD patients and age-matched and younger healthy people, these behavioral and neuroimaging findings tease apart effects of aging and the disease processes in PD on implicit learning. All three groups were tested during the early acquisition phase of a higher-order sequence learning task that recruits both frontostriatal and MTL regions (Schendan *et al.*, 2003). With normal aging, the ability to regulate neural activity in the MTL and frontostriatal circuits changes, which affects implicit sequence learning performance. With Parkinson's disease, an additional dysfunction occurs in the MTL, which further affects this ability.

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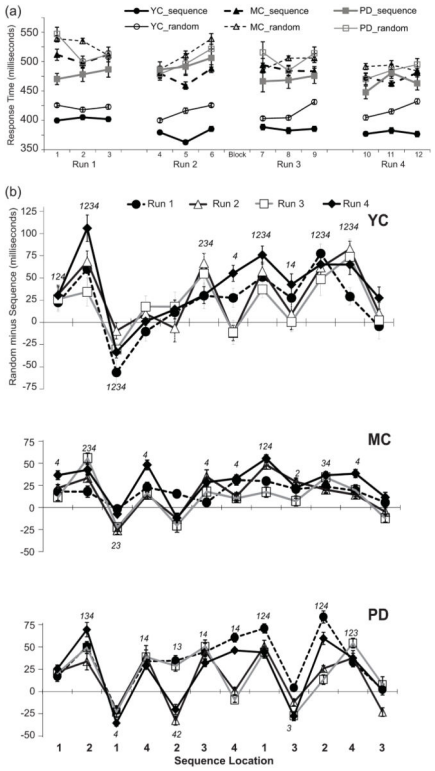

Figure 1.

Performance. YC = Younger control group, MC = Healthy control group matched to Parkinson's disease patients, PD = Parkinson's disease patients. Error bars show SE. (a) Median RTs in each block of each run. (b) Higher-order associative learning in each run for each group. The Sequence is shown along the x-axis. On the y-axis, a significant difference score (Sequence - Random) at a location can be found only if this location and the preceding two or more locations in the repeating Sequence were learned. For example, a significant difference at the final occurrence of 4 (i.e., penultimate location in the Sequence) indicates that the higher-order sequence 3-2-4 had been learned; if only the transition pair 2–4 had been learned, no difference could be found because frequency of transition pairs were identical between Sequence and Random conditions. Numerical labels at each Sequence position designate a reliable RT difference in that run 1–4 (all $p < .05$).

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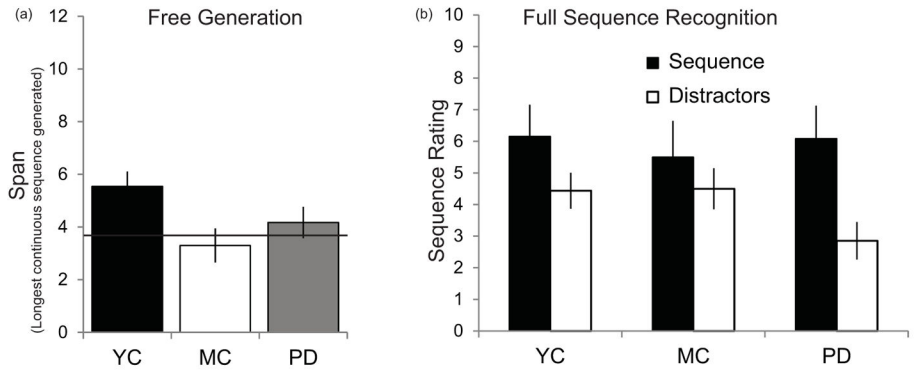

Figure 2.

Memory Tests of Conscious Sequence Knowledge. YC = Younger control participants, MC = Healthy control participants matched to patients with Parkinson's disease, PD = Patients with Parkinson's disease. (a) Free generation results demonstrated that younger people (YC) recalled longer continuous sections of the repeated Sequence than older people (MC). Black horizontal line shows chance level of 3.71 (Rauch, et al., 1997). (b) Full sequence recognition revealed that ratings for the actual Sequence and Distractor sequences differed significantly for the YC and PD groups, and the PD group was significantly more accurate in their ratings of Distractor sequences than the YC group.

