NEURAL BASIS OF AGING AND IMPLICIT ASSOCIATIVE LEARNING

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ABSTRACT

Cognitive neuroscience of aging research examines neurobiology in relation to cognitive stability and decline in adults of different ages. Because most research has focused on a set of cognitive functions, namely explicit forms of learning and memory (e.g., episodic and working memory), relationships between aging, the brain and implicit forms of learning have remained relatively ignored. To broaden our current understanding, this dissertation examined the neural and cognitive bases of implicit associative learning and how these differ in young versus old adults. The first study revealed that healthy older adults are poorer than younger at such unintentional learning of probabilistic, associative regularities, and that age deficits appear late, but not early, in training. The second study examined the neural mechanisms of implicit associative learning, by assessing relationships between variations of the dopamine transporter gene (DAT1), which influence dopamine transporter expression in the striatum, and implicit associative learning in healthy young adults. Results showed that DAT1 genotype predicted how well individuals learned late, but not early in training for implicit sequential associative events. This finding suggests a role for striatal processes (e.g., striatal dopamine) as training progresses in implicit associative learning of sequential events. The final study examined age differences in functional
brain activity during implicit associative learning in young and old adults, finding age
group differences in the balance of neural learning systems during early and late
training. Both age groups recruited the hippocampus early in training, perhaps because
this structure is relatively preserved with age. But, with training, the young recruited
the caudate whereas the old continued to rely on the hippocampus, since the caudate
shows significant age-related morphological and neurochemical declines. This pattern
of brain activity enabled old to maintain near-young levels of performance only early
in training, but not later. Taken together, these three studies demonstrate that implicit
associative learning of probabilistic regularities is characterized by age deficits that
become more pronounced with practice, presumably due to age-related striatal losses
and related changes in the relative balance of supporting neural systems with age.

(Word limit: 350; Character limit: 2,450 – Words used: 336; Characters used: 2,383)
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CHAPTER I: INTRODUCTION

We are aging, both as individuals and as a worldwide population. A recent report revealed that nearly 500 million people are 65 or older, with that total expected to double in the next twenty years (Dobriansky, Suzman, & Hodes, 2007). In fact, in January of 2011, the first wave of the Baby Boom Generation turned 65. As a result, older adults will soon account for almost 20% of the total population in the United States alone (Goulding, Rogers, & Smith, 2003). Because many cognitive functions decline across the adult lifespan (Park et al., 2002) and cognitive function is central to independent living (Salthouse, 2004), there is a demographic imperative to promote healthy and successful aging. However, the personal and societal implications of an aging population have yet to be fully appreciated; it is unclear whether rapidly increasing numbers of older adults will create opportunity to use the talents of older people or be a huge burden on society. One determining factor will be the extent to which we can learn how to promote cognitive health among older individuals.

Researchers in the cognitive neuroscience of aging are contributing to this goal by studying how the brain changes with healthy old age and whether these age-related brain changes are associated with declines in cognitive function. However, our current understanding of the cognitive neuroscience of aging is limited because most research has focused on a subset of cognitive functions where age deficits are pronounced, namely explicit forms of learning and memory (e.g. episodic and working memory) (Grady, 1998; Jonides et al., 2000; Milham et al., 2002; Nagahama et al., 1997; Rajah
D'Esposito, 2005; Reuter-Lorenz et al., 2000). This dissertation focuses instead on the aging of implicit learning.

Unlike its explicit counterpart (Squire, 2004), implicit learning refers to the non-conscious acquisition of information without intent to learn or explicit awareness of what has been learned (Frensch, 1998). Despite its importance to daily life, the relationship between aging and implicit learning remains understudied, as demonstrated by minimal attention to implicit learning in recent reviews of aging, cognition and the brain (Dennis & Cabeza, 2008; Hedden & Gabrieli, 2004; Spreng, Wojtowicz, & Grady, 2010). The dearth of research in this area may be attributable to its seemingly insignificant public health relevance; because implicit learning is difficult to observe and measure, older adults are unlikely to notice or complain about declines in this form of learning. Despite being a less obvious phenomenon, however, everyday experience suggests that implicit learning is ubiquitous, underlying our sensitivity to daily routines, our ability to gain expertise in professional domains and our ability to absorb and retain information that we cannot articulate (Cleeremans, 2002). For these reasons, implicit learning is arguably more important for adapting to the world than explicit learning, especially among older adults who spend little time in schooling environments that focus on explicit learning.

Another possible explanation for the lack of research on aging and implicit learning is that some studies have reported minimal age declines, if any at all, resulting in the dominant assumption that implicit learning is spared in healthy aging (for a
review, see Prull, Gabrieli, & Bunge, 2000; Zacks, Hasher, & Li, 2000). Yet, this claim is also misleading. First, it treats implicit learning as if it is unitary, when in fact there are many kinds that differ in the nature of the regularity to be learned and in the underlying neural substrates (Squire, 2004). Second, although reports of age invariance in implicit learning might make it appear on the surface that there are no age-related differences in brain activity, older brains often show different patterns of brain activity than younger brains, even when performance is equivalent across age group (Reuter-Lorenz & Cappell, 2008). It is particularly likely that this would occur for implicit learning, because some underlying brain regions in young adults or animals show robust healthy age-related declines, most notably the striatum (Raz, 2000). Finally, age deficits have been reported in some investigations of implicit learning, especially when training is extended (Howard et al., 2004a) or when more complex, probabilistic regularities are studied (Howard, Howard, Dennis, & Kelly, 2008), as is the case with the learning to be studied here.

The focus of this dissertation is on implicit probabilistic associative learning, or the sensitivity to detect subtle statistical regularities within our complex environments (Seger, 1994). The ability to learn probabilistic relationships enables us to anticipate and process future events more efficiently, and is central to high-level cognitive skills that are necessary across the lifespan, such as language, social intuition, and decision-making. For example, in language, people become sensitive to probabilities associated with different words in speech streams (Conway, Karpicke, & Pisoni, 2007; Kuhl,
2004; Saffran, Aslin, & Newport, 1996) without being able to explicitly state the grammatical rules underlying this relationship. Similarly, in social interactions, people come to learn that certain nonverbal cues, such as teary eyes, are associated with sadness more often than joy (Lieberman, 2000). Finally, implicit associative learning can guide decisions made during driving, in that people will learn to brake faster for predictable events in traffic, such as traffic lights, than unpredictable events, such as pedestrians in the roadway (Hunt & Aslin, 2001; Stadler, 1992).

It has been difficult to study the aging of implicit associative learning in the laboratory for three main reasons. First, explicit awareness can develop with practice, even for learning tasks that are thought to be implicit, and some popular tasks are influenced by deliberate strategies. Because it is already known that explicit forms of associative learning are age sensitive (Old & Naveh-Benjamin, 2008), it is essential to examine age differences in implicit associative learning independent from explicit knowledge or conscious strategy use. Second, strong motor demands involved in many tasks used to study implicit associative learning can make it difficult to dissociate motor from cognitive learning (Lungu, Wachter, Liu, Willingham, & Ashe, 2004). Moreover, in these motor-based tasks, event timing is often tied to the learner’s response rate in that each response triggers the onset of the next stimulus. This approach is problematic in studies of older adults, who characteristically make slower and more variable responses that in turn may influence learning (Salthouse, 2000). Finally, many popular implicit associative learning tasks rely heavily on feedback or
other goal-directed actions for responding to stimuli (Gluck, Oliver, & Myers, 1996; Price, 2009). This presents a challenge in aging research because young and old adults have been found to differ in how they learn from feedback information (Frank & Kong, 2008; Marschner et al., 2005; Mell et al., 2005; Simon, Howard, & Howard, 2010). Additionally, most feedback-guided tasks are based on delayed judgments, making it difficult to parse age-related declines in learning from age-related declines in the ability to employ that learning to make subsequent judgments.

This dissertation addresses these limitations, by using the recently developed Triplets learning Task to investigate implicit associative learning (TLT; Howard et al., 2008). This task mimics real-world regularities, but shows no evidence of explicit learning even after extended practice. It also has greatly reduced motor demands and does not depend on reward-based feedback. In this task, participants view open circles that are presented horizontally on a computer screen. An event occurs when one of the open circles fills in solid red or green. Each discrete trial or “triplet” consists of three, sequentially-ordered events comprised of two red cues and a green target. Participants are instructed to first observe the two red cues and respond only to the location of a third green target, using corresponding buttons. This design yields a continuous, performance-based measure of implicit associative learning (c.f., Turk-Browne, Scholl, Chun, & Johnson, 2009).

One advantage of using the TLT to study implicit associative learning is the freedom in the type of statistical regularities that can be studied, two of which will be
highlighted here. First, structural relationships among the sequential events, or triplets, can be varied. Triplets in the task may have rule-based structure, where a given cue’s location is predictive of the target event’s location; for example, second-order structure occurs when the location of the first cue predicts the location of the target while the location of the second cue is random and non-predictive (e.g., event x predicts x + 2). Here, a given cue location always predicts only one highly likely target, and a given target is always highly predicted by only one cue location. Note that the relationship between the first cue and the target can be more or less predictable (e.g., deterministic or probabilistic). In the case of a probabilistic regularity, the first cue predicts one target location for a majority of the trials (high probability condition) and another location for a minority of the trials (low probability condition), whereas for deterministic structure, the first cue perfectly predicts the target. Alternatively, all rule-governed structure can be omitted, by randomly selecting a subset of triplets to occur more frequently (high probability condition) than others (low probability condition). In this way, the cue locations neither individually nor collectively predicted the target response. Second, regardless of the aforementioned structural relationships among the sequential events, the level of predictability, or the proportion of high to low probability triplets, can also be varied.

Participants completing this task are not told of any embedded patterns or predictable structures nor do they notice these statistical regularities even after approximately 6,000 trials of training (Howard et al., 2008). Yet, participants reveal
associative learning via differential responding to high versus low probability triplets over the course of training without awareness of doing so. Specifically, their responses to high and low probability triplets do not differ in either accuracy or response speed at the beginning of training but these behavioral measures diverge with practice, including faster and/or more accurate responses to high probability triplets as well as slower and/or less accurate responses to low probability triplets.

The neural bases of such implicit associative learning are not yet clear. One dominant theory of cognitive neuroscience had been that memory depends on functionally and structurally dissociable systems, with explicit learning involving medial temporal lobe networks and implicit learning involving striatal networks (Gabrieli, 1998; Reber & Squire, 1994; Robbins, 1996; Squire & Zola-Morgan, 1996). However, recent evidence shows dynamic and interactive patterns of neural engagement in learning, suggesting that the aforementioned distinction is not as simple as had once been thought. In fact, the same task, whether implicit or explicit, can involve both the medial temporal lobes and the striatum (Amso, Davidson, Johnson, Glover, & Casey, 2005; Degonda et al., 2005; Rose, Haider, Weiller, & Buchel, 2002; Sadeh, Shohamy, Levy, Reggev, & Maril, 2011; Turk-Browne et al., 2009; Witt, Nuhsman, & Deuschl, 2002), with their relative importance changing as practice progresses. Typically, the hippocampus is involved early in training whereas the caudate is involved as people become increasingly proficient (Poldrack et al., 2001;
Poldrack & Packard, 2003; Schendan, Searl, Melrose, & Stern, 2003), but the specific contributions of these structures to implicit associative learning are still unknown.

For example, the hippocampus is part of the explicit memory system (Squire, 2004), so its involvement may reflect conscious intent to learn, or the use of an explicit, rule-based strategy (Foerde, Knowlton, & Poldrack, 2006). Alternatively, the hippocampus may be necessary for flexible binding of implicit stimulus representations (Cohen & Eichenbaum, 1995; Gluck et al., 1996; Poldrack & Rodriguez, 2003), as seen in other implicit learning tasks (Chun & Phelps, 1999; Frank, O'Reilly, & Curran, 2006). Similarly, it is possible that striatal involvement reflects the strong motor sequencing demands of motor-based tasks (Lungu et al., 2004), or reward/punishment learning in feedback-based judgment tasks (Rostami, Hosseini, Takahashi, Sugiura, & Kawashima, 2009). Thus, it is unclear whether previous findings of hippocampus and caudate involvement are true of implicit associative learning or just idiosyncrasies of the particular tasks used so far.

Moreover, little is known about the age-related changes in the neural bases of implicit associative learning. Importantly, implicit associative learning calls on neural regions that show pronounced age-related declines with healthy aging in both volume and function; namely, structural and physiological deficits are greater in the caudate than the hippocampus (Raz et al., 2005). Thus, it is likely that these structures will be involved differently in the aging brain, such that older adults will engage these
interactive learning systems differently from young adults in implicit associative learning.

The following outline explains how each chapter of this dissertation used the TLT to explore two overarching questions about the neural bases of aging and implicit associative learning. First, what are the cognitive and neural processes of implicit associative learning? Second, how do these change in the course of healthy adult aging? Three studies were designed to address these inquiries, using perspectives from three different areas of cognitive science, namely cognitive aging (Chapter II), cognitive neuroscience (Chapter III) and cognitive neuroscience of aging (Chapter IV).

Chapter II. To determine the conditions under which age differences in implicit associative learning occur. The first major goal of this dissertation was to understand what conditions might influence age-related differences in implicit associative learning. As mentioned above, less is known about the aging of implicit associative learning in comparison to other forms of learning and memory, and current theories of age-related preservation may be misleading. Some behavioral studies reveal age-related preservation (Cherry & Stadler, 1995; Curran, 1997; Dennis, Howard, & Howard, 2006, Expts. 1 & 2; Frensch & Miner, 1994; Gaillard, Destrebecqz, Michiels, & Cleeremans, 2009; Howard & Howard, 1989, 1992; Howard, Howard, Dennis, Yankovich, & Vaidya, 2004b, Expt. 1; Salthouse, McGuthry, & Hambrick, 1999); however, recent work has shown age deficits in learning, especially after extended training with probabilistic regularities (Howard et al., 2004a; Howard et al., 2008; Raz,
Williamson, Gunning-Dixon, Head, & Acker, 2000; Rodrigue, Kennedy, & Raz, 2005; Wishart & Lee, 1997). These mixed findings indicate that it is unclear whether implicit associative learning declines with healthy aging, and if it does, we currently lack knowledge of the conditions under which age differences occur. Because such a picture is needed, the first study of the dissertation aimed to contribute to this characterization by using the recently developed TLT to rule out alternative explanations for age-related declines in implicit associative learning. Specifically, because age differences in previous work might reflect age-related declines in rule-based learning deficits, rather than a more general deficit in learning, the study reported in Chapter II tests whether age differences emerge in implicit associative learning when there is no underlying rule-governed structure to be learned. Our predictions are outlined in greater detail in Chapter II.

