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A variable number of tandem repeats in the 3′-untranslated region of the dopamine transporter modulates striatal function during working memory updating across the adult age span

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Abstract

Dopamine modulation of striatal function is critical for executive functions such as working memory (WM) updating. The dopamine transporter (DAT) regulates striatal dopamine signaling via synaptic reuptake. A variable number of tandem repeats in the 3′-untranslated region of *SLC6A3* (*DAT1*-3′-UTR-VNTR) is associated with DAT expression, such that 9-repeat allele carriers tend to express lower levels (associated with higher extracellular dopamine concentrations) than 10-repeat homozygotes. Aging is also associated with decline of the dopamine system. The goal of the present study was to investigate the effects of aging and *DAT1*-3′-UTR-VNTR on the neural activity and functional connectivity of the striatum during WM updating. Our results showed both an age-related decrease in striatal activity and an effect of *DAT1*-3′-UTR-VNTR. Ten-repeat homozygotes showed reduced striatal activity and increased striatal–hippocampal connectivity during WM updating relative to the 9-repeat carriers. There was no age by *DAT1*-3′-UTR-VNTR interaction. These results suggest that, whereas striatal function during WM updating is modulated by both age and genetically determined DAT levels, the rate of the age-related decline in striatal function is similar across both *DAT1*-3′-UTR-VNTR genotype groups. They further suggest that, because of the baseline difference in striatal function based on *DAT1*-3′-UTR-VNTR polymorphism, 10-repeat homozygotes, who have lower levels of striatal function throughout the adult life span, may reach a threshold of decreased striatal function and manifest impairments in cognitive processes mediated by the striatum earlier in life than the 9-repeat carriers. Our data suggest that age and *DAT1*-3′-UTR-VNTR polymorphism independently modulate striatal function.

Introduction

Aging causes widespread impairment of memory function, including working memory (WM) (Sambataro *et al.*, 2012a), and brain networks underlying WM show decreased age-related function (Sambataro *et al.*, 2009). In particular, the dopaminergic meso-cortico-striatal pathway shows a decline of function during WM updating with increasing age (Podell *et al.*, 2012). Converging evidence indicates that dopamine signaling, mostly in the striatum, is reduced in both presynaptic and postsynaptic compartments with aging, and that this continuous process begins in early adulthood, with a 5–10% rate per decade (Backman *et al.*, 2006).

The meso-cortico-striatal dopamine pathway plays an important role in modulating and regulating higher cognitive functions (Frank *et al.*, 2001; Cools *et al.*, 2004). In particular, computational models suggest that dopamine in the dorsal striatum, including the caudate, plays a critical role in gating information to the prefrontal cortex (PFC) (Hazy *et al.*, 2006), thus allowing this region to rapidly switch to incoming information while maintaining task-relevant representations. This function is central to WM updating, which is the selective updating of old information with new incoming infor-

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mation (Murty *et al.*, 2011). More recently, hippocampus and hippocampal–striatal interactions also have been implicated in learning processes (Schiffer *et al.*, 2012; Brown & Stern, 2014; Murty *et al.*, 2013). Hippocampal function is also regulated by dopamine signaling (Shohamy & Adcock, 2010).

Terminals from the substantia nigra area and ventro-tegmental area release dopamine in synapses in the striatal and the limbic regions, including the hippocampus, respectively. Levels of this neurotransmitter in the striatum depend on the activity of the dopamine transporter (DAT), which mediates the reuptake of dopamine into the presynaptic terminal, thus terminating its action. Genetic variation [40-bp variable number of tandem repeats (VNTR) polymorphism] in the 3′-untranslated region of the DAT gene (*DAT1*) (*DAT1*-3′-UTR-VNTR) has been associated with its variable expression (Vandenbergh *et al.*, 1992). Several alleles have been described in *DAT1*-3′-UTR-VNTR, with those ranging from 9 to 11 repeats being most frequent (Vandenbergh *et al.*, 1992). The 10-repeat allele is associated with greater expression of DAT than the 9-repeat allele, resulting in lower striatal extracellular dopamine levels (van Dyck *et al.*, 2005). Although the 3′-VNTR polymorphism is not the only functional variant in *DAT1*, it has been the most extensively investigated.