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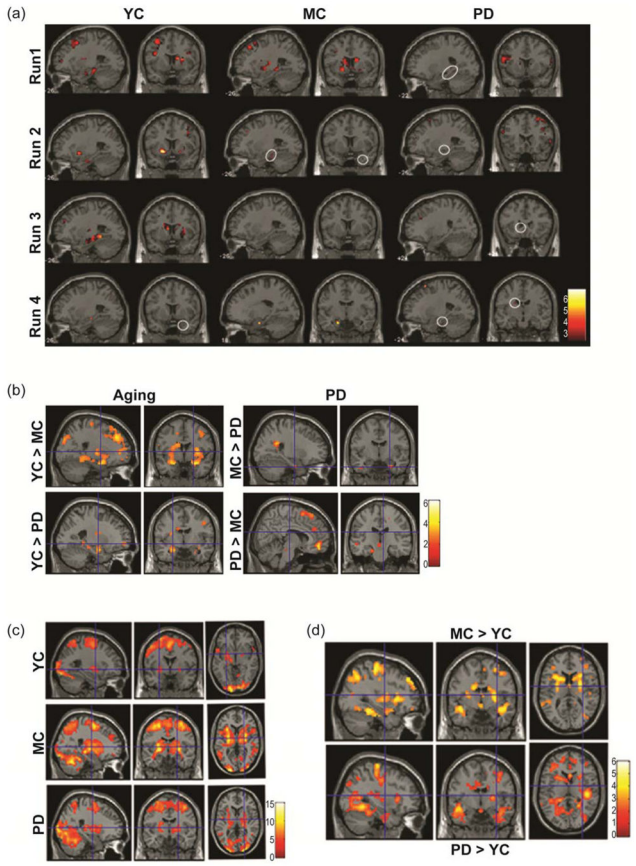

Figure 3. Statistical Parametric Maps

YC = Younger control participants, MC = Healthy control participants matched to patients with Parkinson's disease, PD = Patients with Parkinson's disease. Results displayed in neurological coordinates (left is left).

(a) *Implicit Learning-Related Activation in Each Run of the SRTT.* FMRI results (Sequence > Random; $p_u < .0167$) superimposed on sagittal and axial slices of T1-weighted template image. Circles highlight small clusters of significant voxels within the ROIs. Sagittal and coronal slices show locations of significant learning-related activation in MTL and striatal ROIs, respectively, in each run of each group; note, for comparison between groups, see Figure 3b.

(b) *Learning-Related Activation (Sequence > Random) over All Runs between Groups.* Between group fMRI results ($p_u < .0167$) shown with crosshairs located at the same coordinate in sagittal and coronal slices but at different coordinates for each contrast in order to capture the main significant effects in the ROIs for each set of results. To pinpoint these results with greater anatomical precision, the coordinates, *t*- and uncorrected *p*-values of the peak of the group difference in learning-related activation within each ROI are reported as follows. (left top) YC > MC: MTL (right [30 3 −18] t =2.62, p = 0.0001; left [−18 −21 −21] t =3.62, p = 0.0008); basal ganglia (right caudate [−12 −3 21] t =3.34, p = 0.001; left putamen ([27 −9 6] t =3.19, p = 0.002; [−27 −6 15] t =3.29, p = 0.002); DLPFC (right: [36 33 18] t =3.2, p = 0.003; [27 45 −3] t =2.79, p = 0.006; left: [−36 51 −6] BA11 t =3.91, p = 0.0004). (left bottom) YC > PD: MTL (right: [27 −3 −21] t =2.94, p = 0.004; left: [−24 −6

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−21] t =3.49, p = 0.001; [−30 −21–9] t =2.67, p = 0.007); basal ganglia (right putamen [27 3 −9] t =2.45, p = 0.008; left putamen [−30 −6 6] t =2.5, p =0 .01); right DLPFC ([18 33 36] t =3.01, p = 0.003). (right top) MC > PD: right MTL ([27 −9 −30] t =2.59, p = 0.008). (right bottom) PD > MC: left MTL ([−18 0 −18] t =2.35, p = 0.014; [−18 −21 −21] t =2.71, p = 0.006).

(c) *SRT Task Activation (Random > Baseline) Within Each Group.* One sample t-tests (p_u< . 001). FMRI results displayed on 3 slice-plane views. Both MC and PD groups demonstrate more activation in regions identified as being essential for implicit learning by Schendan, et al. (2003).