Chapter III. To determine the neural regions associated with implicit associative learning in young adults using genetics. The second major goal of this dissertation was to determine whether implicit associative learning was associated with a polymorphism for the dopamine transporter genotype in healthy college-aged adults. The underlying neural networks of implicit associative learning in humans have been explored almost exclusively through research on healthy younger adults using functional brain imaging (e.g., Rose et al., 2002; Schendan et al., 2003) and patient studies (e.g., Knowlton, Mangels, & Squire, 1996; Knowlton, Squire, & Gluck, 1994). However, a remarkable finding emerging from contemporary cognitive neuroscience is
the connection between our genetics and our cognition (Green, Munafo et al., 2008).

Until recently, genotyping was prohibitively expensive, such that direct analysis of genetic variations in healthy samples was not practical. But now, genotyping costs have fallen, allowing social sciences to incorporate genetics into the study of cognition. However, only a handful of studies have examined the genetics of implicit forms of learning (Frank, Moustafa, Haughey, Curran, & Hutchison, 2007; Keri et al., 2005; Negash et al., 2007). Thus, this second study examined relationships between a polymorphism of the dopamine transporter gene (DAT1), which influences dopamine transporter expression in the striatum, and two forms of implicit associative learning that differ in the regularity to be learned and in striatal involvement. Specifically, we examined associative sequence learning (using the Triplets Learning Task), which recruits the dopamine-dependent striatal system, and spatial contextual learning (using the Spatial Contextual Cueing Task), which recruits medial temporal brain networks. Based on evidence that DAT1 expression is higher in the striatum than the medial temporal lobes (Lewis et al., 2001), the study reported in Chapter III tests the hypothesis that DAT1 genotype would influence associative sequence learning but not spatial context learning in college-aged adults. This study also provided converging evidence for the neural processes underlying implicit associative learning in the TLT in a healthy younger sample.

Chapter IV. To determine the age-related differences in the neural bases of implicit associative learning over the course of training. The third goal of this
dissertation was to characterize the neural regions that support implicit associative learning in healthy young and old adults. This study was motivated by our findings of spared early but impaired later implicit associative learning in healthy older adults (reported in Chapter II), and by our findings that striatal processes become increasingly important over the course of implicit associative sequence learning (reported in Chapter III). However, these behavioral studies alone cannot link age-related deficits in learning to age-related differences in neurobiology. There is reason to suspect that striatal mechanisms of implicit associative learning are different for healthy young and old adults as this brain region undergoes robust declines in both volume and function among healthy older adults (Aizenstein et al., 2006; Raz et al., 2005), which can compromise both neural circuitry and cognition in tasks relying on this region (Hedden & Gabrieli, 2004). Yet, few studies have investigated age-related changes in the underlying neural networks supporting implicit associative learning and further, the existing findings are mixed (Aizenstein et al., 2006; Bennett, Madden, Vaidya, Howard, & Howard, 2010; Daselaar, Rombouts, Veltman, Raaijmakers, & Jonker, 2003; Dennis & Cabeza, 2010; Fera et al., 2005; Rieckmann, Fischer, & Backman, 2010). Using functional neuroimaging, we probed the neural networks supporting implicit associative learning in healthy young and old adults to obtain more direct evidence for the hypotheses that the hippocampus and caudate underlie learning in the TLT. Further, we explored whether these regions show age group differences in activation. We predicted that implicit associative learning would be spared early in
training, but impaired later on in older adults, based on earlier work from our lab and the findings reported in Chapter II. We also predicted age differences in the relative balance of supporting brain regions, and that these differences in brain activations contribute to age-related learning deficits over time. Our predictions are outlined in greater detail in Chapter IV.

In sum, humans take advantage of predictabilities in real-life events to coordinate their thoughts and behavior (Schank & Abelson, 1977). As such, implicit associative learning is central to one’s daily activities and quality of life throughout the lifespan. It enables adults of all ages to learn new skills, which is particularly important in light of rapid technological advances that change ways of doing routine tasks (e.g., cell phones, internet, iPods). Behavioral, genetics and functional neuroimaging data presented here will help to reveal how and why such learning may differ for healthy young and older adults by characterizing patterns of healthy age differences in implicit associative learning, and by determining the cognitive and neural bases for this decline. This understanding is an important step toward fostering independent living and successful aging, especially now as the world population continues to age.
CHAPTER II: AGE DIFFERENCES IN IMPLICIT LEARNING OF PROBABILISTIC, UNSTRUCTURED SEQUENCES

This Chapter has been slightly modified from publication: Simon, Howard and Howard (2011). Age differences in implicit learning of probabilistic, unstructured sequences. The Journals of Gerontology Series B, Psychological Sciences and Social Sciences, 66, 32-38.

Our world is largely stable, in that over time, people, places, and things show up in predictable sequences. Learning about such environmental regularities involves becoming sensitive to the order in which events typically occur, regardless of whether or not the sequences have rule-governed structure. For example, in structured language, people can learn different probabilities between phonemes to segment speech into word-like units (Kuhl, 2004) and in unstructured social interactions, people can learn that subtle sequences in facial expressions are associated with one emotional outcome more than another (Lieberman, 2000). Such implicit learning often occurs without intent and explicit knowledge of what has been learned (Frensch, 1998).

Much of the research on aging and implicit learning has focused on learning deterministic associative regularities (i.e., those where an event perfectly predicts subsequent events, with studies typically showing no age differences) (Cherry & Stadler, 1995; Daselaar et al., 2003; Dennis et al., 2006, Experiments 1 & 2; Gaillard et al., 2009; Howard & Howard, 1989, 1992; Salthouse et al., 1999). However, most sequences we encounter in daily life are not deterministic, but rather are probabilistic,
such that an event predicts subsequent events with some uncertainty. Fewer studies have investigated how aging influences probabilistic learning and the available evidence is inconclusive. Although some studies suggest age invariance (Aizenstein et al., 2006; Fera et al., 2005), most studies reveal age-related deficits in learning, especially as training increases (Bennett, Howard, & Howard, 2007; Howard et al., 2004a; Howard & Howard, 1997; Howard, Howard, Dennis, & Yankovich, 2007; Howard et al., 2004b).

Here we investigate implicit associative probabilistic learning using the Triplets Learning Task (TLT; Howard et al., 2008). The TLT was designed to mimic some characteristics of widely-used serial reaction time tasks (Howard & Howard, 1997; Nissen & Bullemer, 1987), while omitting motor sequencing and enabling precise control over event timing. In the TLT, participants view four open circles that become solid red or green in discrete, sequentially ordered, three-event trials or ‘triplets’. On each trial, participants observe two red cues and only respond to a third green target by pressing corresponding buttons. This provides a continuous performance-based measure of learning without motor sequencing, since responses are only made to the target, not to each event. Triplets were originally designed to incorporate the rule-based structure of a probabilistic serial reaction time task (see Howard & Howard, 1997) in that the location of the first or second cue predicted the location of the target (Howard et al., 2008). Further, a given cue location always predicted only one highly likely target, and a given target was always highly predicted by only one cue location.
As in the probabilistic serial reaction time task, learning is revealed in the TLT by people responding more quickly and accurately to high relative to low probability triplets with practice, without any explicit knowledge of what has been learned.

Only one published study has used the TLT to study aging (Howard et al., 2008). Results showed that older adults can learn the subtle, probabilistic associative regularities embedded in the TLT, but not as well as young. Age deficits were attributed to declines in implicit probabilistic learning, such that older adults are impaired in their ability to form associations among events that are probabilistically related (Howard et al., 2008). However, an alternative interpretation of previous findings is that age-related declines in learning may result from the presence of rule-based regularities within the triplets. Because age-related declines have previously been observed in second language acquisition, which calls on such rule-based, structured probabilistic learning (Hakuta, Bialystok, & Wiley, 2003), age differences in the TLT may have been due to declines in non-conscious sensitivity to this rule-governed structure, rather than to a general deficit in learning probabilistic associations.

To examine this possibility, the present study used a version of the TLT in which all rule-governed structure was eliminated by randomly selecting a subset of triplets to occur more frequently than others. Thus, the predictive relationships between the cue locations and target were arbitrary. If previously observed age differences in probabilistic learning were due to age-related deficits in sensitivity to rule-based
structure, then old adults should learn as well as young in this version of the TLT. If instead, as we predict, previously observed age differences reflect generalized age-related declines in probabilistic associative learning, then age differences favoring young adults should persist.

Methods

Participants

Fifteen young (M = 19.0 ± .9 years; range 18-21 years; 6 male) and 15 healthy old adults (M = 71.3 ± 6.0 years; range 66-87 years; 5 male) were recruited from Georgetown University and the community by advertisements in the Washington Post Health Section. Participants received either monetary compensation or course credit. The Georgetown University Institutional Review Board approved the experimental procedures, and all participants gave informed consent. To screen for dementia, older participants completed the Mini-Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975), and all scored ≥ 27, though scores were not obtained for two old adults.

Materials and procedure

Stimuli were programmed, generated and presented using E-Prime (Psychological Software Tools, Inc., Pittsburgh, PA). Participants viewed four open circles on a computer screen. Each trial or “triplet” consisted of two consecutive cue events (circles filled in red) followed by the target (a circle filled in green). Participants were asked to observe the first two events and respond to only the third, target event
location, by pressing one of four corresponding buttons with their dominant hand. Red
cues were displayed for 120 ms and the green target remained in view until participants
made a correct response to its location, with 650 ms separating the correct response
and the first cue on the following trial.

The version of the TLT used here has three important differences compared to
earlier experiments. First, we used a shortened version of the TLT, reducing the
original version from 6,000 to 750 trials (Howard et al., 2008). Second, repetitions
(e.g. 111, 222) and trills (e.g. 141, 232) were not presented during the learning trials
since previous studies have shown that responses to these stimuli reflect pre-existing
response tendencies (i.e. perceptual and motor priming) (Cleeremans & McClelland,
1991) or negative recency (Boyer, Destrebecqz, & Cleeremans, 2005), in addition to
learning. Finally, a randomly chosen set of 16 triplets occurred with high probability
(HP) and the 32 remaining triplets occurred with low probability (LP). In other words,
rather than selecting the high probability triplets to conform to rules reflecting first- or
second-order sequential structure, as in previous TLT studies (Bennett, Romano,
Howard, & Howard, 2008; Howard et al., 2008), here the 16 HP triplets were chosen at
random. As a result of this random selection, the four possible target positions did not
occur equally often. For counterbalancing purposes, each participant received one of
five different random assignments of HP triplets, and the two age groups received the
same assignments. The frequency of HP to LP triplets was approximately 9-to-1
throughout training.
Participants were not informed of any regularities; their only instruction was to respond as quickly and accurately as possible to the target event on each trial. Participants completed three epochs of 250 trials that were divided into 5 blocks of 50 trials each, with breaks provided intermittently (total task ~30 minutes). Mean reaction time and accuracy were displayed to participants at each break, in an attempt to match the two age groups’ overall accuracy at approximately 92%; based on accuracy, participants were instructed to “focus more on speed,” “focus more on accuracy” or “speed and accuracy are about right.”

A sensitive measure of explicit awareness followed completion of the TLT. Participants viewed a random sample from the 64 possible triplets that included HP and LP triplets, in addition to novel triplet combinations (i.e., trills and repetitions). Participants were presented with 64 trials that included, on average, 15 HP triplets (M: 14.96 ± 3.37), 31 LP triplets (M: 31.12 ± 3.69), 12 trills (M: 12.00 ± 3.03) and 6 repetitions (M: 5.93 ± 2.39). Triplets were presented one at a time on the computer screen with the same within-triplet timing rate as in training. Participants were asked to judge whether each triplet had occurred “more often” or “less often” by responding with a button press of “2” or “1,” respectively, and to guess if unsure.

We matched the recognition task to the learning phase by displaying each of the triplets in colored events that simulated the testing phase (i.e., two red and one green event), as compared to earlier work which displayed triplets in the recognition task as a series of black events (Bennett et al., 2008; Howard et al., 2008). This ensures that any
inability to distinguish between high and low frequency triplets in the present study cannot be due to changes in perceptual processes between the TLT and recognition tasks.

Results

*Implicit probabilistic learning.*

Median reaction times (RT) were determined for correct responses for each triplet type for each participant in each block. Overall accuracy was high (~ 94%), so few trials were omitted. These medians were then averaged across blocks to obtain a single mean RT for each participant for HP and LP triplets for each of the 3 epochs, as displayed in Figure 1. An ANOVA was performed on logarithmic transformations of these values to control for the effect of general slowing in older adults. A similar procedure was used to calculate the mean accuracy for each participant for each triplet type.

To examine implicit learning, Group (young, old) × Triplet Type (HP, LP) × Epoch (1–3) mixed-design ANOVAs were conducted separately for accuracy and log-transformed reaction time measures. For accuracy, learning of triplet frequency was revealed as a main effect of Triplet Type, $F(1, 28) = 25.5, p < .0001, r_{\text{effect}} = .69$, with more accurate responses to HP ($95.5 \pm .03\%$) versus LP ($91.9 \pm .07\%$) triplets. The only other effect to approach significance was that of age group; old adults were only marginally more accurate than young adults, $F(1,28) = 3.77, p = .06, r_{\text{effect}} = .34,$
showing that, as intended, the feedback provided after every block resulted in similar overall accuracy for the two age groups.