On the basis of this background, we hypothesised that caudate function during WM updating would be modulated by genetically determined variation in DAT expression levels, such that 9-allele repeat carriers (expressing lower levels of DAT, and thus putatively higher striatal dopamine levels) would show greater memory-dependent striatal activity than 10-repeat homozygotes. We also hypothesised that the physiological age-related decline in caudate responses during WM updating would also be modulated by genetically determined variation in DAT expression levels. Furthermore, given the aforementioned role of the hippocampus and hippocampal–striatal interactions in learning, and the modulatory effect of dopamine signaling on hippocampal function, we further predicted that *DAT1*-3′-UTR-VNTR would also modulate hippocampal function and hippocampal–striatal interaction during WM updating across the adult life span. We believe that this line of research will contribute to a better understanding of the neurobiology underlying individual variability in the function of the nigrostriatal dopaminergic system. In particular, this study explores the role of *DAT1*-3′-UTR-VNTR and age-related changes in striatal function. To this end, we studied healthy volunteers across the adult life span while they performed a WM updating paradigm, a cognitive process that we have previously shown to significantly activate the cortico-striatal system (Murty *et al.*, 2011).

Materials and methods

Subjects

Fifty-four right-handed healthy Caucasian subjects (mean age ± SD, 37.6 ± 15.7 years; age range, 20–77 years; 36 males) were recruited for this study (Table 1), and genotyped for *DAT1*-3′-UTR-VNTR (10-repeat homozygotes, $n = 29$; 9-repeat carriers, $n = 25$). The imaging data of 47 participants were included in a previous article (Podell *et al.*, 2012). All subjects had normal or corrected to normal visual acuity. Handedness was assessed with the Edinburgh Questionnaire (Oldfield, 1971). Exclusion criteria included a past history or the presence of any medical, neurological or psychiatric disorders according to DSM-IV (following a structured clinical interview, SCID-IV) (First *et al.*, 1996), drug treatment (except birth control pills in young women and hormonal substitution therapy in postmenopausal women), and past head trauma with loss of consciousness. Older subjects underwent a thorough neuropsychological assessment to evaluate cognitive status and exclude gross cognitive decline (Mini Mental State Examination > 28, Clinical Dementia Rating = 0). All participants gave written informed consent to take part in the experiment, which was approved by the Intramural Review Board of the National Institute of Mental Health, and carried out in accordance with the principles of the revised Helsinki Declaration.

Task

All participants performed a WM task designed to isolate the selective updating component of WM processes, as described previously (Murty *et al.*, 2011) (Fig. S1). Briefly, each trial consisted of three phases. Participants were required to memorise four digits, each presented in a blue box for 3.8 s (encoding phase). Subsequently, four black boxes were shown in the same position as the blue boxes, and contained digits and/or asterisks. Participants were instructed to hold the same digit as presented before in the box when an asterisk was shown, and to encode the new digit when it appeared in the box. Series of black boxes were presented three to five times for 2.2 s each (experimental phase). Finally, participants were presented with four digits located within red boxes for 3 s, and they were required to indicate with a button press whether the presented whole set of digits matched the set that they were currently maintaining in memory (response phase). Each trial consisted of three different memory conditions based on the manipulation of the experimental phase: maintenance (MAIN), overwriting (OVR), and updating (UPD). In the MAIN phase, black boxes contained only asterisks for five serial presentations (11.2 s), so that participants were required to hold the same set throughout the trial. During OVR, three to five series of digits (6.6–11.2 s) within black boxes were presented, so that participants continually cleared all of the digits that they were maintaining, and encoded four new digits. During this condition, the number of presentations of black boxes was varied to avoid the predictability of the last presentation. During UPD, participants were shown five series of digits (11.2 s) within black boxes containing one to three digits with the rest of the boxes containing asterisks, so that they had to selectively update information from the original sequence of digits. Participants were presented with nine MAIN, nine OVR and 27 UPD trials (nine trials of one-digit to three-digit presentations, respectively) over three

