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Effects of aging and dopamine genotypes on the emergence of explicit memory during sequence learning

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ABSTRACT

The striatum and medial temporal lobe play important roles in implicit and explicit memory, respectively. Furthermore, recent studies have linked striatal dopamine modulation to both implicit as well as explicit sequence learning and suggested a potential role of the striatum in the emergence of explicit memory during sequence learning. With respect to aging, previous findings indicated that implicit memory is less impaired than explicit memory in older adults and that genetic effects on cognition are magnified by aging. To understand the links between these findings, we investigated effects of aging and genotypes relevant for striatal dopamine on the implicit and explicit components of sequence learning. Reaction time (RT) and error data from 80 younger (20–30 years) and 70 older adults (60–71 years) during a serial reaction time task showed that age differences in learning-related reduction of RTs emerged gradually over the course of learning. Verbal recall and measures derived from the process-dissociation procedure revealed that younger adults acquired more explicit memory about the sequence than older adults, potentially causing age differences in RT gains in later stages of learning. Of specific interest, polymorphisms of the dopamine- and cAMP-regulated neuronal phosphoprotein (*DARPP*-32, rs907094) and dopamine transporter (*DAT*, VNTR) genes showed interactive effects on overall RTs and verbal recall of the sequence in older but not in younger adults. Together our findings show that variations in genotypes relevant for dopamine functions are associated more with aging-related impairments in the explicit than the implicit component of sequence learning, providing support for theories emphasizing the role of dopaminergic modulation in cognitive aging and the magnification of genetic effects in human aging.

1. Introduction

Convergent data from brain and behavioral levels underscore that memory is a multifaceted function that involves multiple brain circuitries and cognitive processes (Cohen & Squire, 1980; Reber & Squire, 1994; Squire & Zola-Morgan, 1991; for reviews see Squire, 2004, 2009). A prominent model proposed by Cohen and Squire (1980) posits a basic distinction between declarative and non-declarative memory, and similar views have proposed to distinguish between explicit (verbally reportable) and implicit (not verbally reportable) learning (*e.g.*, Reber, 1989; for reviews see Cleeremans, 1997; Frensch & Rünger, 2003). In this view, declarative or explicit memory refers to memory contents and episodes that can be consciously recalled and is primarily implicated by the hippocampus and adjacent areas, which are commonly referred to as medial-temporal lobe (MTL). Non-declarative or implicit memory, in contrast, subsumes a number of different types of memory that are not dependent on the MTL and mostly inaccessible by conscious recall. Motor skill acquisition is an example of non-declarative/implicit memory and the striatum has been found to be a key component of the neural network underlying this ability (Doyon & Benali, 2005).

One of the commonly applied paradigms for studying motor skill acquisition is the serial reaction time (SRT) task (Nissen & Bullemer, 1987), in which participants learn sequential regularities of successive stimulus locations and their corresponding motor responses. Nissen and Bullemer showed that in this task participants acquired

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motor skills without being aware of what was learned or the learning process itself, and further studies showed that this learning co-occurred with activation in the striatum (Aizenstein et al., 2006; Atallah, Lopez-Paniagua, Rudy, & O'Reilly, 2007; Rauch et al., 1997). Relatedly, studies on the underlying neurochemical processes have suggested that implicit learning is, in part, implicated by dopaminergic receptor mechanisms in the striatum (Karabanov et al., 2010) and is modulated by the gene encoding the dopamine transporter protein (*DAT*, Simon et al., 2011), which is active mostly in the striatum (Heinz et al., 2000). At the same time, it has been reported that motor skill acquisition is preserved in amnesic patients, suggesting that is it hippocampus independent and dissociable from declarative/explicit memory (Nissen & Bullemer, 1987; Reber & Squire, 1994).

1.1. Implicit motor skill acquisition and aging

The distinction between explicit and implicit memory has also been a focus in the research on memory aging. A number of studies have shown little or no implicit memory impairments in older adults (Bo & Seidler, 2010; Fleischman, Wilson, Gabrieli, Bienias, & Bennett 2004; Howard & Howard, 1989; Light & Singh, 1987; for a review, see Rieckmann & Bäckman, 2009). This finding stands in contrast to the apparent age-related deficit in explicit memory, but it is confined to less complex statistical regularities (*e.g.* deterministic and lower order transitions between sequence elements, Howard & Howard, 1997; Howard et al., 2004) and does not apply to the use of chunks (Verwey, 2010; Verwey, Abrahamse, Ruitenberg, Jiménez, & de Kleine, 2011), although in younger adults implicit learning can capture higher order statistics of the sequence structure (Schuck, Gaschler, & Frensch, 2012; Schuck, Gaschler, Keisler, & Frensch, 2012). At the same time, aging is associated with apparent declines in dopaminergic modulation in various extrastriatal (*e.g.*, Kaasinen et al., 2000) and striatal (*e.g.*, Erixon-Lindroth et al., 2005) regions. Furthermore deficiencies of dopaminergic modulation in these brain circuitries contribute to various common cognitive impairments in old age (see Bäckman, Nyberg, Lindenberger, Li, and Farde, 2006; Li, Lindenberger, and Bäckman 2010, for an empirical review; see Li, Lindenberger, and Sikström, 2001, for a theoretical integration) and have been linked to deficiencies in striatal mechanisms underlying learning (Eppinger, Schuck, Nystrom, & Cohen, 2013). Additionally, longitudinal (Raz et al., 2005) and cross-sectional (Walhovd et al., 2011) research on changes in regional brain volumes has shown that the extent of volume shrinkage in the striatum is comparable to the decline in hippocampal volume.

In light of the above-mentioned relations between implicit learning and striatal dopamine on the one hand and the partially spared implicit learning abilities in older adults during sequence learning on the other hand, we investigated the effects of aging and dopamine-regulating factors on the implicit and explicit aspects of sequence learning in the SRT task. Therefore our key research question was whether genetic variations that influence dopamine functioning in older adults influence their learning and memory in the SRT task.

1.2. The DAT and DARPP-32 genes and motor skill acquisition

A recent receptor imaging study showed that sequence learning is modulated by striatal dopamine receptor density (Karabanov et al., 2010). It is thus of specific interest to investigate genotype effects of genes relevant for striatal dopamine function and how the genotype effects interact with age. To study the impact of dopamine-regulating factors, we took the candidate gene approach (see Green et al., 2008). Specifically, we investigated the impact of genetic variations in two genes known to be associated with striatal dopamine signaling, the dopamine- and cAMP-regulated neuronal phosphoprotein (*DARPP-32*, also known as the protein phosphatase 1 regulatory subunit 1B, *PPP1R1B*; location: 17q12) gene (Brené et al., 1994) and the dopamine transporter (*DAT*, *i.e. SLC6A3*; location: 5p15) gene (Vandenbergh, Persico, & Hawkins, 1992). The *DARPP-32* gene is particularly involved in integration of dopaminergic signal transmission in striatal dopamine receptors (Svenningsson et al., 2004). The DARPP-32 protein is highly expressed in striatal medium-sized spiny neurons and has a broad spectrum of effects on D1 as well as D2 receptors (Yger & Girault, 2011). Animal research has shown that manipulations of DARPP-32 implicate motor behavior in rodents (Bateup et al., 2010) as well as the occurrence of L-DOPA induced involuntary movements in a rodent model of Parkinson's Disease (Santini et al., 2007). In humans, it has been shown that a common haplotype that also includes the single nucleotide polymorphism (SNP) rs907094 of this gene is associated with striatal activation and volume (Meyer-Lindenberg et al., 2007) as well as performance in a reinforcement learning task (Frank, Moustafa, Haughey, Curran, & Hutchison, 2007; Hämmerer et al., 2013). Specifically, homozygotes of the *DARPP-32* rs907094 A allele ("A/A" carriers) performed better than carriers of the G allele (*i.e.*, A/G or G/G, henceforth "any G"). In addition to these effects, a recent paper has shown that the *DARPP-32* rs907094 polymorphism is related to attentional regulation (Li, Passow et al., 2013). Given the complexity of DARPP-32's effects on the dopamine system, a recent summary also concluded that "the contribution of DARPP-32 in human behavior remains poorly understood" (Yger & Girault, 2011). The animal research that highlighted a role of DARPP-32 in motor functions and its known role in DA function in general indicate that there is a further need to study the effects of DARPP-32 on human behavior. Moreover, research has shown that the expression of DARPP-32 increases with advancing age (Colantuoni et al., 2008), and hence *DARPP-32* provides an interesting candidate gene in the study of aging and motor skill acquisition. Finally, DARPP-32 is often assumed to be an integrator of neural dopaminergic signal transmission (Svenningsson et al., 2004), and hence its interactions with other DA-relevant genes are of particular interest.