(d) *SRT Task Activation (Random > Baseline) Between-Groups.* Two sample t-tests (p_u< . 0167). FMRI results displayed on 3 slice-plane views. Note, for c and d: Crosshairs located at the same coordinate in sagittal, coronal, and axial slices for each group; crosshairs located at different coordinates between each group in (c) and between pairwise group contrasts in (d) in order to capture the main significant effects in the ROIs for each set of results.

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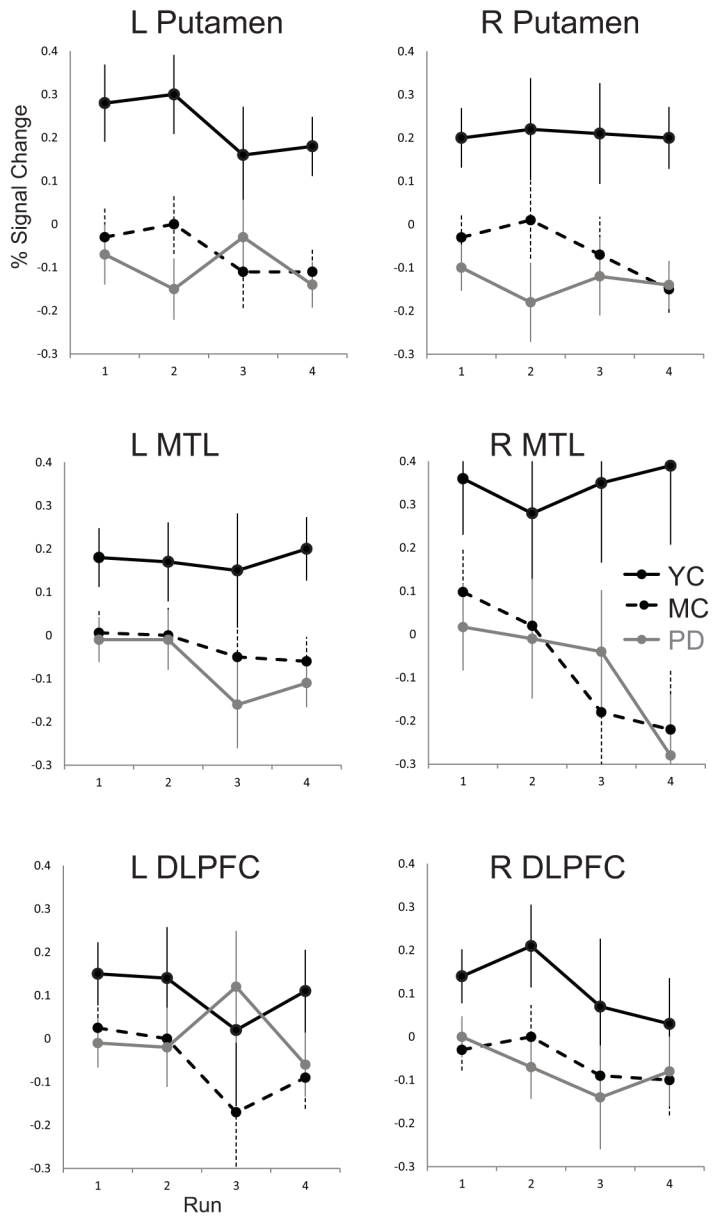

Figure 4.

Signal Changes for Sequence-Specific, Learning-Related Activation in each Run, ROI, and Group. YC = Younger healthy control participants (black solid line), MC = healthy control participants age-matched to patients with Parkinson's disease (black dashed line), PD = Patients with Parkinson's disease (gray solid line). Line graphs of percent signal change difference scores (Sequence minus Random) reveal changes in learning-related activation across runs for each group in the ROIs. Standard error bars provided for each data point.