TABLE 1. Demographics and behavioral data of the sample

	Nine-repeat carriers	Ten-repeat homozygotes	Difference, *P*-value
n	25	29	
Age (years), mean ± SD (range)	40 ± 17.1 (22–68)	35.8 ± 13.7 (20–77)	0.23
Male gender, no.	18	18	0.44
Handedness (years) (EHI), mean ± SD	85.4 ± 30.1	70.5 ± 53.5	0.23
IQ score, mean ± SD	111.7 ± 10.1	111.4 ± 10.1	0.90
Education (years), mean ± SD	16.9 ± 2.2	16.9 ± 2.1	0.98
UPD accuracy (% correct), mean ± SD	82.6 ± 0.8	81.1 ± 1	0.60
WM accuracy (% correct), mean ± SD	97.7 ± 4.6	95.3 ± 7.5	0.16
OVR accuracy (% correct), mean ± SD	88.9 ± 7.3	88.4 ± 5.1	0.80

EHI, Edinburgh Handedness Inventory; *n*, sample size; SD, standard deviation.

experimental runs, with each run lasting for 5 min 20 s. The trial order was pseudo-randomised within each run. Between trials, a fixation crosshair was presented at interstimulus intervals of 3.1 ± 1.2 s. Performance was recorded through a fiber optic response box, which allowed measurement of behavioral data as the number of correct responses (accuracy) and reaction time (RT).

Image acquisition

Each subject was scanned on a GE Signa (GE, Milwaukee, WI, USA) 3-T scanner. A gradient echo blood oxygen level-dependent (BOLD)–echo planar imaging pulse sequence was used to acquire 160 images per each run. Each functional image consisted of 26 interleaved 4-mm-thick axial slices (repetition time, 2000 ms; echo time, 28 ms; matrix, 64 × 64; gap, 1 mm; field of view, 24 cm; flip angle, 90°).

Image processing

Images were processed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm). Briefly, images were slice time-corrected, realigned to the first image of the scan run with INRIALIGN, spatially normalised into a standard stereotactic space (MNI template) to a 3 × 3 × 3 voxel size with an affine and non-linear transformation, and smoothed with an 8-mm full-width half-maximum isotropic 3D Gaussian kernel. The data were temporally high-pass-filtered with a cut-off frequency of 1/128 Hz to remove the effects of scanner signal drifts. Each individual dataset was then carefully screened for data quality via inspection for image artefacts and excessive head motion (>3 mm of head motion or 2° of head rotation). Predictor variables were generated by convolving a canonical hemodynamic response function with a series of discrete event onset times, time-locked to the presentation of encoding, experimental (UPD, OVR, or MAIN) and response phases. Incorrect trials were modeled separately, and not included in the analyses. A voxel-wise general linear model with all of the predictors and subject-specific estimated movement extent as covariates was fitted to each subject's preprocessed images.

In the first-level analyses, linear contrasts were computed by producing *t*-statistical parameter maps at each voxel for the experimental phase during correct OVR, MAI and UPD conditions relative to a fixation baseline, and UPD > OVR to evaluate the neural response associated with selective updating only. Although all MAIN, OVR and UPD trials involve some aspects of WM, MAIN is a simple, delayed, matched-to-sample task, in which the subject is requested only to maintain four digits. On the other hand, UPD requires selective and specific updating of the WM representation while the subject partially maintains some digits. OVR trials also involve WM updating, but this updating is non-selective and non-specific. The contrast UPD > MAIN can identify those brain regions that are involved in selective updating while the WM component is controlled for. As the aim of our study was to identify the selective and specific gating of information to the WM store, a component of executive function hypothesised to depend on meso-cortico-striatal signaling (Murty *et al.*, 2011), we focused our analyses on the UPD > OVR contrast only.