Figure 1. Log-transformed mean of median reaction times. The log transformations of mean of median reaction time (RT), in milliseconds, over epochs for HP (High Probability) and LP (Low Probability) triplets by age group. Error bars represent the standard error of the mean.

The lack of significant main effects (p’s > .06) or interactions (p’s > .13) with age for accuracy simplified interpretation of the RT data (e.g., no speed-accuracy trade offs). As is typical for reaction time, a main effect of Group, $F(1, 28) = 44.32, p < .0001, r_{\text{effect}} = .78$, revealed that young adults responded significantly faster overall than old adults. Skill learning was revealed by a main effect of Epoch, with overall RT decreasing across epochs, $F(2,56) = 11.72, p < .0001, r_{\text{effect}} = .54$. Learning of probabilistic triplet frequencies was also revealed via a main effect of Triplet Type, $F(1, 28) = 64.30, p < .0001, r_{\text{effect}} = .83$, as well as a Triplet Type x Epoch interaction, $F(1, 28) = 5.73, p < .01 r_{\text{effect}} = .43$ with faster responses to HP versus LP triplets that
increased over epochs. A significant Group x Triplet Type interaction, $F(1, 28) = 4.88$, $p < .05 \, r_{\text{effect}} = .41$, showed that this difference in responding to HP and LP triplets was greater for young than old adults. No other interactions were significant, $p$’s > .72.

Given the lack of a 3-way interaction on the analysis of the RT data, the Group x Triplet Type interaction provides inconclusive evidence that there are age differences in probabilistic, associative learning. Moreover, two aspects of the task further limit the interpretation of this result. First, the large overall group difference in RT makes it problematic to directly compare the magnitude of the difference between high and low events across the ages (see Curran, 1997). Second, and more importantly, in using arbitrary, non-rule based regularities, it was not possible to equate target frequency without adding additional constraints on the triplets. So, the previous interaction may reflect frequency-based responding to the target events, in addition to probabilistic, associative learning. In other words, because the four possible target positions did not occur equally often for each participant, faster RTs for HP triplets versus LP triplets, and any age-related differences therein, may be influenced by target frequency as well as by triplet frequency.

Therefore, similar to Howard et al. (2008), we used a measure of sequence-specific associative learning that was not influenced by overall RT. Namely, for each participant on each epoch, we determined the median RT for each unique triplet (e.g., 134) and we then correlated those reaction times with the number of times that each individual triplet actually occurred (i.e., the triplets’ actual frequency of occurrence).
To remove any contribution of simple target frequency, partial correlations were computed between triplet frequency and median reaction time, with target frequency as a covariate. If a participant had learned nothing about triplet probabilities, this correlation would be 0, whereas if (s)he had learned a lot, the correlation would be highly negative (i.e. triplets occurring with high probability would be responded to more quickly) (Howard et al., 2008). For ease of interpreting these values, we multiplied each correlation by -1 to obtain an associative learning score, such that higher scores reflect greater sequence learning.

The means of the associative learning scores for each age group for each of the three epochs are shown in Figure 2. A mixed-design Group x Epoch ANOVA revealed main effects of Epoch, showing that the associative learning scores increased with practice, $F(2, 56) = 4.39, p < .05, r_{effect} = .47$, and a marginal effect of Group, in that young adults tended to have higher associative learning scores than the old, $F(1,28) = 3.45, p = .07, r_{effect} = .33$. Most important, a Group x Epoch interaction revealed that age differences in favor of the young varied across epochs, $F(2,56) = 3.16, p = .05, r_{effect} = .38$. Post-hoc t-tests revealed that the associative learning scores were significantly greater for the young than the old only on Epoch 3, $t(28) = 3.67, p < .01, r_{effect} = .33$. Subsequent single sample t-tests indicated that associative learning scores were significantly greater than 0 for all three epochs in both young (Epoch 1: $t(14) = 4.66, p < .001, r_{effect} = .61$; Epoch 2: $t(14) = 6.48, p < .0001, r_{effect} = .75$; Epoch 3: $t(14) = 11.10, p < .0001, r_{effect} = .90$) and old adults (Epoch 1: $t(14) = 3.75, p < .005, r_{effect} =$
Figure 2. Associative learning scores by epoch and age group. Mean partial correlations between triplet frequency and median reaction time, after controlling for target frequency, for Epochs 1-3 collapsed across individuals in each age group. Error bars represent the standard error of the mean.

Implicitness. Because our purpose is to study implicit learning, it is important to show no evidence of explicit knowledge about HP versus LP frequencies. To assess explicit judgments of triplet frequencies on the recognition paradigm, a Group (young, old) × Triplet Type (HP, LP, repetitions and trills) mixed-design ANOVA was conducted on mean recognition ratings for each triplet type. Recognition data were lost for 1 young and 1 old participant, leaving 14 participants in each group for this analysis. As shown in Figure 3, there was a significant main effect of Triplet Type, $F(3,78) = 15.74, p < .0001, r_{\text{effect}} = .97$, with no age group differences, $F(1, 26) = .009, p > .92, r_{\text{effect}} = .01$, and no interactions ($F(3,78) = 1.03, p > .39, r_{\text{effect}} = .19$. Most
important, post hoc analysis showed that HP and LP triplet ratings did not differ from each other, $t(27) = -0.62$, $p > .54$, $r_{\text{effect}} = .01$. This is strong evidence for the implicitness of learning, in that people responded faster to HP than LP during training, but did not give higher recognition ratings to one than the other during recognition.

**Figure 3. Mean recognition ratings by triplet type and age group.** A rating of 2 indicated that a triplet was believed to occur more often whereas a rating of 1 indicated that a triplet was believed to occur less often. Error bars represent the standard error of the mean.

In addition, using 2 x 2 chi-square analyses conducted separately for each person, we found that no participants revealed awareness of triplet frequency. HP and LP triplets were equally sorted as occurring more often ($p > .11$ in all cases), indicating implicit learning. Moreover, associative learning was independent of recognition task judgments, in that across subjects, triplet ratings on the recognition task (i.e., HP – LP) did not correlate with associative learning scores in any of the three epochs (Epoch 1: $r = .122$, Epoch 2: $r = .262$, Epoch 3: $r = -.199$; $p$’s > .18).

Finally, the main effect of triplet frequency in the ANOVA reported above was due to the fact that both HP and LP triplets were judged as occurring more frequently.
than trills (HP: $t(27) = -3.47, p < .005, r_{effect} = .31$; LP: $t(27) = -3.77, p < .005, r_{effect} = .34$), and repetitions (HP: $t(27) = -5.53, p < .0001, r_{effect} = .53$; LP: $t(27) = -5.80, p < .0001, r_{effect} = .55$). The fact that participants did differentiate trills and repetitions from HP and LP triplets suggests that they understood the recognition task and were not responding randomly; thus, this recognition task is sensitive to explicit knowledge when it is present.

**Discussion**

The present study examined age differences in implicit learning of probabilistic, non rule-based sequences, using a modified version of the Triplets Learning Task (TLT). Results revealed that both young and old adults learned, but that the old learned less than the young, particularly at the end of training. As discussed below, this difference cannot be attributed to age-related motor impairments, such as slower and/or more variable responding. Nor can it be attributed to declarative knowledge of the sequences, or to deficits in learning new rule-governed sequences. Instead, contrary to the commonly held view that implicit learning and memory are spared in aging (Hedden & Gabrieli, 2004; Zacks et al., 2000), our findings support the view that healthy aging is accompanied by a decrease in at least one form of implicit learning, namely in the ability to learn sequential probabilistic associations. We argue below that these age differences may reflect age-related declines in a striatal-based learning system.
The present results join earlier findings using the TLT in enabling us to rule out three alternative interpretations for the age deficits observed. First, age differences in learning found here and in the earlier TLT study (Howard et al., 2008) are not due to general age-related declines in motor movements (Smith et al., 1999). The absence of motor sequences reduces motor demands and variability, which not only minimizes confounds associated with motor impairments but also enables cognitive contributions to sequence learning to be differentiated from motor ones (see Lungu et al., 2004).

Second, age differences cannot be due to age differences in event timing. Older adults are often slower and often experience different event timing in motor learning tasks because each event typically follows the preceding *response* by a fixed interval (Howard et al., 2007). However, event-timing within a trial is fixed in the TLT, and therefore, identical for the two age groups. Finally, age differences cannot be due to deficits in explicit, declarative learning. Results from our sensitive post-training recognition measure, and subsequent non-significant correlations between ratings of triplet frequency and associative learning scores, indicated that learning is implicit, consistent with previous TLT studies (Bennett et al., 2008; Howard et al., 2008). This is important because explicit contamination, such as the application of deliberate strategies, may confound age differences found on tasks that are intended to measure implicit learning (Howard & Howard, 2001).

The present results go beyond earlier findings with the TLT in that they also enable us to rule out effects of age differences in rule-governed learning. We
eliminated all rule-governed structure in the task used here and thereby dissociated rule-based implicit learning from more general implicit association-based processes (Cleeremans, 1993). As a result, the present study demonstrated that both young and old adults are able to learn non-rule-based sequential structure, in addition to the rule-based first- or second-order structure demonstrated in previous studies (Bennett et al., 2008; Howard et al., 2004a; Howard & Howard, 1997; Howard et al., 2008). However, the fact that age differences in learning probabilistic sequences emerged with practice, points to a fundamental deficit in older adults’ ability to learn subtle sequential associative regularities over time.

The present findings also go beyond earlier work by revealing more about the time course of implicit probabilistic learning and the age differences therein. For example, the earlier TLT study only examined associative learning scores for the second half of testing, after 3,000 trials of training. In contrast, we examined the first 750 trials of training separated into three epochs. Though several theories have proposed distinct associative learning stages, with stimulus representations being formed in early trials and habit learning occurring in later trials (Anderson, 1982; Karni, 1996), few have examined how aging influences these separate learning phases. Here, we did not observe age deficits in associative learning during the first epoch, but by the third and final epoch, older adults revealed less learning than their younger counterparts. This finding is consistent with previous implicit sequence learning studies that have shown the greatest divergence between age groups after extensive
practice (Bennett et al., 2007; Dennis, Howard, & Howard, 2003; Howard et al., 2004a; Howard & Howard, 1997; Howard et al., 2008; Howard et al., 2007; Howard et al., 2004b; Negash, Howard, Japikse, & Howard, 2003). Interestingly, the two studies that revealed age invariance in probabilistic learning had relatively little training (Aizenstein et al., 2006; Fera et al., 2005). Both had fewer trials than the present study and reported only small learning effects that would make detecting age differences challenging. Thus, one possible explanation for the lack of age differences in those studies is that training was insufficient to reveal age deficits in learning.

A possible explanation for the changing patterns of age differences with training is that different brain structures may be involved as training progresses. Studies of probabilistic, associative learning in young adults and animals show that the medial temporal lobe governs responding in the early stages of learning whereas performance becomes increasingly dependent on the striatum over the course of training (Poldrack & Packard, 2003; Schendan et al., 2003). Similarly, preliminary functional neuroimaging data of the TLT in young adults revealed early learning-related hippocampal activation, whereas the caudate was recruited with more practice (Simon, Barnes, Vaidya, Howard, & Howard, 2008). The striatum typically comes to dominate probabilistic associative learning because these structures are good at integrating probabilistic information gradually over time (Packard & Knowlton, 2002; Seger, 2006; Shohamy, Myers, Kalanithi, & Gluck, 2008). However, this brain region shows substantial age-related changes in structure and function in healthy older adults.
(Backman, Nyberg, Lindenberger, Li, & Farde, 2006; Gunning-Dixon, Head, McQuain, Acker, & Raz, 1998; Raz et al., 2003) that may compromise learning in tasks that rely on this system (Cabeza, Nyberg, & Park, 2005), such as implicit probabilistic associative learning. Thus, age differences observed during the third training epoch of the present study may be due to greater age-related declines in the striatum relative to the medial temporal lobe in healthy older adults (Jernigan et al., 1991; Jernigan et al., 2001; Raz et al., 2005). In other words, when responding relies on the relatively intact medial temporal systems early on, there is age invariance in performance, but when responding gradually shifts to the age-impaired striatal system, age differences emerge. This is consistent with findings that striatal-based information integration learning reveals age deficits (Filoteo & Maddox, 2004). Though this conclusion may, at first, seem paradoxical, given that older adults did demonstrate learning in all three training epochs, several studies of older adults have revealed increased reliance on extrastriatal brain regions during implicit associative learning when striatal processes are impaired (Aizenstein et al., 2006; Fera et al., 2005; Rieckmann et al., 2010).

In summary, the present experiment revealed that both young and old adults are sensitive to repeating unstructured, non-rule based sequences. However, there are age differences in this sort of implicit probabilistic learning, in that older adults revealed less learning than the younger group with age differences being carried by later training, perhaps reflecting age-related deficits in the striatal associative learning
system. Of note, these age differences were observed with only 30 minutes of testing, using an abbreviated version of the TLT. Shorter training is more practical and is often preferred in functional imaging studies or behavioral studies of older adults or patient groups. The exact mechanisms underlying this age-related deficit in implicit probabilistic learning are yet to be determined, but such an understanding is important for building and testing theories and for developing interventions for older adults that maximize learning.
CHAPTER III: DOPAMINE TRANSPORTER GENOTYPE PREDICTS IMPLICIT SEQUENCE LEARNING

This Chapter has been slightly modified from publication: Simon, Stollstorff, Westbay, Vaidya, Howard and Howard (2010). Dopamine transporter genotype predicts implicit sequence learning. *Behavioural Brain Research, 216*, 452-457.