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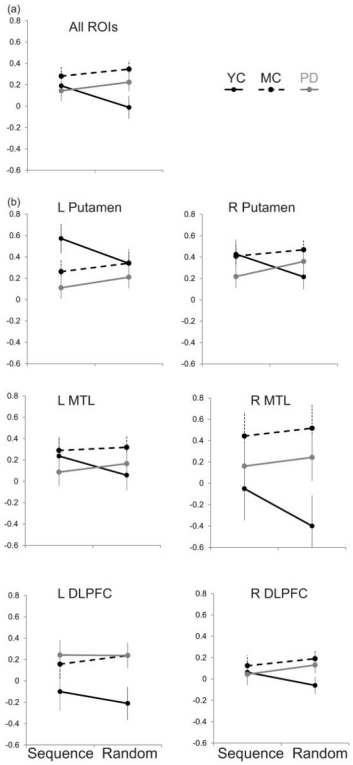

Figure 5.

Signal Changes between Sequence and Random Conditions between Groups. YC = Younger healthy control participants (black solid line), MC = healthy control participants age-matched to patients with Parkinson's disease (black dashed line), PD = Patients with Parkinson's disease (gray solid line). Line graphs of percent signal change averaged across runs for sequence and random conditions reveal condition x group interactions between younger (YC) and older (MC and PD) groups. (a) Condition by Group over all ROIs. (b) By Region of Interest. YC group consistently shows a pattern of increased sequence relative to random conditions, whereas MC and PD groups demonstrate the opposite pattern of increased activation during the Random as opposed to the Sequence condition. Standard error bars provided for each data point.

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Table 1

Demographics and Clinical Neuropsychological Tests

	YC (*n*=14)	MC (*n*=12)	PD (*n*=12)
Age (years)	30 ± 6.9	58 ± 7	59 ± 7.4
Education (years)	17.0 ± 1.42	16.5 ± 3	16.0 ± 2.6
Gender	5M/8F	5M/7F	5M/7F
Handedness		1-LH	3-LH
MMSE		29 ± 1.60	29.5 ± .76
DRS		143 ± .75	143 ± .50
ANART		121.2 ± 7.1	122.7 ± 3.7
Digit Span - Forward		7.3 ± .9	8 ± .6
Digit Span - Backward		5.8 ± 1.2	5.6 ± 1.4
Digit Symbol*		76.7 ± 14	67.8 ± 7.6
Symbol Search		34.4 ± 6	31 ± 4.2
Trails A		32 ± 12	32 ± 7.8
Trails B†		62.9 ± 11.5	80.55 ± 37
BDI-II		2 ± 2	8 ± 7
STAI-State		26.7 ± 6.6	31.5 ± 5
STAI-Trait		27 ± 4.6	33.1 ± 11.5

Note. YC = Younger control participants. MC = Healthy control participants age-matched to patients with Parkinson's disease. PD = Patients with Parkinson's disease. M = male. F = female. LH = left hand. All values represent raw, nonstandardized scores (± *SD*). Mini Mental Status Examination (MMSE) is from Folstein *et al*. (1975); Dementia Rating Scale (DRS) is from Mattis (1988); American National Adult Reading Test (ANART) is from Gruber and Sliwinski (1991); Digit Symbol Substitution, Symbol Search, Digit Span Forward, and Digit Span–Backward measures of the third version of the Wechsler Adult Intelligence Scale are from Wechsler (1997); Trails A and B tests are from Reitan and Wolfson (1993); Beck Depression Inventory-II (BDI-II) is from Beck (1997); Spielberger State–Trait Anxiety Inventory (STAI) measures are from Spielberger et al. (1983).

*$p < .07$

†$p < .08$, one-tailed

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Table 2

Learning-Related Activation in Each Run for MTL ROIs (Sequence > Random; Small Volume Correction)

MTL		*MNI coordinate*			*Runs*				
Group	***Hemi***	***x***	***y***	***z***	***All***	***1***	***2***	***3***	***4***
Hh, pHg									
YC	L	−33	−18	−27	2.31††	2.85*	2.27*	2.04†	
MC	L	−33	−18	−27	2.16††				
YC	R	33	−18	−27	2.76*	2.50*	2.54*	2.50*	
MC	R	33	−18	−27			1.96*		2.16††
PD	R	33	−18	−27		1.82†			
YC	L	−24	−27	−18	3.36**	3.41**		2.77*	1.90†
MC	L	−24	−27	−18		1.84†	2.21*		
PD	L	−24	−27	−18		1.96†			1.84†
Amyg									
YC	L	−21	3	−15	2.67*	2.14††	2.94*		
MC	L	−21	3	−15		1.82†			
PD	L	−21	3	−15	1.84†	2.60*	2.11††		
YC	R	21	6	−21	3.59**	1.80†	2.59*	2.30*	
Hh									
YC	L	−30	−24	−9	3.65**	3.22**	1.68†	3.36**	2.37*
MC	L	−30	−24	−9	1.90†	2.60*			
YC	R	30	−24	−6	2.32*	3.10*	1.70†	2.35*	
MC	R	30	−24	−6		3.27**			