The contrasts of the selective updating of WM content (UPD > OVR) responses in correct trials were then analysed with second-level random effects analyses across subjects. These contrast images allow the isolation of selective and specific gating of information to WM, which is thought to be mediated, at least in part, by meso-cortico-striatal dopamine signaling (Murty *et al.*, 2011; Podell *et al.*, 2012), and is also modulated by cognitive aging (Podell *et al.*, 2012). These statistical images were entered into second-level random effects models to identify age-related, genotype-related and age-by-genotype-related changes in brain activation, and corrected for false discovery rate (FDR) and small volume to control type I error alpha = 0.05. On the basis of our *a priori* hypothesis based on the role of the caudate and its connectivity with the hippocampus in learning and dopamine-dependent modulation of their activity, we used small volume correction (SVC) for these regions identified by use of the WFU-PickAtlas (http://www.fmri.wfubmc.edu). In detail, a simple correlation analysis was performed to assess the effect of increasing age on brain activation during UPD > OVR across adulthood on the whole brain. Age was used as a continuous variable to examine the age-related effects across the whole adult lifespan (Sambataro *et al.*, 2009, 2010). Two-sample *t*-tests were used to evaluate the effects of *DAT1*-3′-UTR-VNTR on brain activity. To explore how *DAT1*-3′-UTR-VNTR modulated the age-related decline in updating responses, we compared genotype effects implicitly masked by the age-related decline in UPD > OVR contrast ($P < 0.005$). Then, we extracted the BOLD signal change from significant clusters, and compared the correlation coefficients between age and BOLD signal change across genotype groups by using the Fisher *r*-to-*z*′ transform. To explore whether there was a relationship between brain activation and UPD accuracy, we performed a linear regression analysis between the signal change from the UPD > OVR contrast extracted from significant clusters bilaterally in the caudate head and the performance computed as the difference between UPD and OVR accuracy across all subjects. Furthermore, to explore whether *DAT1*-3′-UTR-VNTR modulated the relationship between caudate activation and UPD accuracy, we compared the correlation coefficients between accuracy and BOLD signal change across genotype groups by using the Fisher *r*-to-*z*′ transform. To isolate the brain areas with both age-related and genotype-related differences in activation during UPD relative to OVR, we created a conjunction map of the age-related decline and *DAT1*-3′-UTR-VNTR 9 > 10/10 effects for UPD > OVR contrast images. Contrast images were thresholded with $P < 0.05$, so that the resulting conjoint probability of the conjunction maps was 0.0025 (Sambataro *et al.*, 2012b).

Psychophysiological interactions (PPIs) were also computed for each subject by using the bilateral anatomical caudate (identified by use of the WFU-PickAtlas) as the seed region to assess changes in functional coupling of these regions with other brain regions during updating as compared with overwriting, and the resulting images were analysed for genotype and age effects as in the general linear model analyses. Correction for multiple comparisons and small volume was implemented on the hippocampal formation (hippocampus and parahippocampus), given its well-known role in learning and the *DAT1*-3′-UTR-VNTR-dependent modulation of its activity (Bertolino *et al.*, 2008). To explore the correlation between caudate–hippocampal connectivity and updating performance, we correlated PPI scores from significant clusters in the hippocampus with UPD accuracy. We also compared caudate–hippocampal connectivity and UPD accuracy correlation differences between genotype groups by using a one-tailed Fisher *r*-to-*z*′ transform. To further ascertain the relationship between caudate activation and caudate–hippocampal connectivity, we estimated the correlation between these two measures across all subjects.

Genotyping

DAT1 (*SLC6A3*) genotyping was performed on DNA derived from the peripheral lymphocyte fraction of the venous blood. Genotyping of the *DAT1* 40-bp repeat (VNTR) polymorphism (rs28363170) in the 3′-untranslated region was determined with forward 5′-TGT