Contemporary cognitive neuroscience increasingly incorporates genetics data to understand cognition (Green, Munafò et al., 2008). However, our current understanding of genetic influences is limited because most research has focused on conscious, deliberate cognitive control (e.g., Fossella et al., 2002), while the genetics of non-conscious, implicit phenomena has been understudied. Implicit associative learning refers to the acquisition of information about environmental regularities without intending to learn or becoming aware of what has been learned (Frensch, 1998). Here, we investigated the effects of a polymorphism in the gene (SLC6A3) coding for the dopamine transporter (DAT1) on two different forms of implicit associative learning, namely sequence learning and spatial context learning.

Implicit sequence learning involves learning sequential dependencies among events, which is necessary for skills such as language (Kuhl, 2004) and social intuition (Lieberman, 2000). To investigate sequence learning, we used the Triplets Learning Task (TLT; Howard et al., 2008). On each trial, participants encounter three sequentially presented stimuli, two red cues followed by a green target, each appearing in one of four spatial locations. Participants watch the red cues but respond only to the
third, green target. Participants are unaware that certain series of events or “triplets” occur with greater frequency (high probability, HP) than others (low probability, LP). Nonetheless, with practice, they reveal sequence learning in the form of greater improvement in speed and/or accuracy for HP than LP triplets. Studies of Parkinson’s and Huntington’s disease patients with dopamine depletion and striatal pathology, respectively, have shown sequence learning deficits (e.g., Jackson, Jackson, Harrison, Henderson, & Kennard, 1995; Willingham & Koroshetz, 1993), indicating the involvement of the striatum and striatal dopamine system in this form of learning. Moreover, neuroimaging studies of implicit sequence learning in healthy young adults reveal striatal involvement that increases as practice continues (Rauch et al., 1997; Reiss et al., 2005; Rose et al., 2002; Schendan et al., 2003).

Distinct from sequence learning, implicit spatial context learning refers to learning of regularities in spatial layouts, such as how context predicts where a target is likely to occur. For example, spatial context learning can help people anticipate where the stoplight is likely to be at an intersection. The Spatial Contextual Cueing Task (SCCT; Chun & Jiang, 1998) measures this type of learning. Participants search for a target amongst distractors in displays with spatial configurations that either repeat across trials or are novel. Repeated configurations provide contextual guidance for locating the target. Implicit spatial context learning is revealed by faster and/or more accurate responding to repeated vs. novel configurations without explicit awareness. Functional imaging of healthy subjects has shown medial temporal lobe (MTL)
involvement during spatial context learning in the SCCT (Greene, Gross, Elsinger, & Rao, 2007; Preston & Gabrieli, 2008). In addition, impaired contextual learning has been observed in amnesics with MTL damage (Chun & Phelps, 1999; Manns & Squire, 2001).

Sensitive recognition tasks and post-experimental interviews with participants have reliably demonstrated that both the TLT and SCCT measure implicit associative learning (e.g., Bennett et al., 2008; Chun & Jiang, 1998; Chun & Phelps, 1999; Howard et al., 2008). Even after being told that regularities were present, participants cannot accurately describe their nature, nor can they discriminate between triplets that occur with HP vs. LP in the TLT and/or arrays that repeated vs. those that were novel in the SCCT.

Previous work has demonstrated double dissociations between implicit sequence learning and implicit spatial context learning by differentiating the brain regions involved. Specifically, reduced sequence learning but not spatial contextual learning was revealed in healthy older compared to younger adults (Howard et al., 2004b), consistent with findings that healthy aging is associated with greater volume declines in the striatum than in the MTL (Raz et al., 2005). This result was supported by Negash et al. (2007) who found that spatial contextual learning, but not sequence learning, was reduced in older adults with mild cognitive impairment, a condition associated with MTL pathology, compared to age-matched controls. In addition, Negash showed that healthy older individuals carrying the ApoE-e4 allele, which is
also associated with MTL atrophy (Lehtovirta, Laakso, Frisoni, & Soininen, 2000), showed reduced spatial contextual learning, but not sequence learning, compared to non-e4 carriers. Thus, implicit sequence learning and implicit spatial context learning are dissociable and appear to be differentially influenced by individuals’ genetic profiles.

The present study examined relationships between these two forms of implicit associative learning and a polymorphism in DAT1, which influences DAT expression levels (Lewis et al., 2001). Although DAT1 is present throughout the brain (Dahlin, Xia, Kong, Hevner, & Wang, 2007; Lewis et al., 2001), it is expressed most abundantly in the striatum and plays a key role in striatal dopamine transmission, in that it is the primary means of clearing extracellular dopamine from the synaptic cleft (Madras, Miller, & Fischman, 2005), thereby regulating synaptic dopamine concentrations (Deutch & Roth, 1999). DAT1 displays a polymorphic 40-base pair variable number of tandem repeats in the 3’ untranslated region. The most common variations, the 9- and 10-repeat alleles, result in individual differences in DAT availability in the brain (Vandenbergh et al., 1992). Most studies have found that the 10-repeat allele is associated with more DAT availability (Brookes et al., 2007; Fuke et al., 2001; Heinz et al., 2000; Mill, Asherson, Browes, D'Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005). It has been suggested that individuals who carry two copies of this allele (i.e. 10/10 homozygotes) have higher DAT density and therefore less dopamine in the synapse than 9-repeat carriers (Swanson et al., 2000). However,
other studies have reported greater DAT density in 9-repeat carriers (Jacobsen et al., 2000; van de Giessen et al., 2009; van Dyck et al., 2005), and no DAT density differences between genotypes (Krause et al., 2006; Martinez et al., 2001).

If, indeed, 10/10 homozygotes have more DAT expression and less dopamine, we would expect to see decreased learning in tasks facilitated by higher levels of striatal dopamine. Thus, we sought to determine the functional influence of DAT1 genotype on striatal-based implicit sequence learning vs. MTL-based implicit spatial context learning. Based on evidence that DAT1 expression is higher in the striatum than the MTL (Lewis et al., 2001), we expected that DAT1 genotype would influence sequence learning but not spatial context learning. Specifically, we hypothesized that 9-repeat carriers would show greater sequence learning than 10/10 homozygotes in the later stages of sequence learning, given evidence that striatal involvement increases with training (e.g., Rauch et al., 1997). In contrast, we expected that DAT1 genotype would be unrelated to spatial contextual learning, which relies on the integrity of the MTL.

Methods

Participants.

Participants were 37 Georgetown University students who received either monetary compensation or course credit for their participation. Participants were grouped according to their DAT1 genotype. Participants in the 9-repeat/9-repeat homozygous group (n=7) and the 9-repeat/10-repeat heterozygous group (n=11) were
combined, henceforth called 9-repeat carriers, and compared to 10/10 homozygotes (n=19). The genotype groups did not differ in age (9-repeat carriers: $M = 20.2 \pm 1.2$; 10/10 homozygotes: $M = 20.2 \pm 1.2$), years of education (9-repeat carriers: $M = 14.1 \pm 1.0$; 10/10 homozygotes: $M = 14.4 \pm 1.0$) or gender (9-repeat carriers: 15 females; 10/10 homozygotes: 12 females) ($p$'s > .17). Based on self-report, participants were without psychiatric disorder (e.g., ADHD) and did not use drugs known to influence cognitive functioning (e.g., dopaminergic medications). The Georgetown University Institutional Review Board approved all experimental procedures, and all participants gave informed consent.

*Genotyping for DAT1.*

DNA was extracted from Oragene saliva kits (DNA Genotek Inc., Ottawa, Ontario, Canada). The 40 base pair VNTR polymorphism in the 3’ UTR of DAT1 was genotyped by PCR as previously described (Daly, Hawi, Fitzgerald, & Gill, 1999) using the following primers; Forward: 5’-TGTGGTGTAAGGAACGGTCCTGAG-3’ Reverse: 5’-CTTCCTGGAGGTCACGGCTCAAGG-3’. PCR was performed using the AccuprimeTM Taq DNA polymerase system (Invitrogen) with the following PCR program: 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 68°C for 1 min. The PCR products were then run out on a 2% agarose gel stained with ethidium bromide. A 100 bp DNA ladder was then used to identify the various repeat alleles by size: 7-repeat (360bp), 8-repeat (400bp), 9-repeat (440bp), 10-repeat (480bp), and 11-repeat (520bp).
**Triplets Learning Task (TLT).**

On a computer screen, participants viewed four open circles that filled in either red or green in discrete, sequentially ordered, three-event trials or “triplets” (see Figure 4a). For description purposes below, the four stimulus locations are referred to as 1, 2, 3, and 4, with 1 as the leftmost position and 4 as the rightmost position, though these numbers never appeared on the screen. Participants were asked to observe the first two consecutive red events and then to respond only to the third, green target event location, by pressing one of four corresponding buttons with their dominant hand. Red events were displayed for 120 ms each, and the green target remained in view until participants made a correct response to its location, with 650 ms separating the correct response and the first cue on the following trial (Howard et al., 2008).

A randomly chosen set of 16 triplets occurred with high probability (HP) and the remaining possible 32 triplets occurred with low probability (LP). Repetitions (e.g. 111, 222) and trills (e.g. 141, 232) were not presented because studies have shown that they reflect pre-existing response tendencies, in addition to learning (Boyer et al., 2005; Cleeremans & McClelland, 1991; Remillard & Clark, 2001). The frequency of HP to LP triplets was approximately nine-to-one. Each participant received the same set of HP and LP triplets, but their order of presentation was randomized within each block. Participants were not informed of any regularities; their only instruction was to respond as quickly and accurately as possible. Participants completed three testing sessions, each consisting of 5 blocks of 50 trials. During breaks after each block,
participants viewed their mean reaction time and accuracy scores. These scores were presented in an attempt to maintain performance around 92% accuracy. Depending on their accuracy in the preceding block, participants were instructed in one of three ways via a message on the screen: “focus more on speed,” “focus more on accuracy” or “speed and accuracy are about right.”

*Figure 4.* Sample displays from the Triplets Learning Task (A) and Spatial Contextual Cueing Task (B).

**Spatial Contextual Cueing Task (SCCT).**

Participants viewed 12-item stimulus arrays that each contained a single target, a horizontal letter T (rotated 90°) and 11 distractors, which were rotated letter L’s (0°, 90°, 180°, or 270°) that were made to look more like the target by offsetting the point of intersection by 3 pixels (Chun & Phelps, 1999; Expt. 2) (see Figure 4b). These visual arrays were presented in white against a gray background and were randomly generated by placing the 12 items into cells of an invisible grid (6 rows X 8 columns), with items repositioned by 63 pixels along each axis to avoid colinearity. Target
location was balanced for distance from the screen’s center and screen half (left/right); no target appeared in the four center or corner cells.

On each trial, a white fixation dot appeared for 1 second, followed by a visual array that remained on the screen for up to 10 seconds until a response was made. Participants were asked to locate the target and to respond to its orientation as quickly and as accurately as possible by pressing the “z” (if the tail of the T was facing left) or “/” (if the tail of the T was facing right) on the keyboard, making no more than 1 or 2 errors per block. Once a response was detected, participants received auditory feedback of a high-pitch tone for a correct response or a low-pitch tone for an incorrect response. If no response was made, a low-pitch tone sounded after 10 seconds.

A spatial contextual regularity is embedded in this task, such that half the visual arrays repeated across blocks. In these repeated arrays, the distractor array predicted the location of the target, but not its orientation (i.e., the correct response). In other words, regularities in spatial arrays provided contextual guidance for the location of the target, but not whether the target was facing left or right. The remaining arrays for each block were randomly generated for each trial, creating a novel configuration that only appeared once throughout the experiment. Each participant received the same set of repeated and novel arrays, but their order was randomized within each block. Following 12 practice trials, participants completed 30 blocks of 12 trials each, with 6 repeated and 6 novel trials per block (Bennett, Barnes, Howard, & Howard, 2009). Participants were encouraged to take short breaks after each block.
Procedure.

Participants first performed the TLT, followed by the SCCT within a single testing session. Including breaks, total testing time was approximately one hour. On a separate testing day, typically the next day but no more than a week later, participants completed two other unrelated implicit learning tasks that are not reported here. At this time, the experiment concluded with an interview as a probe of participants’ declarative knowledge in all the implicit learning tasks. Four increasingly specific questions were asked: (1) Did you notice anything about the tasks you have performed? (2) Did you notice any repeating patterns within the tasks? (3) Did you use any particular strategies? (4) There were, in fact, regularities in the tasks you just completed. Knowing this, could you describe any of these regularities? Both the TLT and SCCT have consistently yielded evidence of implicit learning on sensitive recognition tasks (Bennett et al., 2009; Howard et al., 2008); therefore, we did not include such measures after each task in the current study in order to keep learning implicit in all tasks completed.

Results

Implicit sequence learning (TLT).

Median reaction times (RT) were determined for correct responses for each triplet type for each participant on each block. Overall accuracy was ~93%, so few trials were omitted. These data were then averaged across blocks to obtain a single mean RT for each participant for HP and LP triplets for each of the 3 testing sessions.
A similar procedure was used to calculate the mean accuracy for each participant for each triplet type.

To assess potential group differences in implicit sequence learning, Genotype (9-repeat carriers, 10/10 homozygotes) × Triplet Type (HP, LP) × Session (1–3) mixed-design ANOVAs were conducted separately for accuracy and reaction time measures. Genotype varied between-subjects, and Triplet Type and Session varied within-subjects. For accuracy, sequence learning was revealed as a main effect of Triplet Type, $F(1, 35) = 8.54$, $p = .006$, $r_{\text{effect}} = .44$, with more accurate responses to HP vs. LP triplets. There was a main effect of Session, $F(2, 70) = 4.87$, $p = .01$, $r_{\text{effect}} = .35$, demonstrating that the feedback provided after every block guided participants to overall performance levels of 92% accuracy, as intended. No interactions reached significance ($p$'s > .26). The lack of a main effect or interaction with Genotype showed that feedback served to successfully match the groups on overall accuracy, which aids interpretation of reaction time data, in that there are no speed-accuracy trade offs.