The second gene we investigated, *DAT*, is also implicated in striatal dopaminergic neurotransmission and regulates the reuptake of dopamine from the synaptic cleft (Heinz et al., 2000). It has been shown that the various number tandem repeat (VNTR) in exon 15 affects gene expression (Fuke et al., 2001). The VNTR 9-repeat allele ("9-repeats") is associated with lower protein availability *in vitro* (Miller & Madras, 2002; VanNess, Owens, & Kilts, 2005) and *in vivo* (Cheon, Ryu, Kim, & Cho, 2005; Heinz et al., 2000; Jacobsen & Staley, 2000; van de Giessen et al., 2009). This decreased availability of DAT likely leads to increased availability of striatal dopamine in the synaptic cleft. In line with these findings, evidence from behavioral genetic studies shows that the *DAT* VNTR 9-repeat allele is associated with better working memory (Brehmer et al., 2009) and episodic memory (Li, Papenberg et al., 2013; Schott et al., 2006), although some studies did not replicate such an association (Boonstra et al., 2008; Rommelse et al., 2008). Of particular interest, Simon et al. (2011) reported an association between implicit learning and the *DAT* VNTR genotype, with 9-repeat carriers learning more than 10/10 homozygotes in an sequential triplet learning task.

In summary, we investigated two dopamine relevant genes. One gene, *DAT*, has been shown to affect implicit learning in younger adults. The second gene, *DARPP-32*, is not well studied in humans, but is known to affect motor behavior in animals, is increasingly expressed with advancing age and plays a particular role in the integration of DA signaling processes. Hence *DAT* and *DARPP-32* are ideal candidate genes to investigate interactive

effects of DA-relevant genes and age on motor skill acquisition. Based on the above reviewed literature, we expected that the investigated genes would influence implicit learning and motor function in younger and older adults.

1.3. Age-related magnification of genetic effects and changes in the organization of memory systems

There are, however, two additional and important considerations about the above described links between dopamine-relevant genes, aging and implicit learning in the SRT task. Firstly, the previous studies have revealed that associations between genetic variation and cognitive phenotypes are stronger in older as compared to younger adults (Li et al., 2010; Li, Papenberg et al., 2013; Nagel et al., 2008; Papenberg et al., 2013; Schuck et al., 2013). It has been proposed that these findings reflect an nonlinear function relating brain resources and cognitive function (Lindenberger et al., 2008). Such a nonlinear function is particularly evident in the case of dopamine, where an inverted U-shape function relating dopamine signaling in frontal cortex and memory has been reported (Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007; for reviews, see Arnsten, 1998; Seamans & Yang, 2004; for a computational account, see Li & Sikström, 2002). Thus, we expected a potential effect of dopamine-relevant genotypes on learning and performance in the SRT task to be stronger in older as compared to younger adults.

Secondly, for two reasons the link between striatal dopamine and knowledge measures from the SRT might be not as clear as the initially cited research suggests. The first reason is that the SRT task is not process pure. It has been shown that participants may also acquire partial explicit memory during the course of sequence learning (*e.g.*, Ferdinand, Rünger, Frensch, & Mecklinger, 2010; Pascual-Leone, Grafman, & Hallett, 1994; Rünger & Frensch, 2008) and hippocampal activity has been found during implicit learning tasks (Gheysen, Van Opstal, Roggeman, Van Waelvelde, & Fias, 2010). Moreover, recent work by Rose, Haider, and Büchel (2010) and Wessel, Haider, & Rose (2012) revealed that the transition from implicit to explicit memory is preceded by increased activity in the brain area that is linked to implicit learning, *i.e.* the striatum. Accordingly, some authors have focused on the interactions of implicit and explicit forms of memory instead of their dichotomous distinction (Destrebecqz et al., 2005; Haider & Frensch, 2005; Perruchet, Bigand, & Benoit-Gonin, 1997; Song, Marks, Howard, & Howard, 2009; Willingham & Goedert-Eschmann, 1999). A number of studies using incidental serial motor learning have indeed shown that implicit and explicit components of learning both develop and contribute to performance in incidental learning tasks. The existence of explicit sequence knowledge in some participants is most clearly shown by post-experimental verbal recall questionnaires (Rünger & Frensch, 2010) or a process–dissociation approach (PDP) based on post-experimental sequence generation tasks (Destrebecqz & Cleeremans, 2001). In addition, Ghilardi, Moisello, Silvestri, Ghez, and Krakauer (2009), Moisello et al. (2009), and Moisello et al. (2011) have shown that different aspects of behavior *during* task performance are related to implicit and explicit components, where kinematic changes and anticipatory movements were related specifically to the latter, but RT decreases were also observed in subjects without declarative knowledge. Hence, this research underpins the notion that implicit and explicit components contribute to SRT performance (RTs), but might be differentially reflected in different aspects of performance and post-experimental questionnaires. Relatedly, a recent study by Bornstein and Daw (2012) applied computational learning models to account for RT data from the SRT task and showed that models that incorporated both a fast and slow learning processes fitted the data best. Moreover, the faster learning process correlated with striatal activity indicating habitual associative responses, whereas the slow process correlated with hippocampal activity reflecting episodic memory processes about the structure of the sequence (model-based learning). While knowledge in the SRT might initially be largely implicit, explicit knowledge might gradually emerge with ongoing training (Perruchet et al., 1997; Rose et al., 2010). Such evolving explicit knowledge can be reflected in certain aspects of performance, such as anticipatory key presses. Whether the additional striatal involvement preceding the first signs of explicit knowledge that was reported by Rose et al. (2010) is related to more implicit knowledge that eventually leads to explicit knowledge or to the emergence of explicit knowledge directly, however, is still unclear.

The second reason that complicates the link between striatal dopamine and knowledge measures from the SRT is that aging does not only impair the dopamine system, but might also influence the differentiability of the neural bases of implicit and explicit memory. Two recent imaging studies (Dennis & Cabeza, 2011; Rieckmann, Fischer, & Bäckman, 2010) have found that in older adults the MTL plays a role in implicit learning in addition to the striatum and the striatum plays a role in explicit memory in addition to the MTL. In contrast, younger adults in these studies showed mainly striatum activation during implicit learning and MTL activity during explicit learning. Hence, the division of labors between the MTL and the striatum for explicit and implicit learning may become less distinct with aging, a phenomenon that is consistent with predictions by theoretical accounts of dopamine aging (Li, Lindenberger, & Frensch, 2000; Li et al., 2001; Li & Sikström, 2002) and further evidence from older adults and patients with striatal damage (Moody, Bookheimer, Vanek, & Knowlton, 2004; Papenberg et al., 2011). Consequently, we expected that the dopamine-relevant genetic factors also influence the explicit component of sequence learning, particular in older adults. In the present study we investigated these expectations by employing methods to disentangle implicit and explicit memory that develops during the SRT task.