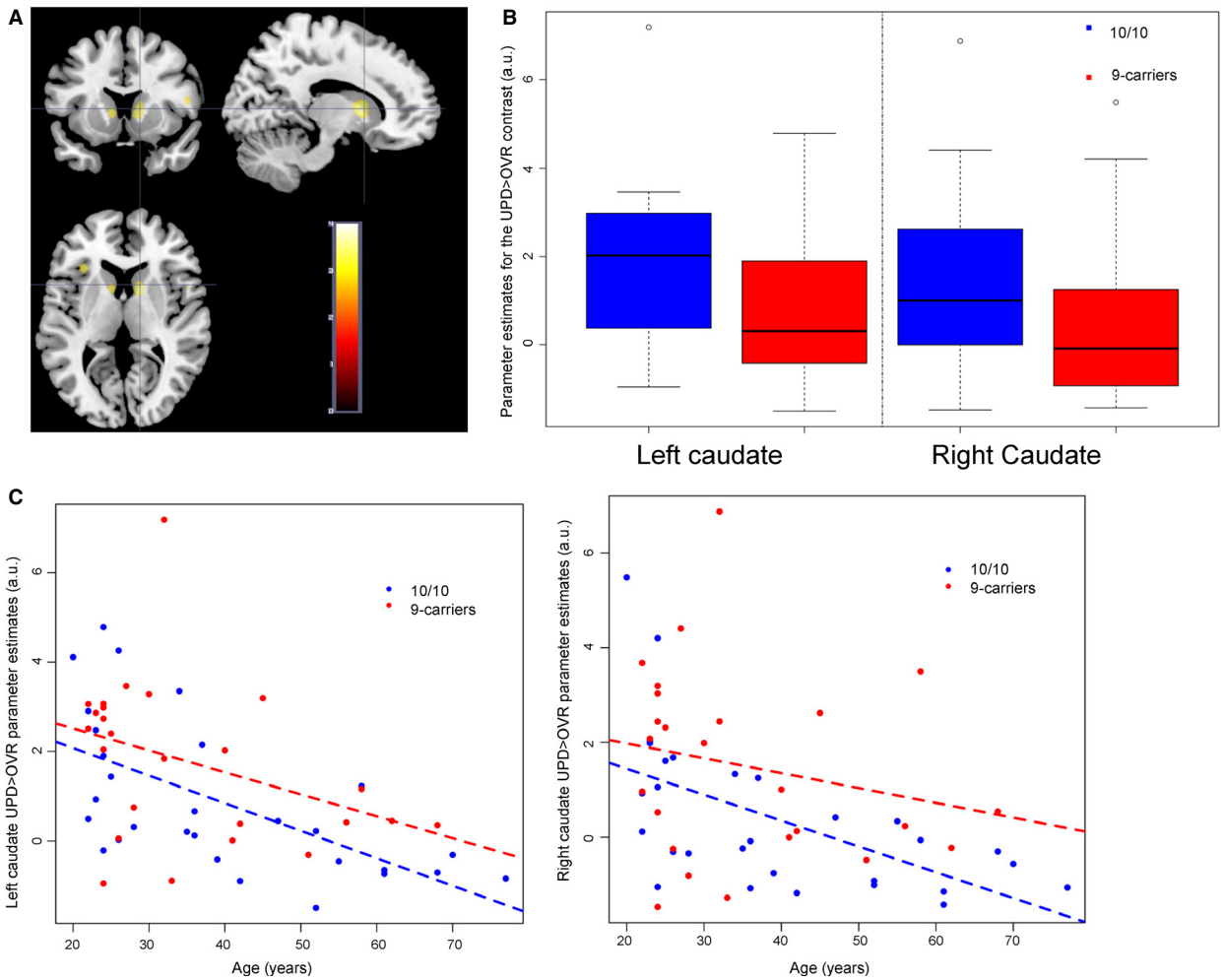

FIG. 1. Effect of *DAT1*-3′-UTR-VNTR and age on UPD > OVR brain responses. (A) On the left, statistical maps show greater activation on the UPD > OVR contrast in the caudate regions that show an age-related decline in 9-repeat carriers than in 10-repeat homozygotes. Statistical *t*-maps (thresholded at $P < 0.005$ for visual display) are overlaid on the MNI brain template. (B) Boxplots of the cluster-averaged parameter estimates from the UPD > OVR contrast in the left caudate (peak cluster coordinates: $x = 12$, $y = 9$, $z = 6$, $k = 48$) and right caudate (peak cluster coordinates: $x = -9$, $y = 9$, $z = 3$, $k = 27$), respectively. In the boxplot, the black band indicates the median, the bottom and the top of the box indicate the first and third quartiles, the whiskers indicate the minimum and the maximum values within 1.5 times of the interquartile range, and the circles indicate the outliers. (C) Bilateral caudate activation during updating (parameter estimates extracted from UPD > OVR contrast as in B) declined with age similarly in the left ($r = -0.52$, $P < 0.001$) and the right ($r = -0.41$, $P < 0.005$) hemispheres in both *DAT1*-3′-UTR-VNTR groups. Nine-repeat carriers are represented in red, and 10-repeat homozygotes in blue.