For reaction time, skill learning can be seen in Figure 5, with overall RT (regardless of Triplet Type) decreasing across sessions, $F(2,70) = 35.53$ $p < .0001$, $r_{\text{effect}} = .71$, reflecting overall skill and practice. Sequence learning was seen as a main effect of Triplet Type, $F(1, 35) = 98.85$, $p < .0001$, $r_{\text{effect}} = .86$, with faster responding to HP relative to LP triplets. This sequence learning effect (or separation of HP and LP trials) increased with practice, as indicated by the significant Triplet Type × Session interaction, $F(2,70) = 3.59$, $p = .03$, $r_{\text{effect}} = .31$. Finally, and most important, there was
a Genotype × Triplet Type × Session interaction, $F(2,70) = 5.19, p = .008, r_{\text{effect}} = .36$, indicating that there are group differences between genotypes which vary with practice. No other main effects or interactions reached significance ($p$’s > .26).

*Figure 5. Mean of median RTs by genotype for the TLT.* Mean of median reaction time (in milliseconds) over sessions for high- and low-probability triplets for 9-repeat carriers and 10/10 homozygotes. Error bars represent the standard error of the mean.

To explore this interaction more fully, we calculated sequence-specific learning scores, i.e., mean difference RT scores (LP – HP), which are shown in Figure 6a. Single sample t-tests indicated that these learning scores were significantly different from 0 for all three sessions (Session 1: $t(37) = 4.93, p < .0001, r_{\text{effect}} = .41$; Session 2: $t(37) = 7.99, p < .0001, r_{\text{effect}} = .65$; Session 3: $t(37) = 7.55, p < .0001, r_{\text{effect}} = .62$), indicating sequence-specific learning. Most importantly, as predicted, when these scores were compared between the genotypes at each session, learning was
significantly greater for the 9-repeat carriers than the 10/10 homozygotes in Session 3 only, \( t(35) = 2.25, p = .03, r_{\text{effect}} = .13 \).

Figure 6. Mean of median RT difference scores by genotype and by individuals for the TLT. A) Mean of median reaction time difference scores (in milliseconds) between triplet types (i.e., low probability minus high probability) across sessions for each genotype. Error bars represent standard error of the mean. B) Mean of median reaction time difference scores (in milliseconds) for individual subjects for Session 3, with subjects ordered by the magnitude of their difference score.

The same effect can be seen in Figure 6b, which illustrates individual RT difference scores from Session 3. Using a median split on these difference scores, we classified any subject with a difference score greater than or equal to 27.4 as being a high learner and any subject with a difference score less than 27.4 as being a low learner (see Figure 3b). Of the 19 high learners, 13 were 9-repeat carriers and 6 were 10/10 homozygotes and of the 18 low learners, 5 were 9-repeat carriers and 13 were
10/10 homozygotes, $\chi^2(2) = 6.11$, p=.01. Thus, at the individual level, most 9-repeat carriers were high learners whereas most 10/10 homozygotes were low learners.

Implicit spatial learning (SCCT).

For each participant, median RT for correct trials and percentages of correct responses were calculated separately for each array type for each block and averaged into three 10-block sessions. Similar to TLT, mean of median RTs (see Figure 7) and mean accuracy were analyzed separately in Genotype (9-repeat carriers, 10/10 homozygotes) × Array (repeated, novel) × Session (1–3) mixed ANOVAs, with Genotype varied between-subjects, and Array and Session varied within-subjects. For accuracy, visual search skill learning was seen as a significant main effect of Session, $F(2,70) = 5.72$, $p = .005$, $r_{\text{effect}} = .38$, showing that accuracy improved with practice. No other main effects or interactions reached significance ($p$’s > .12). Overall, accuracy was high ($M = 94.9\%$, $SD = .05$).

Figure 7. Mean of median RTs by genotype for the SCCT. Mean of median reaction time (in seconds) over sessions for new and repeated configurations for both 9-repeat carriers and 10/10 homozygotes. Error bars represent the standard error of the mean.
For mean of median RT, shown in Figure 4, visual search skill learning was revealed by a significant main effect of Session, $F(2, 70) = 94.01, p < .0001, r_{effect} = .85$, demonstrating that responses became faster with practice. Spatial context learning was revealed by a significant main effect of Array, $F(1,35) = 18.00, p = .002, r_{effect} = .58$; participants responded faster to repeated vs. novel arrays. Finally, as predicted, no main effects or interactions with genotype approached significance, $p’s > .35$, indicating that contextual learning did not differ for 9-repeat carriers and 10/10 homozygotes.

Because there was no Array × Session interaction, we conducted an ANOVA across blocks (1-10) within the first session only, to ensure that the main effect of Array was reflecting learning. This revealed a significant Array × Block interaction, $F(9, 324) = 2.04, p < .05, r_{effect} = .23$, with the Array effect significant on Block 10, $t(36) = 2.32, p < .05, r_{effect} = .13$, but not on Block 1, $t(36) = 1.66, p > .05, r_{effect} = .07$. No main effects or interactions with Genotype were significant ($p’s > .12$). Thus, learning occurred within the first session for both groups.

*Implicitness.*

Comments from the post-experimental interviews were examined for insight into the regularities that may have been detected or the strategies people used. These revealed no evidence of declarative knowledge. No one could accurately describe the regularities from the TLT or the SCCT. In addition, no explicit strategies for these two learning tasks were reported.
Discussion

The present study investigated whether DAT1 genotype contributed to individual differences in implicit learning of sequential regularities and spatial contexts in healthy young adults. As predicted, DAT1 was related to sequence learning, with greater learning for the 9-repeat carriers than 10/10 homozygotes over time. In contrast, there were no significant group differences in spatial contextual learning. Because response speed and accuracy were nearly identical between the groups for both learning tasks, overall performance differences cannot account for the observed implicit sequence learning differences, suggesting specificity in how DAT1 contributes to neural and behavior functions. To our knowledge, this is the first study to reveal a relationship between DAT1 and implicit associative learning, indicating that some individual differences in implicit learning of sequential regularities are influenced by genotype.

Reduced sequence learning in 10/10 homozygotes likely reflects differences in striatal dopamine between DAT1 genotypes. Indeed, our results are consistent with the assumption that 10/10 homozygotes have greater levels of striatal DAT availability when compared to 9-repeat carriers (Brookes et al., 2007; Fuke et al., 2001; Heinz et al., 2000; Mill et al., 2002; VanNess et al., 2005) and that this higher gene expression leads to decreased levels of synaptic dopamine in that region (Swanson et al., 2000). Thus, our findings demonstrate the importance of dopamine for implicit sequence learning, even in healthy young adults. Reductions in striatal dopamine have...
previously been found to impair implicit sequence learning, as revealed by learning deficits in Parkinson’s disease patients (Siegert, Taylor, Weatherall, & Abernethy, 2006). Similarly, children with ADHD, a disorder well-characterized by dopamine dysfunction (Dougherty et al., 1999; Krause, Dresel, Krause, Kung, & Tatsch, 2000), have shown sequence learning impairments in comparison to healthy controls (Barnes, Howard, Howard, Kenealy, & Vaidya, In press). Moreover, older adults show reduced implicit sequence learning relative to young adults (Howard et al., 2004a; Howard & Howard, 1997; Howard et al., 2008) that may result from age-related decreases in striatal dopamine (Backman et al., 2006; Rieckmann & Backman, 2009; Volkow et al., 1998). In fact, when using the same version of the TLT used here, older adults performed similarly to the present study’s young 10/10 homozygotes in that the learning deficit was revealed only in the third training session (Simon, Howard et al., 2010).

If 9-repeat carriers are indeed learning more than 10/10 homozygotes, then they would be predicted to respond faster on HP triplets and slower on LP triplets than 10/10 homozygotes. This is because as people learn the predictive relationships within the triplets, they increasingly anticipate the correct target on trials with HP triplets. In contrast, on trials with LP triplets, people come to anticipate a different target than actually occurs. Thus, to avoid making too many errors (participants were guided to 92% accuracy), participants must first inhibit the expected, incorrect response. The results shown in Figure 2 are consistent with this, but with the group difference carried
primarily by the LP trials. This pattern of results may be related to ceiling performance on the HP trials, in that participants were responding around 340 ms by Session 3 ($M$: 339.46, $SE$: 7.8), which is faster than what has been reported previously for HP triplets in the TLT (Bennett et al., 2008; Howard et al., 2008). Thus, responses to the LP triplets may provide a more sensitive measure of triplet contingency learning in the present study. This interpretation is also consistent with reports that 9-repeat carriers have increased incidence of response inhibition when compared to 10/10 homozygotes (Colzato, Pratt, & Hommel, 2010; Kim, Kim, & Cho, 2006).

Though some studies of healthy young adults have revealed significant behavioral differences as a function of DAT1 genotype (Brehmer et al., 2009; Garcia-Garcia, Barcelo, Clemente, & Escera, 2010), most work has revealed minimal or non-existent behavioral differences between genotypes (Bertolino et al., 2006; Dreher, Kohn, Kolachana, Weinberger, & Berman, 2009; see also Rommelse et al., 2008, for a review). Here, we demonstrated a significant difference in sequence learning between DAT1 genotype that was not driven by just one or two subjects. This indicates that the TLT may be among the more sensitive measures to reveal behavioral effects of DAT1 genotype, perhaps because the task recruits fewer regions outside the DAT-rich striatum than previously employed tasks.

To our knowledge, only one previous study has examined the relationship between genotype at a polymorphism relevant to the dopamine system and implicit associative learning. Keri et al. (2005) used a Weather Prediction Task that has been
characterized by implicit striatal-based processes during early learning and explicit MTL-based processes later on (Knowlton et al., 1994). Results revealed that a polymorphism of the dopamine D3 receptor (DRD3) gene, which is found in higher densities in the ventral striatum relative to the MTL (Meador-Woodruff, 1998), was associated with early, striatal-based learning, but not later, MTL-based learning. These findings, together with the present study, suggest that relationships between implicit associative learning and dopaminergic genes can provide additional insight into the neurochemical and neuroanatomical mechanisms of implicit associative learning. For example, our results provide evidence that contextual cueing and sequence learning involve different neural substrates (Barnes et al., In press; Bennett et al., 2008; Howard et al., 2004b; Negash et al., 2007). Because DAT1 has greater expression in the striatum relative to the MTL (Lewis et al., 2001), our findings add to a body of work showing that implicit sequence learning requires striatal brain networks as training progresses, whereas implicit spatial context learning does not, reflecting instead the integrity of the MTL (e.g., Chun & Phelps, 1999).

Future research will be needed to overcome some limitations in the current study. First, the sample here is relatively small. Because the rate of false positive results is higher with smaller samples and less power (Green, Munafò et al., 2008), replication with a larger sample is in order.

Second, we included no measures of explicit knowledge of the presented spatial or temporal patterns, other than a brief post-experimental interview. Even so,
extensive evidence, using similar participant populations and the same versions of the TLT and the SCCT as those used here, indicates that even when sensitive recognition tests are given, people are unable to discriminate between predictable and unpredictable items, thus showing no explicit knowledge (Bennett et al., 2009; Howard et al., 2008). Further, people do not report adopting conscious, deliberate strategies for stimulus selection (Bennett et al., 2008; Chun & Jiang, 1998; Howard et al., 2004b).

This stands in contrast to other learning tasks, like Weather Prediction (Knowlton et al., 1994), in which participants often develop explicit knowledge with practice and use hypothesis-testing strategies that make it difficult to dissociate implicit from explicit learning (Meeter, Myers, Shohamy, Hopkins, & Gluck, 2006).

Third, we did not counterbalance task order; participants always completed the TLT before the SCCT. As a result, participants may have been more fatigued for the second task. However, this is unlikely to explain our pattern of results. Both implicit associative learning paradigms were relatively short (~30 minutes each), and thus, training was not significantly longer than typical testing sessions. Moreover, both groups demonstrated strong learning effects in both sequence and spatial context learning that are comparable to previous studies of similar populations (see Bennett et al., 2009; Howard et al., 2008). Further, it seems unlikely that genotype would interact with fatigue to confound the results presented here.

The present study extends evidence for genetic influences on cognition beyond the domain of explicit processes to implicit forms of learning. Future studies should
explore the contributions of multiple dopaminergic genes for possible gene-gene interactions on implicit learning. For example, if there is a dopamine dosage effect, alleles that decrease dopamine availability might combine to further impair implicit sequence learning. Alternatively, there may be specificity in how different dopamine genes influence different forms of implicit learning, depending on the regions of the brain where genes are preferentially expressed and protein expression patterns. Sequence learning may be more sensitive to DAT1 genotype, whereas other forms of learning (e.g., reward-based learning) may be more sensitive to polymorphisms in other genes relevant to dopaminergic function (e.g., DARPP-32, DRD2) (Frank et al., 2007).

In sum, our findings revealed an association between a polymorphism of DAT1 and striatal-based implicit sequence learning, such that the presence of at least one 9-repeat allele was beneficial for detecting sequential regularities over time. In contrast, no relationship was observed between DAT1 genotype and implicit spatial context learning, a form of learning associated with the MTL. How these implicit learning differences influence real-world behaviors should be a topic of future research. The continued study of genetic influence on cognitive functioning among healthy adults may provide more insight into the neurochemical and anatomical correlates of memory, including various forms of implicit learning and processing.
CHAPTER IV: NEURAL BASES OF AGING AND IMPLICIT ASSOCIATIVE LEARNING

This Chapter has been slightly modified from submission: Simon, Vaidya, Howard and Howard (under review). Neural bases of aging and implicit associative learning.

Here, we examined the neural bases of implicit associative learning, and how these vary with adult age. Despite the importance of implicit learning in adapting to the world, its aging has been studied much less than its explicit counterpart. This neglect is likely due to the dominant view that implicit learning is relatively unaffected by age (Dennis & Cabeza, 2008; Hedden & Gabrieli, 2004). Yet, this assumption is misleading; even though older adults can acquire new implicit associations, evidence from a range of tasks suggests that older adults rarely attain the level of performance of young adults, and further, that the magnitude of age differences increases with training (Filoteo & Maddox, 2004; Maddox, Pacheco, Reeves, Zhu, & Schnyer, In press; Raz et al., 2000). This finding is particularly true for probabilistic tasks, in which older adults’ learning asymptotes while young adults continue to learn (Bennett et al., 2007; Ciomek, Song, Howard, & Howard, 2007; Howard & Howard, 1997; Howard et al., 2008; Howard et al., 2004b; Simon, Howard et al., 2010). The current study asks why this is the case, by examining the neural bases of this form of learning in aging.