1.4. Hypotheses and aim of study

In summary, the present study investigated the effects of human aging and polymorphisms of the *DAT* gene (VNTR) and the *DARPP-32* gene (the SNP rs907094) on implicit and explicit knowledge development during the SRT task. The previous studies have shown that older adults are less impaired in implicit than in explicit memory, but age differences in the detailed dynamics of the development of explicit knowledge as well as in the effects of dopaminergic genotypes on the different memory components are unknown. Moreover, previous research has indicated that the striatum might not only be implicated in implicit but also explicit memory and the dopaminergic neuromodulation account of cognitive aging (Li et al., 2000, 2001; Li, Naveh-Benjamin, & Lindenberger, 2005; Li & Sikström, 2002) predicts that with advanced age, a stronger link between striatal dopaminergic functions and explicit memory might occur. Additionally, the resource modulation hypothesis predicts that any genetic effects should be stronger in older as compared to younger adults. In line with these theories and data, we expect (a) larger age differences in the development of explicit as compared to implicit memory during the SRT task, (b) effects of dopamine-relevant genotypes on implicit and explicit components, (c) larger genetic effects in older adults in general and (d) larger genotype effects on the explicit component in older as compared to younger adults.

To test these predictions, we assessed participants' memory performance in the SRT task in terms of learning-related RT reductions and error rates, as well as post-learning measures of explicit and implicit sequence memory derived from the process-dissociation

procedure (PDP) and explicit verbal recall. We expected better performance for younger adults with respect to learning-related reductions in RTs and error rates after extended practice. The above-mentioned research by Bornstein and Daw (2012) suggested that learning that reflects the structure of the sequence (model-based learning) is slower, thus may only be observed later in the process. Furthermore, findings from the studies by Ghilardi et al. (2009)and Moisello et al. (2011) showed that participants without explicit knowledge developed less RT improvements and show less anticipatory movements. Hence, more explicit memory in younger adults might also be reflected in larger RT gains in the later part of training and more errors in trials where the stimuli did not follow the learned sequence. Moreover, in line with the evidence reviewed above, we predicted age-differences in post-experiment sequence memory for explicit (verbal recall and explicit PDP score) but not for implicit memory (implicit PDP score). Concerning dopamine genotypes, we anticipated effects on motor aspects of learning (*e.g.*, RT reduction) and implicit memory. We also anticipate these effects to be magnified in older adults. Lastly, based on neuroimaging results showing a dedifferentiation of the striatal and MTL memory systems and research showing that the transition from implicit to explicit memory implicates the striatum, we also anticipate an effect of dopamine-relevant genotypes on the explicit component of sequence learning, and that if such effects exist, they would be larger in older adults.

2. Material and methods

2.1. Participants

One hundred fifty-seven subjects of Caucasian origin participated in the study. The following participants were excluded from analysis: one older adult whose mean error rate exceeded 3.3 SDs from the age-group mean (> 23% errors), one younger participant whose genotyping failed and 5 participants (2 older and 3 younger) who had a rare *DAT* genotype (1 DAT VNTR 8/10 and 4 DAT VNTR 10/11). The effective sample was composed of 70 older (mean age: 65.8 years, range: 60–71, 35 female) and 80 younger (mean age: 25.1 years, range: 20–30, 38 female) adults, see Table 1. All participants were screened for neurologic, psychiatric, and other medical conditions using a questionnaire. The local ethics committee at the Max Planck Institute for Human Development approved the study. All participants gave written consent to the procedures and the collection of saliva samples and received 27 Euro as compensation for participation. In addition to the experimental task described below, the participants reported their years of education and we obtained information on two marker tests of fluid (perceptual speed) and crystallized (verbal fluency) intelligence. The age groups did not differ with respect to years of education ($t(113)=1.10$, $p=.2738$). Similar to the previous results based on representative population samples (Li et al., 2004), older adults performed worse than younger adults in the perceptual speed measure (identical pictures), $t(147.1)=-20.11$, $p<.0001$, but better than younger adults in the crystallized intelligence test (spot-a-word test), $t(146)=4.65$, $p<.0001$. Data from performance in a virtual spatial navigation task of this sample is reported elsewhere (Schuck et al., 2013). Table 1 summarizes the sample characteristics.

2.2. Genotyping

Saliva samples were collected using Oragene OG-250 collection kits (DNA Genotek, Ontario, Canada), and DNA extraction was conducted using standard procedures.

Genotyping of *DARPP-32* SNP rs907094 was carried out in a 384-well microtiter plate format using a commercially available 5′-exonuclease allelic discriminiation assays (based on "TaqMan" chemistry, Applied Biosystems [Foster City, CA, USA]). TaqMan oligonucleotide probes for genotyping were designed and synthesized by the manufacturer (assay ID: C___7452370_1_) and experimental conditions followed the manufacturer's instructions. In short, genotyping was performed on 384-well microtiter plates in 5 μl reaction volumes. For each reaction we combined 10 ng DNA template, 5 × TaqMan genotyping assay and 5 × TaqMan Genotyping Master Mix. Thermal cycling was done on a PTC-240 PCR instrument using the following cycling conditions: pre-amplification phase at 50 °C (2 min), initial denaturation at 95 °C (10 min), followed by 45 cycles of denaturation at 95 °C (15 s), annealing and extension at 60 °C (60 s). For the *DAT* VNTR, we genotyped the 40-base-pair VNTR in the 3′ untranslated region following previously published procedures (Lim et al., 2006). Product amplification was achieved by polymerase chain reaction (PCR) on 96-well microtiter plates in 10 μl reaction volumes. For each reaction we combined 1.5 μM of each primer, 50 ng/μl of DNA template, 0.25 mM dNTPs, 0.25U Taq polymerase and Q solution (QIAGEN Ltd; Hilden, Germany). Thermal cycling was done on an MJ Research Thermo Cycler PTC-240 using the following cycling conditions: initial denaturation at 94 °C (3 min), followed by 35 cycles of denaturation at 94 °C (45 s), annealing at 70 °C (90 s), and extension at 72 °C (35 s), followed by a final extension step at 72 °C (6 min). Genotypes were called after visualization of amplification products on a Shimadzu MCE-202 MultiNA instrument (Shimadzu Corporation, Kyoto, Japan) using the DNA 500 kit following the manufacturer's protocol. In this assay, the 9-repeat allele ran at approximately 430 bp, while the 10-repeat allele ran at approximately 470 bp.

Concerning the *DARPP-32* SNP rs907094, participants were grouped into "A/A" and "any G" carriers (Frank, Doll, Oas-Terpstra, & Moreno, 2009). The frequencies of the genotypes among the older adults were 56.3%, 35.2% and 8.5% for the A/A, A/G and G/G alleles and 57.5%, 33.8% and 8.8% among the younger adults. The *DAT* VNTR genotypes were grouped into "any 9" and "10/10" carriers (cf. Li et al., 2012). The frequencies of older adults carrying the different alleles were 49.3% for 10/10, 43.7% for 9/10, and 7% for 9/9. The distribution of genotypes among the younger adults was 50%, 41.3% and 8.8% for 10/10, 9/10 and 9/9 repeats, respectively. The observed counts genotypes did not differ from that expected according to Hardy–Weinberg equilibrium; all χ^2s < 1.3; all ps > 0.05 (Rodriguez, Gaunt, & Day, 2009).

2.3. Procedure

2.3.1. Serial reaction time task

Implicit and explicit learning was assessed with a modified SRT task (Nissen & Bullemer, 1987) that was followed by a processes-dissociation procedure (Destrebecqz & Cleeremans, 2001) and a verbal recall questionnaire. To ensure an incidental learning situation, the words "memory" or "learning" were not used in the experiment description and participants were only informed that the aim of the study was motoric coordination. During the SRT task, participants sat in front of a screen where circles arranged on a horizontal line indicated four possible stimulus locations. Four reaction buttons were arranged roughly below these four locations (we used the F3, F4, F9 and F10 keys on a turned-around keyboard). The participants were asked to use the middle and index fingers of the left and right hands and keep their fingers rested on the buttons during the entire experiment. For each trial, a circle was filled at one of the four possible locations and the participants were asked to press the spatially corresponding key on the keyboard. Participants were instructed to respond to each target as quickly as possible. Errors were indicated by a red exclamation mark appearing in the center of the screen. The response–stimulus interval was 250 ms. The entire experiment consisted of 30 blocks, each containing 8 repetitions of the 12 element sequence, totaling to 2880 trials. After each block participants could take a short break and received feedback about their performance. To avoid potential age differences in error rates we used differential feedback between blocks (cf. Bennett, Howard, & Howard, 2007). Depending on a participant's mean accuracy in the last block, a statement prompted either to "speed up the responses a little in the next block" (> 96% correct), to "slow down the responses a little in the next block" (< 90% correct) or to "continue as before" (if the error rate was between 4% and 10%).