GGTGTAGGGAACGGCCTGAG-3′ and reverse 5′-CTTCCTGGA GGTCACGGCTCAAGGTCA-3′ primers. Polymerase chain reaction amplification of the 40-bp repeat VNTR alleles was performed as described elsewhere (Szekeres *et al.*, 2004). Polymerase chain reaction products were separated by 2% agarose gel electrophoresis, visualised by ultraviolet transillumination, and fragment-sized by comparison with the Invitrogen 50-bp DNA ladder. Participants genotyped for *DAT1*-3′-UTR-VNTR were subdivided into 9-repeat carriers (9/9, $n = 3$; 9, $n = 22$) and 10-repeat homozygotes (10/10, $n = 29$). Allele frequencies in this sample did not deviate from Hardy–Weinberg equilibrium (exact test, $P = 0.2$).

Results

Behavioral results

Task performance, age, gender, IQ and education did not differ significantly across genotype groups (all *P*-values > 0.2). Age correlated significantly with IQ ($r = 0.46$; $P = 0.001$) and education ($r = 0.43$; $P = 0.001$). In spite of this, there was impaired UPD performance with increasing age, as measured by reduced accuracy ($r = -0.43$; $P = 0.001$) and longer RTs ($r = 0.42$; $P = 0.001$). Within each genotype group, an increase in age significantly correlated with reduced accuracy (10/10, $r = -0.421$, $P = 0.023$; 9, $r = -0.488$, $P = 0.013$) and longer RTs (10/10, $r = 0.459$, $P = 0.012$; 9, $r = 0.466$, $P = 0.019$). There was no age × *DAT1*-3′-UTR-VNTR interaction effect on behavioral performance ($P > 0.20$).

Imaging results

Effect of task

The UPD > OVR contrast showed robust activation (Table S1) in a bilateral network of brain regions encompassing the basal ganglia, anterior cingulate cortex, thalamus, and cerebellum, and the dorsolateral PFC, ventrolateral PFC (VLPFC), premotor, parietal and occipital cortices, and cerebellum.

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Effect of age

An increase in age was associated with lower activation in the basal ganglia, dorsolateral PFC, VLPFC, anterior cingulate cortex, and premotor and parietal cortices bilaterally (Fig. S2; Table S2).

Effect of DAT1-3′-UTR-VNTR

We found a significant effect of *DAT1*-3′-UTR-VNTR in the caudate bilaterally (left caudate, *xyz* = −9,9,2; right caudate, *xyz* = 9,9,2; $P < 0.05$ FDR SVC; Table S3), with 9-repeat carriers showing greater activation during UPD relative to OVR than 10-repeat homozygotes.

Effect of age and DAT1-3′-UTR-VNTR

We found a significant effect of *DAT1*-3′-UTR-VNTR in the caudate regions that show an age-related decline in UPD > OVR responses (left caudate, *xyz* = −9,9,3; $k = 27$, $Z = 3.20$, $P < 0.05$ FDR SVC; right caudate, *xyz* = 12,9,6; $k = 48$, $Z = 3.44$, $P < 0.05$ FDR SVC; Fig. 1A and B), with 9-repeat carriers showing greater activation during UPD relative to OVR than 10-repeat homozygotes. This difference remained significant even after removal of outliers identified with the interquartile range method. There was a significant positive correlation between caudate activation during UPD relative to OVR with UPD relative to OVR accuracy in the left caudate ($r = 0.42$, $P = 0.002$) and the right caudate ($r = 0.37$, $P = 0.006$). This association was not modulated by *DAT1*-3′-UTR-VNTR (all *P*-vaues > 0.2; Fig. 1C). There was a significant age-related decline in activation of the bilateral caudate, VLPFC, superior parietal cortex, premotor cortex, and cerebellum, as previously reported (Podell *et al.*, 2012). Although there were no significant age × *DAT1*-3′-UTR-VNTR interactive effects ($P > 0.2$), conjunction analysis with the main effect of age and genotype contrasts revealed significant effects in the right caudate and bilateral inferior frontal gyrus and parietal regions (Table S4). We found significant associations between accuracy and activation of the left caudate head ($r = 0.47$, $P = 0.001$) and the right caudate head ($r = 0.42$, $P = 0.001$). This association was not modulated by *DAT1*-3′-UTR-VNTR (all *P*-values > 0.2).