Research with young adults suggests that implicit associative learning involves two interactive learning systems: one based on the medial temporal lobes (MTL) and the other based on the striatum. Specifically, studies show that the hippocampus is
important for rapid association formation early in training, while the caudate is involved in integrating probabilistic information gradually over an extended temporal window (Poldrack & Packard, 2003). This pattern has been reported in the motor-based serial reaction time task (Nissen & Bullemer, 1987), in which people learn to make faster motor responses to repeating sequences vs. those that are randomly determined (Rose et al., 2002; Schendan et al., 2003), as well as in the judgment-based weather prediction task (Knowlton et al., 1994), in which people learn to classify stimuli into two contrasting categories when given probabilistic feedback (Poldrack et al., 2001; Shohamy, Myers, Kalanithi, & Gluck, 2008).

Of note, healthy older adults have pronounced volume declines in the caudate (Gunning-Dixon et al., 1998; Raz et al., 2003) while the hippocampus shows relatively little decline (Head, Snyder, Girton, Morris, & Buckner, 2005; Sullivan, Marsh, Mathalon, Lim, & Pfefferbaum, 1995; Sullivan, Marsh, & Pfefferbaum, 2005; but see also Raz et al., 2004). Because studies show that Parkinson’s patients and animals with striatal damage have increased reliance on their intact hippocampus to support striatal-based learning (Moody, Bookheimer, Vanek, & Knowlton, 2004; Poldrack & Packard, 2003), we propose that age deficits in implicit associative learning appear with practice due to the relative aging of the hippocampus and caudate, and the functional reorganization of neural learning systems that occurs as a result. Early in training, old adults learn implicit associative regularities just as well as young adults because the hippocampus, which is efficient at the rapid association formation needed early in
training, is relatively spared with aging. However, age deficits emerge later because old adults continue to engage the hippocampus throughout training, and this structure is not as well-suited as the caudate for the gradual integration of complex, probabilistic associations (Ashby, Turner, & Horvitz, 2010).

To our knowledge, only five functional imaging studies have examined the neural bases of aging and implicit associative learning. One study found no age group differences in behavioral performance or in task-related brain activations, including striatal regions (Daselaar et al., 2003). This age equivalence was likely due to the use of a “young” adult sample (aged 30-35) that is older than the 20-year-old groups used in other studies, which is important since the striatum shows linear declines starting in early adulthood (e.g., Raz et al., 2003; Reeves, Bench, & Howard, 2002). Of the remaining studies, each reported that older people had reduced striatal activation and increased reliance on regions elsewhere in the brain during learning relative to young adults. In two studies, increased MTL activation was observed in older adults consistent with our hypothesis here (Dennis & Cabeza, 2010; Rieckmann et al., 2010), whereas in two other studies, older adults recruited frontal or parietal cortices (Aizenstein et al., 2006; Fera et al., 2005). However, none of these studies yielded age-related deficits in behavioral learning. This may be a result of brief training, and/or that event timing was slowed down to fit the imaging protocol, which resulted in minimal learning effects in the young that made it hard to detect age deficits. Likewise, some of the above studies used deterministic regularities, which may not produce age
differences in learning (Howard & Howard, In press). Thus, it is not yet known which neural regions contribute to observed age differences in learning implicit probabilistic associations with practice.

Here we used the Triplets Learning Task (TLT; Howard et al., 2008), which uses event timing that is more conducive to learning and provides ample training to examine the time course of behavioral learning and of brain activations in young and old adults. Importantly, studies using this task have shown that both age groups learn equally well early in training, but that age deficits appear over time (Howard et al., 2008; Simon, Howard et al., 2010). Moreover, the TLT has been found to be sensitive to individual differences in striatal function in healthy young adults (Simon, Stollstorff et al., 2010). In this task, participants view open circles that become red or green in discrete three-event sequences or “triplets.” On each trial, participants observe two red cues and respond only to a third green target via corresponding button, providing a continuous, performance-based learning measure. Unbeknownst to participants, triplets have a probabilistic second-order structure, in that the first cue’s location predicts one target location for a majority of the trials and another location for the remaining trials. Such complex probabilistic sequences minimize spontaneous explicit awareness (Reber, 1976), rendering it possible to study the evolution of implicit associative learning with training. In fact, people reveal such learning with practice by faster responses on more predictable trials despite having no explicit knowledge of the embedded regularities, even after 3 hours of training (Howard et al., 2008).
The present study used event-related functional imaging of the TLT to investigate the neural bases of implicit associative learning in healthy young and old adults to examine why old adults rarely attain the level of learning of practiced young adults. Our hypotheses focused on the hippocampus and caudate, and predicted that young and old adults rely on these brain regions differently. Early on, hippocampal activation will be common to both age groups but older adults will reveal reduced caudate activity compared to young. With more training, the young will continue to recruit the caudate whereas the old will not, but will instead continue to recruit the hippocampus due to age-related striatal declines. We expect that this pattern of brain activity will result in age equivalent behavioral learning early on, but not later.

To ensure learning was implicit, we performed a separate behavioral experiment in young adults who completed the same version of the TLT outside of the scanner, followed by two sensitive measures of explicit knowledge.

Methods

Participants. For the fMRI study, 11 young adults (18.8 ± .60 years old, 6 female) and 12 healthy, older adults (67.5 ± 3.2 years old, 9 female) received either course credit or monetary compensation for their participation. Young adults were all students at Georgetown University and old adults responded to advertisements in the Washington Post Health Section. All participants, but one, were right-handed. Due to scanner malfunction, data were lost for 1 young and 1 old adult in the final scan run (late training). The Georgetown University Institutional Review Board approved the
experimental procedures, and all participants gave informed consent. Prior to participation, adults were screened for conditions that would prevent them from being able to enter the MRI environment. These included having a neurological disease or disorder, using drugs known to influence cognition and/or meeting criteria for dementia (i.e. score below 27 on the Mini-Mental Status Exam) or abnormal intelligence status (i.e. scores outside the expected age-range on neuropsychological measures of processing speed, cued recall, free recall, verbal memory, vocabulary and reading ability) (see Table 1 for results).

For the behavioral study, participants were 11 young adults (20.1 ± 1.0 years old 6 female) who received either monetary compensation or course credit for their participation. None had participated in the fMRI study.

**Experimental paradigm.** The TLT was a shortened and simplified version from

<table>
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<th>Test</th>
<th>Cognitive Processing</th>
<th>Young adults</th>
<th>Old adults</th>
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<td>29.3 (0.8)</td>
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<td>67.9 (5.7)</td>
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<td>60.7 (13.7)</td>
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<td>10.4 (3.0)</td>
<td>-2.77*</td>
</tr>
<tr>
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<td>7.0 (1.6)</td>
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<td>Verbal memory</td>
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<td>45.8 (6.4)</td>
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<td>Verbal memory</td>
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<td>19.0 (2.0)</td>
<td>ns</td>
</tr>
<tr>
<td>WAIS-III digit span forward</td>
<td>Working memory</td>
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<td>11.8 (2.0)</td>
<td>ns</td>
</tr>
<tr>
<td>WAIS-III digit span backward</td>
<td>Working memory</td>
<td>7.4 (2.6)</td>
<td>9.0 (2.6)</td>
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<tr>
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</table>

Notes: All scores are given as mean (SD), with neuropsychological test scores based on raw data except where standard scores (SS = age-adjusted standard scores with a mean of 100 and SD of 15) are noted. Independent sample t-tests show group effects (*p < .05, **p < .01, NS = not significant). Two young participants did not complete the WAIS-III logical memory, COWAT-FAS, and USC-REM tests. MMSE = Mini-Mental State Examination, WAB-III = Wechsler Adult Intelligence Scale, 3rd Ed, COWAT-FAS = Controlled Oral Word Association Test-FAS, USC-REM = University of Southern California Repeatable Episode Memory Test, and WJ-III = Woodcock-Johnson, 3rd Ed. In the MMSE, all young adults scored a perfect score (30) whereas older adults' scores ranged from 28 to 30.
that reported in Howard et al. (2008). Participants viewed three open circles on a computer screen, displayed against a gray background (Figure 8). Each trial or “triplet” consisted of the sequential presentation of two cue events (circles filling in red) followed by the target (a circle filling in green). Each red event was displayed for 200 ms and the green target remained in view for 850 ms, with 250 ms separating events (total of 2000 msec/trial). Participants observed the first two red events and responded to only the third, green target event location as quickly and as accurately as possible via corresponding button. Unbeknownst to participants, the first cue’s location probabilistically predicted the target event’s location as described below and the second cue’s location was unrelated, thereby creating second-order structure.

Figure 8. Sample presentation of one triplet from the functional neuroimaging version of the TLT.

Eighteen triplets (out of a possible 27) were presented: 9 occurred with high probability (HP) and 9 with low probability (LP). The frequency of HP to LP triplets was 4:1. Event locations were counterbalanced, such that cue and target events occurred equally often in each location. Trial order was presented in fixed,
pseudorandomized sequence, optimized using OptSeq2 (Dale, 1999). Participants completed three runs, with breaks between each run. Each 6 minute and 30 second run included 108 HP and 27 LP trials that were presented in a rapid event-related design with a temporally jittered inter-trial interval (.5 – 6 sec, mean = 1.36 sec). In the present study, we focused only on Run 1, which will be referred to as early training and Run 3, which will be referred to as late training.

The behavioral sample performed the same task as above, except that explicit knowledge was probed immediately after testing in two ways. First, participants completed a recognition task in which they observed each of the 27 possible triplets, and they were instructed to judge if each triplet had occurred frequently, infrequently or never during training by responding 2, 1 or 0, respectively. Second, participants were interviewed about strategy and their declarative knowledge of triplet structure. Questions ranged from general inquiries about strategies used to improve performance to more specific questions that asked participants to describe any patterns or relationships between red and green events that they might have noticed.

fMRI acquisition. Imaging data were acquired using a 3.0 Tesla MRI system (Siemens Magnetom Trio, Erlangen, Germany). A technician positioned participants in the supine position with a circularly polarized head coil. A mirror mounted on the head coil allowed participants to view stimuli during scanning. Fitted padding minimized head movement.
A high resolution T1-weighted structural scan (MPRAGE) was acquired first, using a 3D MPRAGE sequence with a scan time of 7:23 minutes and the following parameters: TR/TE = 2300/2.94 ms, TI = 900 ms, 90° flip angle, 1 slab, 160 sagittal slices with a 1.0 mm thickness, FOV = 256 x 256 mm², matrix = 256 x 256, resulting in an effective resolution of 1.0 mm³ isotropic voxels. A neurologist later reviewed these images and identified no clinically significant structural abnormalities (e.g., lesions or abnormal growths). Functional imaging was acquired on the same equipment while participants completed the TLT. Functional data were acquired along the AC-PC line using T2*-sensitive gradient Echo Planar Imaging pulse sequence with the following parameters: TR = 2500ms, TE = 30ms, 256 x 256 mm FOV, 64 x 64 acquisition matrix, 90° flip angle and a 0.3mm gap for an effective resolution of 4.0 x 4.0 x 4.0 mm³. Forty-two contiguous 3.7mm thick axial slices were acquired descending in the transverse plane for 154 time points for each run.

Behavioral analysis. To determine if participants showed implicit associative learning, we compared performance on HP vs. LP triplets. Repetitions (e.g. 111, 222) and trills (e.g. 141, 232) were excluded from the analyses reported below because they reflect pre-existing response tendencies (Boyer et al., 2005). Median reaction times (RTs) were determined for correct responses on each triplet type in each block of 27 trials. These medians were averaged to obtain a single mean RT for each individual and the two triplet types. A similar procedure was used to calculate the mean accuracy for each person for the two triplet types.
**fMRI processing and data analysis.** Functional images were analyzed in SPM5 (www.fil.ion.ucl.ac.uk/spm). The first 2 TRs were discarded from the analysis as they had been included for signal stabilization. All participants displayed less than 3mm of motion in any direction within each run, so no data were eliminated due to motion artifact. Images were slice-timed, motion corrected and spatially normalized to the MNI template using each subject’s high resolution structural MPRAGE. Normalized image volumes were then smoothed (8mm full width at half maximum Gaussian kernel) and temporally filtered (128 second high-pass filter). fMRI responses for correct responses on HP and LP triplets were modeled by a canonical hemodynamic response function, and autocorrelations removed signal related to biorhythms. The remaining trial types (trills, repetitions and incorrect trials) were excluded from analysis.

For each participant, an activation map was generated using a linear contrast identifying regions that showed greater response to predictable events (HP > LP) in the first run (early training) and in the third run (late training). Individual-level contrasts were entered into second-level analysis with subjects as a random factor. Given the study’s primary focus on the hippocampus and caudate, these areas were targeted as regions of interest (ROI) using one mask including both regions in both hemispheres, defined anatomically by the automated anatomic labeling (AAL) library (Tzourio-Mazoyer et al., 2002). Correction for multiple comparisons was performed using 3dClustsim (a function from AFNI), based upon Monte Carlo simulation of random
correlated noise distribution to estimate the probability of false positive detection at $p < .05$ ($p < .005$ with a cluster extent of 19 voxels for the gray matter mask of the bilateral hippocampus and caudate combined (Ward, 2000: http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf)). Two second-level analyses were performed separately for early and late training: (1) To test our hypothesis of age group differences in the caudate and not the hippocampus, we compared the response to predictability between young and old adults using a two-sample t-test. (2) To examine whether the hippocampus and caudate engagement varied with the magnitude of behavioral learning, we ran correlations between individual implicit associative learning scores and the response to predictability (activation associated with the HP > LP contrast) within each age group in a voxel-wise manner with the combined anatomical mask. Learning scores were calculated as the difference in performance between HP and LP triplets at both early and late training (i.e., LP triplet RT minus HP triplet RT).