Unbeknownst to the participants, consecutive stimuli locations followed one of two deterministic 12-element second order sequences as used previously (342312143241 or 341243142132, Destrebecqz & Cleeremans, 2001; Reed & Johnson, 1994). These sequences are balanced for stimulus locations and transition frequency while second order conditional probabilities (subsequences of three elements) differ. Except in block 26 (see below), one sequence was repeated 7 times

Table 1
Sample statistics.

Age group	Age	Education (years)	DAT VNTR ($n_{any\ 9}/n_{1010}$)	DARPP-32 ($n_{AA}/n_{any\ G}$)	Spot-a-word*	Identical pictures*
Older ($n=70$)	65.8	13.9	36/35	40/31	25.23 (5.3)	22.20 (3.3)
Younger ($n=80$)	25.1	14.6	40/40	46/34	20.67 (6.7)	34.22 (4.0)

Note: table shows basic statistics about the current sample, including distributional information of genotypes and markers of perceptual speed (identical picture) and crystalline intelligence (spot-a-word). * indicate significant differences between age groups. Details see text.

within a block (*frequent* condition) and the other sequence was shown once (*infrequent* condition). The difference in RTs between the infrequent and the frequent condition reflects the amount of knowledge about the frequent sequence. In this manner, we obtained a continuous estimate of learning-driven RT and error changes. In order to compare our results to a more traditional SRTT design where one block with random sequences is often used to assess learning (*e.g.*, Howard & Howard, 1992), the frequency relations were reversed in block 26. Accordingly, the infrequent sequence was repeated seven times and the frequent condition only once in this block.

2.3.2. Process-dissociation procedure

Following the main task, we conducted a process-dissociation procedure (PDP; Jacoby, 1991) that was adapted for the SRT task (Destrebecqz & Cleeremans, 2001; see also: Destrebecqz et al., 2005; Gaillard, Destrebecqz, Michiels, & Cleeremans, 2009). Using the PDP-procedure to asses implicit and explicit knowledge has been considered as an improvement over recall measures because it is a forced choice test that does not suffer from the reduced sensitivity that has been criticized of recall measures (Shanks & St. John, 1994). Specifically, participants were informed that consecutive stimulus locations followed a repeated sequence. They were then told that in the following task they did not have to react to the stimuli any more. Instead, a button press by the participants caused the corresponding circle to be filled. The participants were now asked to type in sequences under two conditions: In the *exclusion* condition, the task of the participants was to avoid typing the sequence that was repeated during the main task. In the *inclusion* condition, participants were asked to type the sequence that was repeated before. In both conditions, the task lasted for 48 reactions and participants were told to avoid repetitions. The RSI was 250 ms. We analyzed the generation performance in both conditions separately to study the extent of control that participants have over their sequence memory. In line with Jacoby's instantiation of the PDP (Jacoby, 1991), we assumed that performance in the inclusion condition reflects a combination of implicit and explicit knowledge, whereas the inability to suppress knowledge in the exclusion condition is a reflection of familiarity or implicit memory (see Destrebecqz and Cleeremans (2001) and Gaillard et al. (2009) for similar approaches with the SRT task).

2.3.3. Verbal recall questionnaire

After completing the computer-based part, a questionnaire was given to the participants. They were asked to write down a 12-element sequence that repeated during the main experiment. If participants felt that they did not have any memory of the sequence, they were asked to guess based on their intuition.

2.4. Data analyses

The data was analyzed using R (R Development Core Team, 2011) and SAS (SAS Institute, Cary, USA). Unless otherwise noted, we used mixed-effect ANOVAs (SAS PROC MIXED) in combination with Kenward–Rodger degrees of freedom (Kenward & Roger, 1997). ANOVAs contained combinations of the between subject factors of age group and DA score (or *DARPP-32* (rs907094) and *DAT* VNTR, see below) and the within subject factors of condition (*frequent* vs. *infrequent*/*exclusion* vs. *inclusion*) and acquisition phase as appropriate. Post-hoc *t*-tests were conducted within PROC MIXED and Bonferroni adjusted as appropriate. Exact *p*-values up to a level of $p=.0001$ are reported. For the analyses involving reaction times, we used individual medians in each factor cell (Luce, 1991).

To estimate the amount of explicit and implicit knowledge reflected in the PDP and verbal recall questionnaire data, we computed a knowledge score for each participant based on the probability of the match between the reported and the correct sequence given chance (see Rünger and Frensch (2008) for a similar approach). To this end, we first simulated 10^5 sequences that had approximately the same statistical constraints as the empirical data.[1] From these sequences we derived the baseline distributions for the numbers of correct triplets and the length of the longest correct subsequence. Next, we computed the overlap of each participant's sequences with the correct sequence separately for the recall and PDP tests. Since the PDP test contained 48 responses, we divided the responses in 4 sequences of 12 elements each and consider only the 12 elements with the best match. This overlap was transformed into knowledge scores by taking the probability of this overlap as determined by the above-mentioned simulation. Finally, the probabilities were linearized according to common practices by taking their negative logarithm (*e.g.*, Bortz, 1999). The resulting scores lie between 0 and 1, where 1 reflects the highest amount of knowledge, *i.e.* getting all 12 items correct (the probability of this event to occur by chance is 0.00001, and the log-score is accordingly $-\log_{10\text{^}5}(0.00001)=1$). The most likely event to occur by chance (getting 2 triplets correct), had a log-score of 0.11. Analyses of the training RT and error data involved five factors (age group, acquisition phase, condition, *DARPP*-32 rs907094 and *DAT* VNTR, see below). To reduce model complexity and because our hypothesis concerned main effects and first level interactions (especially regarding gene-gene and age group by gene interactions), we used a stepwise inclusion approach for higher-level interactions. Specifically, if first level interactions were significant we included the second level interactions involving these lower-level interactions. If these were significant, we included the higher-level interactions involving the significant lower level interactions. Because perceptual speed might have a large influence on the SRTT, which is not of interest in the current study, the identical pictures score was used as a covariate in all analyses of reaction times and errors. For the ANCOVAs dealing with the training data reported below, the time course of the data was binned into the first and the second acquisition phase of learning (*i.e.*, blocks 1–12 and blocks 13–25). In order to avoid any effects of the reversal in block 26, we consider only blocks 1–25. Block 26 will be considered in a separate analysis.

[1] Number of repetitions: 13.8% vs. 14.7% in the empirical and the generated sequences, respectively, $t(173.09)$, $p=.7808$; mean frequencies of single buttons (mean maximum frequency of button): 3.68 vs. 3.68, $t(176.07)=0.06$, $p=.9511$.

3. Results

3.1. General SRT performance

As expected, younger adults had shorter reaction times (main effect age group: $F(1, 136)=29.61$, $p<.0001$) and made more errors (3.2% and 4.6% for older and younger participants, respectively, $F(1, 147)=5.43$, $p=.0212$) than older adults. General performance improvements across practice were reflected in decreasing RTs and errors across acquisition phases, *i.e.*, we found main effects of acquisition phase for both measures, $F(1, 73.5)=157.14$, $p<.0001$, and $F(1, 119)=11.87$, $p=.0008$. The main effects of *DARPP-32* rs907094 and *DAT* VNTR on RT level were not or only marginally significant, $F(1, 152)=2.64$, $p=.1096$ and $F(1, 161)=2.74$, $p=.0999$ and the corresponding effects on the error level failed to reach significance, $ps>.16$. However, carrying non-beneficial alleles on both genes resulted in generally slower RTs, as reflected in the 2-way interaction *DARPP-32* × *DAT*, $F(1, 153)=4.27$, $p=.0404$. No corresponding effect was found for errors, $F(1, 184)=0.02$, $p=.8796$. Regarding RTs, there were trends for interactions between the genotypes and the age group. Specifically, the effect of *DARPP-32* tended to be larger in older adults, reflected in the marginal age group × *DARPP-32* interaction, $F(1, 158)=2.98$, $p=.0861$. Furthermore, the gene–gene interaction between *DARPP-32* and *DAT* also tended to be larger among older adults, as reflected in the marginal three-way age group × *DARPP-32* × *DAT* interaction, $F(1, 154)=3.37$, $p=.0683$. No corresponding effect was found for errors, $ps>.3582$. Hence, the two dopamine-associated polymorphisms influenced the RT speed in older but not younger adults. Fig. 1A depicts the interaction effect of genotypes on RT level separately for the two age groups.