Psychophysiological interactions (PPIs)

PPI analysis of the caudate revealed greater UPD-dependent connectivity of this region with the hippocampus in 10-repeat homozygotes than in 9-repeat carriers (Fig. 2). Also, the caudate–hippocampal PPI connectivity correlated positively with UPD accuracy in 10-repeat homozygotes ($r = 0.38$, $P = 0.04$), but not in the 9-repeat carriers ($r = -0.07$, $P = 0.74$), with a trend for significance difference between these correlations ($P = 0.1$). There was no significant age-related or *DAT1*-3′-UTR-VNTR modulation of age-related decline in PPI results. There was no correlation between caudate activation and hippocampal PPI ($P > 0.2$) on either side.

Discussion

In this study, we investigated the effects of *DAT1*-3′-UTR-VNTR genetic variation on brain regions underlying WM updating and how these effects could modulate age-related changes. Accuracy and RTs were similar across genotype groups, thus reducing the possibility that behavioral measures could account for genotype-related neural differences. Two main findings emerged. First, we found that *DAT1*-3′-UTR-VNTR modulated striatal activity and its connectivity with the hippocampus during WM updating across the adult age range. Second, we found that the activity of the striatum during WM updating decreases with increasing age, and that the rate of decline is similar in carriers of *DAT1*-3′-UTR-VNTR genetic variants.

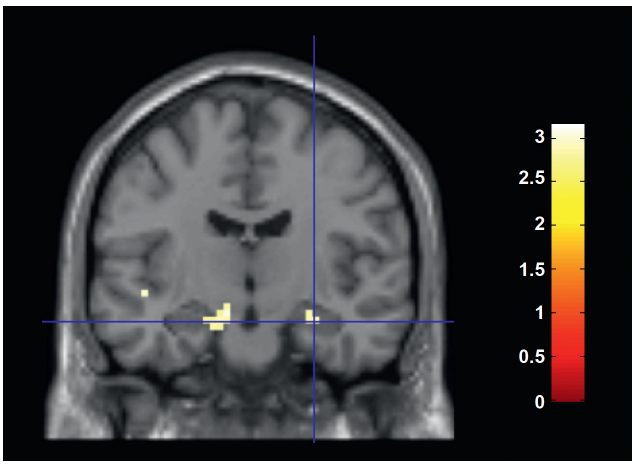

FIG. 2. Effect of *DAT1*-3′-UTR-VNTR on UPD > OVR psychophysiological responses. Ten-repeat homozygotes showed greater updating-dependent connectivity between the caudate and hippocampus than to 9-repeat carriers. Statistical *t*-maps (thresholded at $P < 0.005$ for visual display) are overlaid on the MNI brain template.

Our results suggest that genetic variation in *DAT1*-3′-UTR-VNTR affects brain activation and connectivity during WM updating across the adult life span. In younger subjects, Schott *et al.* (2006) found that *DAT1*-3′-UTR-VNTR modulated neural activity in the midbrain during recognition memory. Also, in a similarly aged population, Bertolino *et al.* (2009) found that this genotype modulated the PFC during WM, with 9-repeat carriers having greater activation in the ventral, dorsal and medial PFC than 10-repeat homozygotes. We found that 9-repeat carriers have greater striatal activation during updating than 10-repeat homozygotes. These effects of *DAT1*-3′-UTR-VNTR were primarily located in the head of the caudate nucleus, which has projections to the dorsolateral PFC documented previously through a variety of approaches, including diffusion tensor imaging (Lehericy *et al.*, 2004; Leh *et al.*, 2007; Draganski *et al.*, 2008), morphometry (Cohen *et al.*, 2008), functional connectivity (Choi *et al.*, 2012), and structural covariance studies in humans, as well as tracing studies in monkeys. These regions have been implicated in WM function (Lewis *et al.*, 2004; Dahlin *et al.*, 2008; McNab & Klingberg, 2008), including updating (Murty *et al.*, 2011). In our study, caudate activation correlated positively with performance, further confirming its role in WM updating. We did not find any effect of *DAT1*-3′-UTR-VNTR on hippocampal activity. Previous findings showed the effects of *DAT1*-3′-UTR-VNTR on implicit sequence learning (Simon *et al.*, 2011). The learning of the sequence dependencies is a process that depends on striatal activity (Jackson *et al.*, 1995). Subjects carrying 9-allele repeats show greater sequence learning over time than 10-repeat homozygotes (Simon *et al.*, 2011), adding to the evidence for a modulatory role of *DAT1*-3′-UTR-VNTR in striatal function.