Finally, as an exploratory analysis, we examined whether any regions in the rest of the brain differed by age group, using a two-sample t-test similar to that in (1) but without any mask. We used an uncorrected threshold, $p < .005$ with extent threshold of 10 voxels, in order to balance Type I and Type II error rate (Lieberman & Cunningham, 2009).

All reported coordinates were converted from MNI to Talairach space using the algorithm mni2tal (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach).
Results

Behavioral results

*fMRI Study*. Data were analyzed using a Group (fMRI Young, fMRI Old) x Triplet (HP, LP) x Training Stage (Early, Late) mixed-design ANOVA. Group varied between-subjects, and triplet type and training stage varied within-subjects. Overall accuracy was high (Young: $M$: 99.0%, $SD$: .03; Old: $M$: 98.4%, $SD$: .04), and the ANOVA for accuracy revealed no significant main effects or interactions, $p$’s > .24. This aids interpretation in that there were no age-related speed-accuracy trade-offs. As expected, the ANOVA for RT produced a main effect of group, $F$ (1, 19) = 33.32, $p$ < .001, revealing that young adults responded significantly faster than old adults (Young: 372.82 ± 50.19; Old: 483.02 ± 43.75). A main effect of training stage, $F$ (1, 19) = 11.79, $p$ < .005, revealed significantly faster responses over time (Early: 439.64 ± 71.71; Late: 420.34 ± 72.66), and a main effect of triplet type, $F$ (1, 19) = 3.61, $p$ < .001, revealed associative learning or significantly faster responses to HP than LP triplets (HP: 418.59 ± 70.73; LP: 442.26 ± 72.92). The triplet type x training stage interaction was not significant, $F$ (1, 19) = 2.09, $p$ = .15\(^1\); however, the critical group x triplet type x training stage interaction was significant, $F$ (1, 19) = 5.84, $p$ < .05, showing age group differences in associative learning over time. RT difference scores,

\(^1\) Due to the lack of triplet type x training stage interaction, we conducted a separate ANOVA across epochs (1-3) within the first session only to ensure that the observed main effect of triplet type was reflecting learning. This additional test revealed a significant triplet type x epoch interaction, $F$(2, 42) = 7.42, $p$ < .0005, and no interactions with group ($p$’s > .08), indicating that associative learning occurred quickly for both age groups.
shown in Figure 9, revealed that young adults had significantly higher learning scores than older adults only late in training, \( t(19) = 2.05, p = .05 \).

We further evaluated this age deficit in late learning by examining each individual’s implicit associative learning scores, i.e., LP triplet RT minus HP triplet RT. Using a median split, we classified any subject with a difference score \( \geq 20.2 \text{ ms} \) as being a high learner and any other subject as a low learner. Of the high learners, 8 were young and 3 were old whereas of the low learners 2 were young and 8 were old, \( \chi^2 (2) = 5.8, p = .02 \). Thus, late in training, most young adults were classified as high learners and most old adults as low learners.

Figure 9. Mean of median RT difference scores by age group. Low – High Probability triplets in milliseconds over early and late learning for young (black) and old (white) adults in the fMRI study and young adults in the separate behavioral study (cross-hatch). Error bars represent the standard error of the mean.

Though neuropsychological measures had been collected to characterize our sample as normal, we also correlated these scores with individual implicit associative
learning scores from early and late training to determine whether age-related differences in processing speed, free recall, or cued recall were related to age differences observed in late associative learning. There were no significant correlations between individual RT difference scores at early or late training on any of the neuropsychological measures, either within or across groups (p’s > .21).

**Behavioral Study.** Participants were highly accurate for both the HP and LP triplets across training stages (M: 99.2%, SE: 0.02). To assess potential differences in implicit associative learning inside and outside of the scanner, mixed design ANOVAs were conducted separately for RT and accuracy: Group (fMRI Young, Lab Young) x Triplet Type (HP, LP) x Training Stage (Early, Late). There were no significant interactions between the studies for either behavioral measure (p’s > .23), indicating similar learning between the two experiments (see Figure 9).

**Measures of explicit knowledge.** Young adults in the separate behavioral study also completed two sensitive measures of explicit knowledge. Post-experimental interviews revealed no strategies related to learning and no evidence of explicit knowledge, in that no one reported that the first cue predicted the target or that some triplets occurred more often than others. To assess explicit judgments of triplet frequencies on the recognition task, a one-way repeated measures ANOVA was conducted on the mean recognition ratings for each triplet type (HP, LP and those that never occurred). Data were lost for 1 participant, leaving 10 participants in this analysis. There was no relationship between judgment and triplet category, F(2,18) =
.46, \( p > .05 \), such that all triplet types were rated as having occurred equally often during training (HP: 1.19 ± .17, LP1.14 ± .20, never occurred: 1.21 ± .25).

To examine individuals, 3 x 3 chi-square analyses for each person compared judgments (more often, less often, never) for the three triplet categories (HP, LP, never occurred). As expected, no individual had explicit knowledge of triplet frequencies, in that ratings did not vary with triplet type (\( p \)’s > .09). Moreover, associative learning was independent of recognition task judgments; triplet ratings on the recognition task (i.e., HP-LP ratings) did not correlate with individual RT difference scores in early or late training (\( p \)’s > .12). In sum, participants unknowingly learn regularities of the sort studied here.

*fMRI results*

*Age group differences in the hippocampus and caudate.* Consistent with our predictions and as seen in Figure 10, in early training we observed two clusters of activity in the left caudate that were significantly greater in young vs. old adults (\( x, y, z = -14, -11, 25; z = 3.31, k = 33 \) voxels; \( x, y, z = -8, 15, 6; z = 3.21, k = 32 \)). Of note, and also as predicted, there were no significant age-related differences in the hippocampus in early training.
Figure 10. Clusters of activity in the left caudate in which younger adults show a greater response to predictability than older adults in early training. Graph shows contrast estimates, extracted from the mean of activated clusters using MARSBAR (±standard error) in young and old adults (*p < 0.05).

The previous analysis does not reveal whether the hippocampus showed a response to predictability in each group, so we examined this using one-sample t-tests separately for young and old adults using the same corrected threshold as above. As predicted, at early training hippocampal involvement was observed, in the left hemisphere in young (x, y, z = -19, -38, 9; z = 3.50, k = 24 voxels) and bilaterally in older adults (x, y, z = 32, -16, -6; z = 3.90, k = 47 voxels; x, y, z = 25, -34, 7; z = 3.60, k = 44 voxels).

In late training, the response to predictability in the hippocampus and caudate
did not differ significantly for young and older adults.

*Correlations with implicit associative learning.* Using the aforementioned anatomical mask of the bilateral hippocampus and caudate, in early training no significant correlations were observed between the neural response to predictability and individual implicit associative learning scores for either age group.

Late training, however, revealed the predicted dissociation, such that individual differences in learning were related to individual differences in caudate response for young adults and in hippocampal response for older adults. That is, as shown in Figures 11a and 11c for young adults two clusters were observed in the bilateral caudate ($x, y, z = 12, 10, -4; z = 4.44, k = 37$ voxels; $x, y, z = -16, 2, 20; z = 3.17, k = 43$ voxels), both showing positive correlations with learning, whereas for older adults no significant clusters were observed in the caudate. In contrast, older adults revealed a positive correlation between learning scores and the hippocampus ($x, y, z = -27, -37, 3; z = 3.23, k = 19$ voxels) as shown in Figures 4b and 4d, that was not observed in the young.
Figure 11. *Regions showing significant correlations between implicit associative learning scores and neural response to predictability.* (A) Young adults and (B) older adults at late training, using a combined mask of the bilateral caudate and bilateral hippocampus (anatomically-defined). The scatter plot is presented to visualize how individual differences in the (C) caudate and (D) hippocampus relate to individual differences in late implicit associative learning within each age group. Contrast estimates were extracted from the mean of activated clusters using MARSBAR (Brett et al., 2002) in young and old adults.

*Whole-brain age group differences.* Early in training, young adults showed a greater response to predictability than older adults in regions often observed during associative learning, including the bilateral caudate, the left dorsolateral prefrontal cortex and the bilateral cerebellum. In contrast, older adults showed a greater response to predictability than younger adults in parietal regions, including the inferior parietal lobule and the pre- and post-central gyri (see Table 2). This is consistent with a
previous study of aging and associative learning that showed compensatory parietal activation in healthy older adults that was related to deficient neural responses in the prefrontal cortex and caudate (Fera et al., 2005).

In late training, young adults showed a greater response to predictability than older adults in the occipitotemporal cortices, whereas old adults showed a greater response than young in bilateral frontal regions (see Table 3). This is consistent with findings that older adults show an age-related reduction in occipitotemporal activity coupled with an age-related increase in frontal activity (Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008).

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>BA</th>
<th>Talairach (mm)</th>
<th>Peak z</th>
<th>Volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young &gt; Old Dorsolateral Prefrontal Cortex</td>
<td>Left</td>
<td>46</td>
<td>-29 29 20</td>
<td>3.74</td>
<td>528</td>
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<tr>
<td>Caudate</td>
<td>Left</td>
<td>-14</td>
<td>-11 25</td>
<td>3.26</td>
<td>344</td>
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<tr>
<td>Caudate</td>
<td>Left</td>
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<td>15 6</td>
<td>3.19</td>
<td>232</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Right</td>
<td>11</td>
<td>-46 -45</td>
<td>3.17</td>
<td>192</td>
</tr>
<tr>
<td>Cingulate</td>
<td>Left</td>
<td>24</td>
<td>-16 35 13</td>
<td>3.06</td>
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<tr>
<td>Caudate</td>
<td>Right</td>
<td>10</td>
<td>20 10</td>
<td>3.05</td>
<td>96</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Left</td>
<td>19</td>
<td>-20 -58 -14</td>
<td>2.98</td>
<td>88</td>
</tr>
<tr>
<td>Old &gt; Young Precentral Gyrus</td>
<td>Right</td>
<td>6</td>
<td>47 5 19</td>
<td>3.85</td>
<td>232</td>
</tr>
<tr>
<td>Inferior Parietal Lobule</td>
<td>Right</td>
<td>1/2/40</td>
<td>-26 37</td>
<td>3.54</td>
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<tr>
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<tr>
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<td>-39 33</td>
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<tr>
<td>Precentral Gyrus</td>
<td>Right</td>
<td>13</td>
<td>-38 31 31</td>
<td>2.88</td>
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</table>
Discussion

This study is the first to examine age differences in hippocampal and caudate involvement during early and late phases of learning of implicit associations. Results revealed three main findings. First, implicit associative learning is not spared in healthy aging but rather is characterized by age-related deficits evident later with practice. Second, this type of learning is supported by different neural regions in young and old adults. Early in training, both age groups showed a similar response to predictability in the hippocampus, but young adults showed a greater response to predictability than older adults in the caudate. Later in training, we observed systematic individual differences in the neural response to predictability that related to variation in the amount of learning within each age group; young adults who showed greater implicit associative learning showed a greater caudate response to predictability, whereas for older adults, the hippocampus showed this relationship. The hippocampus
is not as well suited as the caudate to the gradual acquisition of probabilistic associations over time (Ashby et al., 2010), so we argue that age deficits in learning emerge with practice due to age-related declines in the striatal learning system. To our knowledge, this is the first functional imaging study to examine the neural bases of implicit associative learning in aging when behavior is both spared (early) and impaired (late).

*Age differences in behavioral learning*

Young and old adults both demonstrated learning of probabilistic associations in the TLT (i.e., faster responses to triplet events that occur more often), but age differences in learning emerged with practice in favor of the young despite no changes in task demands (Howard et al., 2008; Simon, Howard et al., 2010). One surprising aspect of our data was that older adults’ learning appeared to get worse from early to late training ($p = .05$). This age-related learning deficit cannot be attributed to age differences in event timing within a triplet, since this did not depend on participant response rate and therefore could not be influenced by older adults’ longer and more variable response times (Howard et al., 2007). Nor could it be due to age-deficits in explicit associative learning (Old & Naveh-Benjamin, 2008) since no explicit knowledge was revealed on separate recognition tests. This age-related learning deficit also cannot be attributed to age differences on neuropsychological measures, as these scores did not correlate with learning in either age group. Finally, age deficits in learning are not a result of fatigue arising from longer training because training was
relatively short in comparison to behavioral studies and further, age deficits have been observed in studies that had extended training via short sessions occurring over a number of days (Howard et al., 2004a). Instead, from our neuroimaging results, we argue that age differences in learning result from a fundamental deficit in the striatal learning system in old adults, a deficit which particularly impairs learning of probabilistic associations of the sort embedded in the triplets task studied here.

**Age differences in neural bases of learning**

Early in training, older adults learn implicit associative regularities just as well as young adults and the hippocampus reveals a similar response to predictable events in both age groups. The hippocampus is efficient at forming new stimulus representations that compress redundant information while dedifferentiating predictive information (Gluck & Myers, 2001), and is likely involved early on as sequences are initially acquired, to flexibly bind the three events that occur within each triplet (Schendan et al., 2003; Wallenstein, Eichenbaum, & Hasselmo, 1998). Early in training, we also observed a greater response to predictability in the caudate in young adults as compared to old adults, even though the groups did not differ behaviorally. This result replicates reports of reduced striatal activation in older adults in associative learning tasks when behavioral learning was age equivalent (Aizenstein et al., 2006; Dennis & Cabeza, 2010; Fera et al., 2005; Rieckmann et al., 2010). Although there is this initial learning period when age groups perform similarly behaviorally but not neurofunctionally, our study extends previous work by providing additional training
that reveals age-related differences in both learning-related brain activity and behavior. This suggests that a caudate response to predictable events is necessary for the gradual acquisition and integration of triplet frequencies over time, or more generally, for learning implicit probabilistic associative events that occur more often with practice (Ashby et al., 2010; Poldrack, 2002).