To further explore the interaction of *DARPP-32* and *DAT*, we combined the two dopamine (DA) genotypes into a gene score, henceforth *DA score* (Bertolino, Blasi, Latorre, Rubino, Rampino, & Sinibaldi, 2006; Papenberg et al., 2013). The gene score contrasts participants with either one or two beneficial genotypes (*i.e.*, *DARPP-32* rs907094 *AA* and/or *DAT VNTR 9/9*; $n=118$, 53 older, 65 younger, henceforth DA+) with participants without any beneficial genotype ($n=33$, 18 older, 15 younger, henceforth DA−). We used this grouping because carriers of no beneficial dopamine genotypes might be particularly susceptible to age-associated cognitive decline (Bäckman et al., 2006). In this analysis we found a main effect of gene score on RTs, $F(1,78)=7.59$, $p=.0073$, whereby carriers of at least one beneficial DA genotype (*i.e.* the DA+ group) yielded shorter reaction times than carriers of no beneficial genotypes (*i.e.*, the DA− group). Furthermore, the magnitude of this gene score effect was larger in older compared to younger adults, as indicated by the age group × gene score interaction, $F(1,81.3)=6.94$, $p=.0101$. Post-hoc *t*-tests showed that the effect of DA score on RTs was evident for older adults, $t(50.7)=3.11$, $p=.0062$, but not for younger adults, $t(40.3)=0.06$, $p=.9509$. No corresponding effects were found for errors, $ps>.6865$. Fig. 1B shows the RTs as a function of condition, DA score, block and age group.

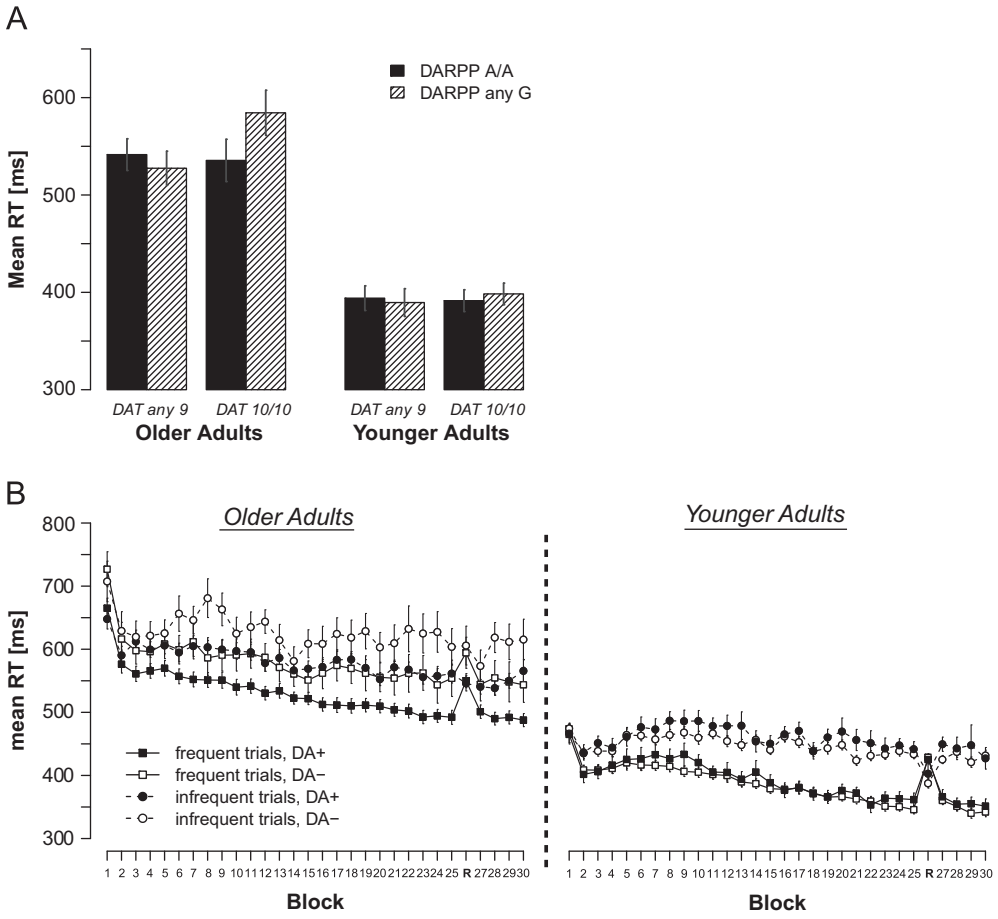

Fig. 1. *Reaction times as a function of training, condition, genotype and age group.* (A) Mean RTs of the frequent condition for the combinations of *DARPP-32* SNP rs907094 and *DAT* VNTR alleles separately for younger and older adults. (B) Younger and older participants' RTs as a function of block, condition and DA score. Bars indicate standard errors of the mean.

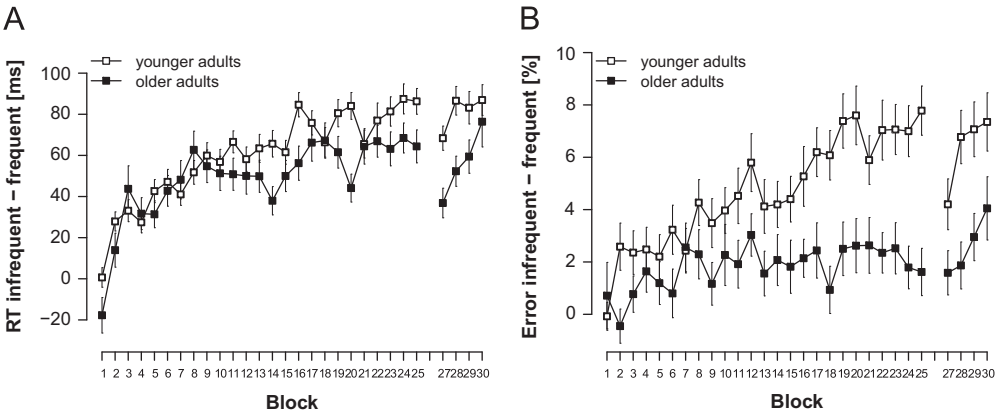

Fig. 2. *Effect of age group on learning-related RT and error differences*. The shown data illustrates the three way interactions between age group, condition and acquisition phase on (A) RTs and (B) errors. Bars indicate standard errors of the mean.

3.2. Learning in the SRT

Memory of the sequence structure was reflected by shorter RTs for the frequent than the infrequent condition, *i.e.* a main effect of condition, $F(1, 93.4)=393.28$, $p<.0001$.

Similarly, participants made fewer errors in the frequent as compared to the infrequent condition, $F(1, 127)=184.79$, $p<.0001$. Moreover, increasing sequence knowledge was reflected by the fact that the RT difference between conditions increased over time (condition × phase, $F(1, 118)=156.21$, $p<.0001$), which was also the case for errors, $F(1, 174)=38.01$, $p<.0001$.

3.2.1. Effects of age on learning in the SRT task

Analysis of the effect of age group revealed that learning-related RT gains of younger adults became larger in the second acquisition phase as compared to older adults' gains, *i.e.* we found a significant 3-way age group × condition × acquisition phase interaction, $F(1, 118)=9.31$, $p=.0028$, see Fig. 2A. Hence, with continuing training younger adults showed more learning in terms of RT benefits. This was also supported by the analysis of the RT increase in Block 26 which indicated a main effect of age group, $F(1, 107)=3.96$, $p=.0492$.