Our findings extend the effects of this genetic variation on striatal activity to WM processes, and specifically to updating. Given the well-known function of DAT, the observed differences are probably mediated by changes in striatal dopamine signaling. In 9-repeat carriers, decreased DAT striatal expression could result in greater striatal dopamine signaling, which plays a pivotal role in WM updating

(Murty *et al.*, 2011). We also found that *DAT1*-3′-UTR-VNTR may affect hippocampal–striatal connectivity, suggesting alternative cognitive strategies in 10-repeat homozygotes to maintain performance. Previous studies have indicated an important role of the hippocampus and its interaction with the striatum in mediating both implicit and explicit learning (Schiffer *et al.*, 2012; Brown & Stern, 2014) across the adult life span (Rieckmann *et al.*, 2010; Dennis & Cabeza, 2011). Decreased availability of striatal and hippocampal dopamine in 10-repeat homozygotes could result in compensatory increased functional coupling between these two regions for optimal performance on the task.

Brain activation decreased in the caudate, VLPFC and parietal cortex with increasing age (Podell *et al.*, 2012). Evidence suggests that the age-related reduction in brain responses could be associated with decreased dopamine signaling (Braskie *et al.*, 2008; Klostermann *et al.*, 2012). Indeed, the concentration of this neurotransmitter, the availability of DAT and dopamine receptor densities decline from early to later adulthood (Backman *et al.*, 2006). Although these changes spanned the same brain regions modulated by *DAT1*-3′-UTR-VNTR, we did not find an age × *DAT1*-3′-UTR-VNTR interaction. This suggests that age-associated reductions in cortico-striatal activation of brain regions underlying updating are not differentially sensitive to variations in DAT levels related to *DAT1*-3′-UTR-VNTR. However, because of dopamine signaling baseline differences, we cannot rule out the possibility that 10-repeat homozygotes may achieve lower levels of activation at a younger age, and thus may show age-related cognitive impairments earlier in life. Consistent with our findings of *DAT1*-3′-UTR-VNTR modulation of the striatum in adulthood, neurodevelopmental studies have shown that *DAT1*-3′-UTR-VNTR can affect WM and general cognition in children as well (Cornish *et al.*, 2008; Soderqvist *et al.*, 2012).

In conclusion, these findings add to the evidence for a role of dopamine in the modulation of cortico-striatal networks underlying cognition during adulthood, particularly WM updating. Further studies investigating longitudinal age-related changes of DAT-dependent effects on neural networks and their relevance to neuropsychiatric disorders are warranted.

Supporting Information

Additional supporting information can be found in the online version of this article:

Fig. S1. Working memory updating task (Murty *et al.*, 2011).

Fig. S2. Effect of age on updating > overwriting brain responses.

Table S1. Effect of task (updating > overwriting: $P < 0.05$ FDR).

Table S2. Age-related decline of updating > overwriting responses ($P < 0.005$).

Table S3. Effect of *DAT1*-3′-UTR-VNTR (9 > 10/10; $P < 0.005$).

Table S4. Conjunction of age-related and *DAT1*-3′-UTR-VNTR effects of updating > overwriting.

Disclosure statement

The authors declare no competing financial or personal interests that can influence the presented work. Dr Fabio Sambataro is a full time employee of F. Hoffman-La Roche Ltd.

Abbreviations

BOLD, blood oxygen level-dependent; DAT, dopamine transporter; *DAT1*-3′-UTR-VNTR, variable number of tandem repeats in the 3′-untranslated region of *SLC6A3*; FDR, false discovery rate; PFC, prefrontal cortex; PPI, psychophysiological interaction; RT, reaction time; SVC, small volume correction; VLPFC, ventrolateral prefrontal cortex; VNTR, variable number of tandem repeats; WM, working memory.

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