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MTL		MNI coordinate			Runs				
Group	*Hemi*	*x*	*y*	*z*	*All*	*1*	*2*	*3*	*4*
YC	R	30	−18	−15	2.80*	2.80*	2.26*	2.54*	
MC	R	30	−18	−15		2.80*			
PD	R	30	−18	−15				1.80†	
YC	R	27	−21	−18	2.80*	2.80*	2.20*	2.43*	
MC	R	27	−21	−18		2.91*			
PD	R	27	−21	−18	1.66$	1.97†	1.78†	1.80†	
STG									
YC	R	27	9	−27	3.04*	1.73†	2.46*		2.10*

Note. YC = Younger control participants, MC= Healthy control participants age-matched to patients with Parkinson's disease, PD= Patients with Parkinson's disease

L: Left hemisphere, R: Right hemisphere, MTL: Medial temporal lobe, Hh: Head of the hippocampus, pHg: Parahippocampal gyrus. Amyg: Amygdala, STG: Superior temporal gyrus

*FDR-corrected $p < .05$.

**FDR-corrected $p < .005$.

†Uncorrected $p < .05$.

††Uncorrected $p < .01$

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Table 3

Learning-Related Activation in Each Run for Frontostriatal ROIs (Sequence > Random; Small Volume Correction)

		MNI coordinate			*Runs*				
Group	***Hemi***	***x***	***y***	***z***	***All***	***1***	***2***	***3***	***4***
STRIATUM									
Caudate – Head									
YC	L	−9	24	0			1.90†		
MC	L	−9	24	0		1.85†	2.52*		
PD	L	−9	24	0	1.82†		1.82†	2.27*	
Caudate - Body									
YC	L	−15	−6	18	2.86*	2.70*	2.31*	1.88†	1.66†
MC	L	−15	−6	18		2.55*			
PD	L	−15	−6	18		2.19††			
YC	R	15	3	15		3.32**	1.99$	1.93*	
MC	R	15	3	15		4.10**			
YC	L	−15	15	12	2.55*	1.73†		1.85*	
MC	L	−15	15	12		3.24**			
PD	L	−15	15	12		2.13††			1.67†
YC	R	15	12	12	2.74*	3.86**	1.70†	2.26*	
MC	R	15	12	12		3.19*			
PD	R	15	12	12	1.96†	1.87†			1.70†
Putamen (Lentiform Nucleus)									
YC	R	30	0	−3	2.88*	2.52*	2.23*	1.97*	
MC	R	30	0	−3		1.71†			

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		MNI coordinate			***Runs***				
Group	***Hemi***	***x***	***y***	***z***	***All***	***1***	***2***	***3***	***4***
YC	R	18	6	−9	2.87*	1.72†	2.73*	1.89†	
MC	R	18	6	−9		2.43*			
YC	L	−27	−9	9	2.97*	2.47*	2.16††	1.94†	
MC	L	−27	−9	9		2.62*			
FRONTAL LOBE									
SFG									
YC	R	36	45	−15		2.42*	2.47*		
PD	R	36	45	−15					2.50*
IFG									
YC	L	−51	21	6	1.93†	4.39**			
MC	L	−51	21	6		1.80†			
PD	L	−51	21	6	1.76†	2.72*			1.71†
YC	R	51	33	15	2.04†	3.13**	3.23**		
MC	R	51	33	15		1.68†			
PD	R	51	33	15	1.83†	1.72†		1.93†	

Note. YC = Younger control participants, MC= Healthy control participants matched to patients with Parkinson's disease, PD= Patients with Parkinson's disease

L: Left hemisphere, R: Right hemisphere. SFG: Superior frontal gyrus, IFG: Inferior frontal gyrus

*FDR-corrected $p < .05$.

**FDR-corrected $p < .005$.

†Uncorrected $p < .05$.

††Uncorrected $p < .01$