Similar to the results for the RT analysis, there was also a triple interaction of age group, condition and acquisition phase for

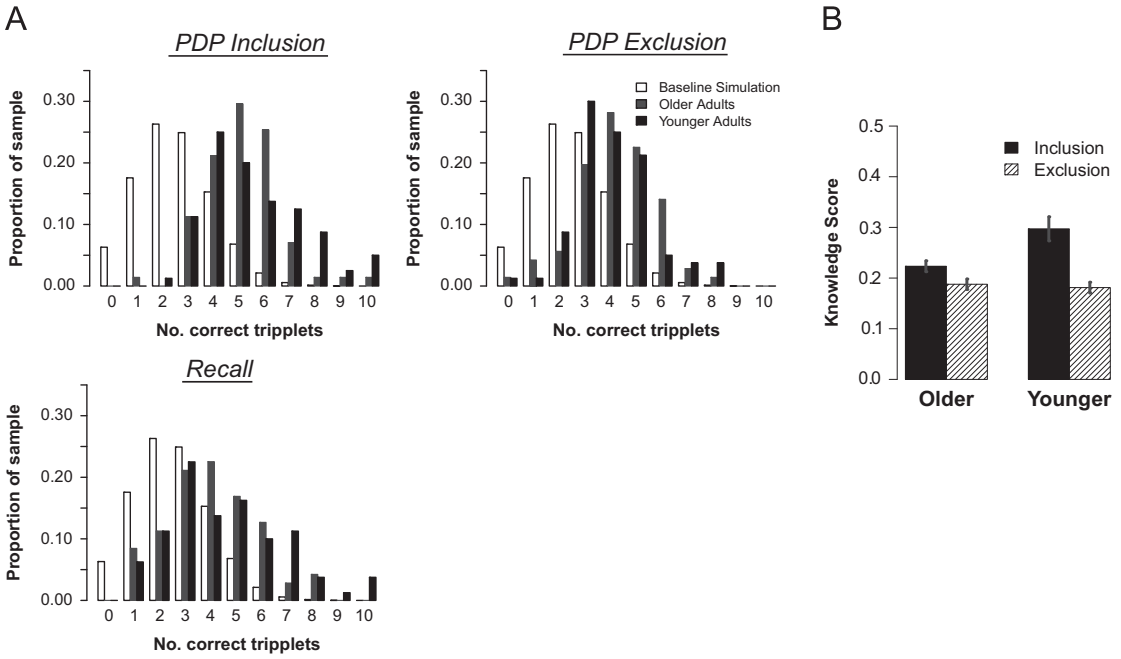

Fig. 3. *Performance on PDP tests.* (A) Normalized histograms for recall, inclusion and exclusion tests. White bars show results from simulation of sequences, see text. Gray and black bars show older and younger adults, respectively. (B) Knowledge scores ($\log_{10^{\wedge}5}$-transformed probabilities) for the generated patterns during the inclusion and exclusion conditions separately for age groups.

errors, $F(1, 174)=33.91$, $p<.0001$, as well as the corresponding lower level interactions, *i.e.* age group interacted with condition, $F(1, 127)=40.8$, $p<.0001$, and with acquisition phase $F(1, 116)=12.88$, $p=.0005$. This triple interaction reflects that younger adults had higher error rates in the infrequent as compared to the frequent condition (8.6% vs. 3.9%, $p<.0001$) and that this different grew larger with training (3.1% vs. 6.2% difference in the early and late acquisition phase, $p<.0001$), but no such pattern can be observed in older adults, albeit their overall higher error rates, see Fig. 2B. Hence, for younger adults we found higher error rates in the infrequent as compared to the frequent condition and an additional increase in this difference over time. This pattern likely reflects younger adults' greater knowledge about the sequence that leads to increased errors in the infrequent sequence, as would be expected based on the above reviewed evidence of more explicit knowledge development in younger adults. Fig. 2 shows the effect of age group on learning (infrequent–frequent condition) across blocks for RTs (panel A) and errors (B).

3.3. Effect of age on implicit and explicit knowledge components of learning in the SRT task: PDP and recall

The above analyses indicted that aging does not only influence performance level, but also learning in the SRT task. Next, we disentangled implicit and explicit components of the acquired knowledge. To this end, we analyzed the performance during the PDP and the recall questionnaire. Fig. 3A shows the distribution of correct triplets for the two age groups in the recall and PDP tests as well as the simulated baseline distribution.

3.3.1. Amount of explicit and implicit knowledge

First, we quantified the amount of explicit knowledge more precisely. As can be inferred from Fig. 3A, more younger adults reported sequences with a high degree of overlap with the correct sequence and this was particularly the case for the inclusion and the recall tests. In the recall test, younger adults had higher (probability-based) knowledge scores than older adults for the number of triplets (mean number of triplets: YA 4.5; OA 3.98), $t(128)=2.11$, $p=.0369$ and the mean maximum length of correct runs (YA 5.3, OA 4.6), $t(141)=2.37$, $p=.0194$. Concerning the results from the PDP, it is generally assumed that inclusion performance reflects explicit plus implicit memory and exclusion performance indicates automatic, implicit memory (Destrebecqz & Cleeremans, 2001; Gaillard et al., 2009). Following Jacoby's framework of dissociating automatic from intentional memory processes using the PDP (Jacoby, 1991) and its adaption to implicit sequence learning (Destrebecqz & Cleeremans, 2001),[2] we computed an explicitness score by subtracting the exclusion from the inclusion score. In line with the above reported results from the recall test,[3] younger adults had also higher explicitness scores than older adults, $t(123)=2.67$, $p=.0087$. This analysis is equivalent to a two-factorial ANOVA with factors age group and condition (inclusion vs. exclusion), which yielded a significant interaction of age group and condition, $F(1, 123)=7.12$, $p=.0087$. This interaction was driven by the fact that older and younger adults did not differ in the exclusion condition, $t(148)=0.08$, $p=1$, but in the inclusion condition, $t(111)=2.79$, $p=.0124$. Hence, younger adults' performance in the inclusion condition reflected high amounts of explicit knowledge, whereas they did not differ in performance in the exclusion condition, which is assumed to reflect implicit knowledge. Whereas both age groups did not differ with respect to implicit knowledge acquired, they differed in the amount of explicit knowledge. This conclusion is further supported when only participants with larger amounts of explicit sequence knowledge are considered. Specifically, we categorized participants as

[2] Destrebecqz and Cleeremans did not use the difference, but only the number of hits in the inclusion condition alone. Regardless if we considered the difference between the inclusion and the exclusion condition or the inclusion condition alone as a measure of explicit knowledge, a significant difference between age groups can be found (see Fig. 3).

[3] As expected, the recall score was mildly correlated with the score from the PDP. Specifically, Kendall's tau correlation between the number of triplets during recall and during the inclusion condition across age groups was $\tau=.22$, $p<.001$, with $\tau=.25$, $p=.004$ and $\tau=.17$, $p=.058$ for younger and older adults, respectively.

having explicit knowledge of the sequence if they reported more than 6 correct triplets in the recall condition (corresponding to a cutoff of $p < .01$, because the event of 6 correct triplets is less likely than 1% to be produced by chance) and compared the number of explicit subjects across age groups. A χ^2-test revealed that a larger proportion of younger than older adults could be categorized as having explicit knowledge (20% vs. 7%), $\chi^2(1)=4.2$, $p=.0393$ (with continuity correction).

3.3.2. Relation of explicit knowledge to RT gains over the course of learning

The analyses above indicate that an important age difference consists in the amount of explicit knowledge that participants acquired. At the same time, analysis of RTs and errors during learning showed that only after a substantial number of repetitions the two age groups start to differ. To connect these two findings, we investigated the relation of the explicit knowledge scores to the RT gains that emerge during learning. If the gradual occurrence of age difference in RT-based learning indicators is a reflection of the emerging explicit knowledge, then one would expect increasingly strong links between these RT differences and the explicit knowledge. Hence, we calculated correlations relating the RT difference between the frequent and infrequent condition to the recall knowledge score separately for each block. This analysis results in a correlation that reflects the influence of explicit memory on learning-related RT reductions for each block. To study the development of the correlations between verbal knowledge and RT gains over time, we submitted these correlations to a linear regression with block as the independent and the correlations as the dependent variables. This analysis revealed that younger adults show a linear increase over blocks in the correlation between explicit memory on RT benefits, $t(23)=6.53$, $p<.0001$, but not in older adults, $t(23)=-1.0$, $p=.317$. Hence, over the course of learning younger adults increasingly employed explicit knowledge to speed up their RTs, whereas older adults did not, indicating that the greater amount of explicit knowledge we observed at the end the experiment is linked to the gradually occurring age effect in RT differences during the learning phase.

3.4. Interactions of dopamine-related genes and age in learning and memory

3.4.1. Effects of DA genes on learning in the SRT task

None of the learning-related RT effects interacted with the DA score, *i.e.* nor any of the interactions involving condition, $ps > .3344$, neither the effect of sequence reversal in Block 26, $F(1, 38)=0.13$, $p=.7232$, was influenced by DA score. The learning-related error pattern showed a trend for an interaction with age group and dopamine-relevant genotypes. Specifically, we found a marginal four way interaction between age group, condition, acquisition phase and DA score, $F(1, 76.8)=3.69$, $p=.0583$ and a significant five-way interaction of age group, acquisition phase, condition, *DARPP-32* and *DAT*, $F(7, 156)=2.41$, $p=.0228$.[4] These interactions reflect the fact that older carriers of beneficial dopamine genotypes (*i.e.*, the DA+ group) yielded a significantly larger error rate in the infrequent as compared to the frequent condition in the second acquisition phase of training (5.3% vs. 2.9%, $t(84.92)=4.35$, $p<.0001$), indicating greater acquisition of the frequent sequence. This effect, however, was not observed in older adults who did not carry beneficial alleles, $t(27.31)=1.6$, $p=.1199$. Moreover, whereas the difference between errors in the infrequent and the frequent condition did not differ between older DA+ and DA− carriers in acquisition phase 1, $t(44.7)=1.16$, $p=.2537$, there was a marginal difference in acquisition phase 2, $t(61.3)=-1.9$, $p=.0618$. Among younger adults, this pattern did not differ between the two gene score groups. As we indicated above, younger adults generally showed an increasing difference between conditions across acquisition phases and this effect likely reflects increasing sequence knowledge, a finding that is in line with the previous reports of an association between explicit knowledge and more anticipatory key presses (Ghilardi et al., 2009). The above analysis showed that older DA+ but not DA− carriers had a similar error pattern than younger adults, with higher error rates in the infrequent condition particularly in the second half of the acquisition phase. Fig. 4A shows the difference between errors in the infrequent and the frequent conditions separately for the DA scores, the acquisition phases and the age groups.

3.4.2. Effects of DA genes on explicit knowledge

To analyze a possible influence of the DA score on the measures of explicit and implicit knowledge, we conducted ANOVAs including main effects for the factors age group and DA score (DA+ vs. DA−) for the recall, inclusion and exclusion tests. The analysis of the recall revealed a significant interaction of DA score and age group, $F(1, 85.9)=6.4$, $p=.0132$. Follow-up analyses showed that among older adults DA+ carriers had significantly higher recall scores than DA− carriers, $t(67)=2.67$, $p=.015$, which is also reflected in a higher number of triplets: 4.2 vs. 3.3, max. 8 vs. 6, $p=.03$. In contrast, this was not the case for younger adults, $t(27.7)=0.07$, $p=1$. In the PDP inclusion condition, we did not find an effect of DA score, $F(1, 44.3)=0.82$, $p=.3689$. Likewise, we did not find such an effect for the exclusion condition, $F(1, 86.4)=2.31$, $p=.1318$. Fig. 4B shows the data from the verbal recall.

Table 2 provides an overview over descriptive statistics and Table 3 over the reported effects.

4. Discussion

Implicit memory is related to dopamine in the striatum and associated with a polymorphism (VNTR) on the *DAT* gene (Simon et al., 2011). Moreover, striatal dopaminergic neuromodulation is largely impaired in older adults and likely related to learning deficiencies (Eppinger et al., 2013). At the same time, however, previous studies have shown that learning-related RT benefits in an implicit learning task are comparable for younger and older adults (*e.g.*, Howard & Howard, 1989), suggesting that implicit learning might be spared from age-related deterioration. In the present research, we investigated the amount of implicit and explicit sequence memory after extensive training in an incidental learning task in younger and older adults. Specifically, we studied the effects of dopaminergic genotypes on motor performance and disentangled contributions from explicit as well as implicit memory to sequence learning indicators (RT and error gains).

Our analyses revealed that older adults had (a) worse motor performance (*i.e.*, overall RT level), (b) less learning-related RT and error differences (*i.e.*, better performance in trials following a frequent as compared to a infrequent sequence) and (c) developed less explicit knowledge (*e.g.*, verbal recall) about the sequence over the course of learning. Implicit knowledge (as indicated by the exclusion PDP score), in contrast, was equivalent between age groups. In particular, we observed that both age groups developed substantial learning-related RT improvements during the first 10 blocks of learning (*i.e.*, 70 repetitions of the sequence), whereby amount and speed of acquisition were comparable to younger adults. After block 10, however, younger but not older adults continued to improve. At the end of learning (25 blocks) they

[4] According to the stepwise inclusion procedure, this term would not have been included in the analysis. It is reported here to further evaluate the marginal interaction with DA score.

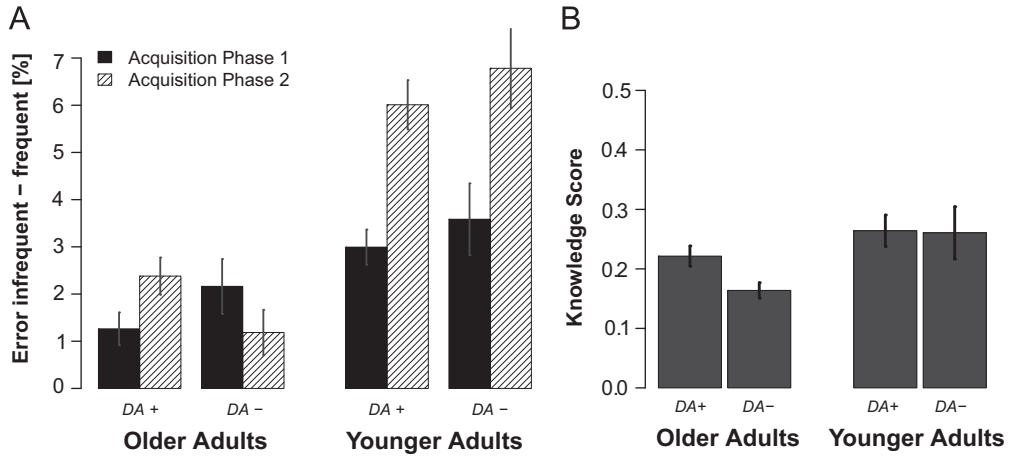

Fig. 4. *Interaction of age and gene effects on verbal recall and errors.* (A) Plot depicts the learning related error effect (more errors in the infrequent as compared to the infrequent condition) for DA score and age group. (B) Knowledge scores for the recalled sequence separately for the DA score levels and age group. Bars indicate standard error of the mean.

Table 2
Descriptive statistics of key dependent measures.

Age group	DA score	Reaction time (ms)				Error (%)				PDP (knowledge score)		Verbal recall (knowledge score)
		Blocks 1–12		Blocks 13–25		Blocks 1–12		Blocks 13–25		Exclusion	Inclusion	
		Random	Sequence	Random	Sequence	Random	Sequence	Random	Sequence			
Older	DA –	645.4	608.7	614.3	560.2	5.32	3.16	3.99	2.80	0.16	0.22	0.16
	DA +	602.3	563.1	569.1	509.1	4.38	3.11	5.33	2.95	0.20	0.22	0.22
Younger	DA –	468.8	422.0	456.0	374.8	7.78	4.19	10.90	4.12	0.19	0.24	0.26
	DA +	457	415.2	442.3	368.9	7.03	4.04	9.86	3.85	0.18	0.31	0.26

Note: table shows descriptive statistics of task performance and markers of implicit and explicit knowledge. Details see text.

Table 3
Summary of findings (inferential statistics).

Factor	Performance	General learning	Implicit knowledge	Explicit knowledge
Age group	RT: $p < .01$ Error: $p = .02$	RT: $p < .01$ Error: $p < .01$	–	Recall: $p = .04$ PDP: $p < .01$
DA Genes	RT: $p < .01/p = .04$		–	
Age group × DA Genes	RT: $p = .01/(p = .07)$	Error: $(p = .06)/p = .02$	–	Recall: *$p = .01$*

Note: table shows a summary of findings of the different measures. All p values reflect the corresponding terms in the ANOVAs as reported in the text (rounded to the second decimal place). Double values given in the DA gene and age group × DA genes rows reflect the DA score and *DARPP-32* × *DAT* factors, respectively.

showed significantly larger RT reductions (see Fig. 2A). Correlation analyses showed that with ongoing training, younger adults increasingly developed and made use of their explicit memory to speed up RTs, whereas older adults did not show this pattern. Hence, an observable age difference in learning related RT gains in an implicit motor skill acquisition task was accompanied by emerging explicit knowledge in younger but not older adults. The emergence of explicit knowledge after the initial 10 blocks of learning is in line with the slower model-based instead of habit-based learning in the computational account of sequence prediction by Bornstein and Daw (2012).

Most notably, our genetic analyses showed that genetic effects of two dopamine related genes interacted with each other as well as with age group, reflecting interactive genetic effects in older but not younger adults. Similar to the effects of age group, these effects modulated (a) motor performance level, (b) learning-related error differences and (c) explicit knowledge. Specifically, a combination of non-beneficial alleles in dopamine-relevant polymorphic DNA sites (SNP rs907094 in *DARPP-32* and a VNTR in *DAT*) was related to slower RTs, less learning related difference between errors in the frequent and the infrequent conditions and less explicit sequence memory (verbal recall) after learning in older but not younger adults.

Our data is of theoretical significance from three perspectives: first, it provides novel evidence to lend further support for the notion that the development of implicit and explicit memory during incidental sequence learning are differentially affected by aging. Second, it underscores the role of dopamine in affecting motor-based sequence learning, particularly the emergence of explicit memory in an incidental learning situation. Third, it substantiates the view that genetic effects on cognition might be magnified in older adults. Regarding the first perspective, our findings of spared implicit sequence memory (Bennett, Madden, Vaidya, Howard, & Howard, 2011; Fleischman et al., 2004; Howard & Howard, 1989, but see Gaillard et al. (2009) for an exception that is methodological close to the present study) as well as emerging age-differences in learning related RT benefits (Bennett et al., 2011; Simon, Vaidya, Howard, & Howard, 2012) are in line with the previous studies. Our analyses extend these previous results by supporting the idea that increasing development and usage of

explicit memory contributes to emerging age differences in RT benefits in the SRT task. Regarding the second perspective, our study supports the crucial importance of dopamine decline for cognitive aging (Bäckman et al., 2006). The presented results suggest that age-related decline in dopamine modulation may underlie all observed age-related deficits in motor performance and explicit memory during sequence learning. Interestingly, the dopamine genotypes investigated in this study related only to our measures of explicit but not of implicit sequence memory, despite the known relation between dopamine and implicit learning in younger adults. This result is surprising in light of previous research that linked dopamine and dopamine-related genotypes to implicit learning. At the same time, our finding adds to a growing literature that supports the notion that implicit and explicit sequence learning engage overlapping brain networks (Aizenstein et al., 2004; Aizenstein et al., 2006) and that explicit memory is also implicated by the striatum (Scimeca & Badre, 2012) and dopamine (Karabanov et al., 2010). Moreover, it is in line with studies that indicated that the striatum plays a specific role in the development of explicit memory in incidental learning situations (Rose et al., 2010). The previous research has also revealed that the correlations of explicit and implicit memory of a sequence with striatal and hippocampal brain activity might change with age (Dennis & Cabeza, 2011; Rieckmann et al., 2010). This research showed that the hippocampus plays a greater role in implicit sequence learning in older adults. The present findings compliment the aforementioned research and indicate that age-related impairments of striatal dopaminergic neuromodulation might lead to an impairment of the development of explicit memory but not implicit memory during incidental sequence learning.

Finally, our behavioral genetic results are in accordance with the previous reports of age-related magnifications of genotype–phenotype associations (Li, Chicherio et al., 2010; Li, Papenberg et al., 2013; Nagel et al., 2008; Schuck et al., 2013). The present study provides further support for the notion that this magnification might be a general phenomenon which results from non-linear cognition-brain resources relationships (Lindenberger et al., 2008). Moreover, our findings also add to the literature documenting that not a single polymorphic locus alone, but an interaction of two alleles which both impact the dopamine system are associated with cognitive phenotypes in older adults (see also Li, Passow et al., 2013; Papenberg et al., 2013). Our results also underline that studying gene-gene interactions and combining functionally similar genes provides a powerful tool to investigate effects on cognitive and neurological phenotypes (Bertolino et al., 2008; Bertolino et al., 2009; Li, Passow et al., 2013). Given the age-related decline of DAT availability (van Dyck et al., 2002) and D2/D3 receptor density (Kaasinen et al., 2000; Rinne et al., 1993), a magnification of genetic effects falls in line with the resource modulation hypothesis (Lindenberger et al., 2008). Due to our small sample size, the difficulty to interpret null findings in younger adults, and explanatory gaps in the links between genetic influences and brain activity, our genetic findings have to be interpreted with caution and further investigations are needed to replicate our results.

Despite the aforementioned support of the present results by data and theory, some aspects of our findings warrant further investigation. First, it is surprising that despite the effect of DA genotypes (*DARPP-32* rs907094 or *DAT* VNTR) on verbal recall (and the weak effect on learning-related error patterns), we did not find larger RT benefits in DA+ compared to DA− carriers. A correlational analysis of RT gains and verbal recall revealed increasingly large correlation among younger but not older adults, although some of the older adults have substantial explicit memory. This suggests that RT benefits observed in younger adults may partially reflect explicit knowledge about the sequence that emerged during the course of learning and indicates differential age effects on error rates and RTs (see also, Seidler, Tuite, & Ashe, 2007 for an example of such effects). It is possible that the differential feedback about speed at the end of each block might also contribute to this finding. The question why older adults who obtained substantial explicit memory over learning did not show RT benefits, however, needs to be a subject of future investigations. On a more general level, the use of a candidate genes approach relies critically on statistical power (Payton, 2009). In our findings, some of the effects that are apparent only in one measure (*e.g.*, the DA score did have an effect only on the recall but not on the PDP measure) or are only marginally significant (learning related error pattern) reflect a potential limitation in statistical power of our study. Another potential reason, however, might be reliability differences between the recall and PDP measures (*e.g.*, Buchner & Brandt, 2003).

In summary, the present study supports the view that (a) implicit learning shows less age-related impairment than explicit learning, (b) older adults show a specific impairment in the development and use of explicit memory from initially implicit memory and (c) dopamine-regulation genetics is linked to older adults' capability to develop explicit memory in incidental learning situations. On a general level, our findings of genotype by age group interactions support the view that aging-related change in underlying neurochemical mechanisms in specific or brain functions in general may affect the extent of genetic effects on implicit and explicit memory. However, replications from future studies involving independent samples are necessary in order to establish the generalizability of these effects. Taken together, these results offer new insights into the process of age-related memory deterioration on the cognitive and neural level. They underscore the notion that studies of age-related memory changes need to differentiate between functions that are related to the striatum and to the hippocampus in younger adults and consider changes on the level of behavior as well behavior-brain, genotype-phenotype relations.

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