Yet, when older adults showed impaired implicit associative learning later in training, we surprisingly did not see group-level age differences in the hippocampus or the caudate. The fact that we could only observe learning-related correlations late in training, but not group activations, is likely due to the variability in the amount of learning between individuals as training progresses. In late training, the caudate response to predictability was related to learning in young adults only. Across subjects, greater learning scores were associated with greater caudate activation to more predictable events. Unlike younger adults, the hippocampus was associated with later learning in older adults only, such that older individuals who demonstrated greater learning in late training also showed a greater hippocampal activity in response to triplets that occurred more frequently. Reduced involvement of the caudate, but not the hippocampus, in older adults confirmed our predictions, which were based on evidence that the hippocampus exhibits less age-related morphological change than the caudate (Raz et al., 2005). Indeed, this claim is further supported by a diffusion tensor imaging study from our lab, which used the same sample of participants studied here. Importantly, Bennett, Madden, Vaidya, Howard and Howard (2010) found age group
differences in the integrity of white matter connections (assessed by fractional anisotropy) between bilateral caudate-DLPFC tracts, but not between bilateral hippocampus-DLPFC tracts.

Taken all together, our results not only reveal age-related preservation of hippocampal function (e.g., Johnson, Schmitz, Asthanaab, Gluck, & Myers, 2008; Rand-Giovannetti et al., 2006), but also compensatory hippocampal activation in the face of striatal declines (Dennis & Cabeza, In press; Rieckmann et al., 2010). This pattern is akin to how Parkinson’s patients and brain-lesion animals compensate for their striatal deficits (Moody et al., 2004; Poldrack & Packard, 2003). In fact, Rieckmann and Backman (2009) have argued that increased MTL activity in older adults might explain why some behavioral studies have observed no age deficits in implicit learning (e.g., Cherry & Stadler, 1995; Salthouse et al., 1999). It is likely that older adults were able to maintain striatal-dependant behavioral learning via the hippocampus in these cases because the embedded regularities were deterministic, rather than probabilistic. Consistent with this view, behavioral age deficits were observed in the present study and in an implicit motor-based task that each had a complex second-order probabilistic structure (e.g., Howard & Howard, 1997), but not in a similar task that contained a complex second-order deterministic structure (Gaillard et al., 2009). Because the striatum is better suited for learning complex, probabilistic associations over time than the hippocampus (Hartley & Burgess, 2005), age-related functional reorganization of learning systems may be unable to adequately
compensate for striatal losses when learning probabilistic regularities, thereby producing age deficits in some forms of implicit associative learning. Future work should address this possibility empirically, by directly comparing the learning of deterministic vs. probabilistic regularities in young and old adults.

No explicit knowledge

Despite having responded faster to high than low probability triplets during training, young adults in a separate behavioral study were unable to discriminate between triplet frequencies in a post-experimental recognition task, consistent with work that had trained young and old participants on up to 6,000 trials (Bennett et al., 2008; Howard et al., 2008; Simon, Howard et al., 2010). While we cannot definitively rule out that explicit knowledge of triplet structure did not develop in our fMRI sample, these behavioral findings using the same TLT task as the fMRI study, suggest that it was unlikely. Further, they indicate that it is possible to test implicit learning in the TLT without use of a dual-task, such as counting tones simultaneously, which is often used to foster implicit learning in other studies (e.g., Grafton, Hazeltine, & Ivry, 1995). Training without a secondary task is better suited for isolating neural regions associated with implicit associative learning as opposed to those imposed by dual task demands, in addition to being less taxing and more practical for older adults.

Moreover, a post-training interview revealed that people did not adopt conscious, deliberate strategies for stimulus selection in the TLT. This stands in contrast to other probabilistic associative learning tasks, like weather prediction,
which participants often use hypothesis-testing strategies that make it difficult to
dissociate implicit from explicit learning (Meeter et al., 2006). Because age differences
in strategy are not likely in the TLT, our results suggest that neural compensation with
age can be non-strategic and does not always reflect deliberate changes in learning
strategy (Reuter-Lorenz & Cappell, 2008).

Conclusions

In sum, older adults show declines in implicit associative learning, contrary to
popular belief, and these deficits likely reflect age differences in the contribution of the
hippocampus and caudate to implicit associative learning. Early on, when responding
involves the relatively preserved hippocampus, there is age invariance in behavior, but
when responding is dominated by the age-impaired striatum later in training, age
differences emerge. Because the ability to acquire new implicit associations is essential
throughout the lifespan, especially later in adulthood when little time is spent in formal
schooling, our findings suggest that older adults may face challenges in acquiring new
skills or adapting to new environments (e.g., graduated assisted living facilities and
internet) even after extended exposure. Thus, future research should focus on
understanding of the cognitive and neural bases of implicit learning in order to foster
independent and successful aging.
CHAPTER V: GENERAL DISCUSSION

This dissertation presented three studies that characterized the cognitive and neural bases of implicit, probabilistic associative learning, and how these differ for younger and older adults. Contrary to the popular view that implicit learning is preserved with old age (Hedden & Gabrieli, 2004; Zacks et al., 2000), Chapters II and IV revealed that healthy older adults are poorer than younger adults at learning probabilistic associative relationships, even though the studies varied in the exact structure present, and that these deficits appeared only late in training. Importantly, this deficit in learning cannot be attributed to age-related deficits in general processing resources, neuropsychological performance or motor movements, nor it is a result of testing parameters (e.g., rule-based structure, use of feedback, variable event timing) or explicit knowledge. Rather, results from Chapters III and IV suggest that impairments in implicit associative learning reflect a fundamental deficit in the function of the striatal learning system.

Though there is evidence that the hippocampus plays a role in early implicit associative learning, the caudate typically comes to dominate learning with practice (Frank et al., 2006; Grafton et al., 1995; Poldrack et al., 2001; Rauch et al., 1997; Rose et al., 2002; Schendan et al., 2003; Shohamy et al., 2008), reflecting the role of the striatum in the incremental learning of probabilistic information over an extended temporal window (Ashby et al., 2010; Seger, 2006). Thus, striatal declines can compromise cognitive and neural function in implicit associative learning tasks.
Accordingly, Chapter III showed that DAT1 genotype, which influences dopamine transporter expression most abundantly in the striatum (Madras et al., 2005), predicted how well young college-aged adults learned implicit associative regularities. Carriers of the 9-repeat allele, which is associated with greater caudate volume and activity (Bertolino et al., 2006; Durston et al., 2005; Jacobsen et al., 2000), showed significantly more implicit associative learning over the course of training than their 10/10 homozygote counterparts. This advantage was specific to late training, when the caudate was expected to dominate learning. Interestingly, Chapters II and IV showed nearly identical learning trajectories, with a learning advantage for younger adults compared to older adults. Using functional neuroimaging, Chapter IV demonstrated that this age-related learning deficit was associated with a deficient caudate response throughout learning in older adults. This finding was interpreted to reflect age-related volume declines in the caudate of healthy older adults (Gunning-Dixon et al., 1998; Raz et al., 2003), as well as decreased white matter integrity in tracts connecting the caudate to frontal regions (Bennett et al., 2010).

However, morphological declines in the striatum may not be enough to explain the aforementioned learning deficits. Indeed, one study of patients with focal lesions in the striatum showed unimpaired implicit associative learning (Exner, Koschack, & Irle, 2002). Neurocomputational models suggest dopamine may be the culprit. Reduced dopamine activity increases neuronal noise (Li, Lindenberger, & Sikstrom, 2001),
which can have functional consequences such as under-recruitment of task-relevant brain regions or non-selective recruitment of additional brain regions (Backman, Lindenberger, Li, & Nyberg, 2010; Samanez-Larkin & D'Esposito, 2008). Of note, the striatum exhibits marked age-related neurochemical declines, including reductions in absolute levels of dopamine (Garnett, Firnau, & Nahmias, 1983), as well as in dopamine receptors (Rieckmann et al., 2011; Rinne, Lonnberg, & Marjamaki, 1990; Volkow et al., 1998; Wang et al., 1996; Wong, Young, Wilson, Meltzer, & Gjedde, 1997) and the dopamine transporter (van Dyck et al., 2002; Volkow et al., 1996). Moreover, the striatal dopaminergic system is strongly implicated in implicit associative learning, as revealed by learning deficits in Parkinson’s disease patients (Jackson et al., 1995; Nagy, Keri et al., 2007; Shohamy, Myers, Grossman, Sage, & Gluck, 2005; Wilkinson & Jahanshahi, 2007), monkeys with nigrostriatal dopamine damage (Aosaki, Graybiel, & Kimura, 1994; Matsumoto, Hanakawa, Maki, Graybiel, & Kimura, 1999) and young adults with lower levels of dopaminergic metabolism (Nagy, Kelemen et al., 2007). In addition, a study of healthy young adults revealed endogenous release of striatal dopamine during implicit associative learning (Badaiyan, Fischman, & Alpert, 2007). Thus, cognitive deficits observed in 10/10 homozygotes (Chapter III) and older adults (Chapters II and IV) during implicit associative learning may be due to neurochemical shifts in the dopamine system.

In fact, the dopamine hypothesis of cognitive aging suggests that many cognitive changes associated with normal aging are related to simultaneous declines in
dopamine availability (Backman et al., 2000; Erixon-Lindroth et al., 2005; Volkow et al., 1998; Wang et al., 1998) which begin in early adulthood (Reeves et al., 2002). A recent report further argues that dopamine losses contribute to striatal dysfunction during cognitive tasks regardless of age (Backman et al., 2010). This view fits with the findings from Chapter III, and earlier work (Badaiyan et al., 2007; Nagy, Kelemen et al., 2007) that striatal dopamine can predict learning in healthy young adults. Because genetically influenced variations in dopamine demonstrated behavioral results similar to declines observed in healthy aging, it suggests that deficits in implicit associative learning may be accounted for by striatal dopamine deficits irrespective of adult age.

Taken together, the aforementioned findings suggest that as learning gradually shifts with practice from the hippocampus to an impaired striatal system (e.g., declines in striatal volume or dopamine), behavioral deficits emerge. However, other factors may also contribute to the observed deficits in implicit associative learning, such as the effectiveness of inhibitory mechanisms. As participants learn more about triplet contingencies in the Triplets Learning Task, they will increasingly anticipate triplets that occur with high frequency. Thus, in order to maintain accuracy on low frequency triplets, participants must suppress this prepotent response, where the learned response conflicts with the actual target location. Since learning is accompanied by a response inhibition for low frequency triplets, individual and group differences in inhibitory function may underlie individual and group differences in learning (Lustig, Hasher, & Zacks, 2007) and/or in the expression of learning. Indeed, both older adults and 10/10
homozygotes have diminished inhibitory systems, including perceptual and response inhibition impairments (Cornish et al., 2005; Hasher, Stoltzfus, Zacks, & Rypma, 1991) that may not rely on striatal functioning (see Aron & Poldrack, 2005). This suggests that deficits can be observed in learning (or at least in its manifestation in performance) even when underlying striatal processes are intact; yet, deficits arising from inhibitory dysfunction may still implicate the dopaminergic system, as dopamine is thought to be a critical neurotransmitter underlying inhibition.

In sum, this dissertation succeeded in demonstrating age declines in implicit associative learning, and in characterizing the role of the striatum and dopamine as potential contributors to this deficit. Now, more focused questions can be asked to build on this characterization, by asking what task features and what neural mechanisms play a role in producing implicit associative learning impairments. This information will be useful for building and testing theories about implicit learning, as well as for developing interventions that could maximize learning throughout the lifespan.

Future Directions

Of central importance to this dissertation was the investigation of the cognitive and neural bases of probabilistic, associative regularities and age differences therein. But task features that produce deficits in learning remain unknown. From the present data, one theory is that implicit associative learning deficits can be predicted by the extent to which the striatal-based learning system is engaged and the amount of
dysfunction within that system. Thus, future work should explore what aspect of the
implicit associative learning recruits the striatum. Sensitivity to striatal function likely
depends upon whether a task uses deterministic versus regularities, which are
completely predictable from previous elements, or probabilistic regularities, which
contain random or unpredictable events. In fact, neuroimaging studies of young adults
showed that caudate activity varies as a function of such regularities. When an
association was 100% predictable, the striatal signal decayed as subjects learned the
deterministic relationship. However, caudate activation was sustained throughout
learning when associations were probabilistic (Delgado, Miller, Inati, & Phelps, 2005;
Seger & Cincotta, 2005; c.f., Bischoff-Grethe, Martin, Mao, & Berns, 2001). No
studies to date have directly compared the neural bases of learning probabilistic versus
deterministic regularities in older adults, though this is a promising future direction for
at least two reasons. First, there is already cross-study behavioral evidence of
interactions between age and structure of the associative regularity; that is, preserved
learning in studies containing deterministic structures (e.g., Curran, 1997; Gaillard et
al., 2009; Howard & Howard, 1992; Salthouse et al., 1999), and impaired learning in
studies using probabilistic associations (e.g., Bennett et al., 2007; Filoteo & Maddox,
2004; Howard et al., 2008; Maddox et al., In press). Second, it is particularly likely that
the aging brain will be more sensitive than the young brain to different regularities due
to suboptimal neural resources (Backman et al., 2006). Recent findings, including
those from Chapter IV, show that older adults rely on different brain regions from
younger adults during some forms of implicit associative learning, potentially to compensate for striatal losses. Older adults showed increased activation of the medial temporal lobes in studies using deterministic regularities (Dennis & Cabeza, In press; Rieckmann et al., 2010; c.f., Chapter IV and Daselaar et al., 2007) and frontal or parietal cortices in studies of probabilistic learning (Aizenstein et al., 2006; Fera et al., 2005). But it is not yet clear why these different neural systems were recruited across studies because they differed in other task features, including amount of training and type of task. Thus, direct comparisons of different regularity types within the same task are needed and can provide insight into age-related changes in the neural mechanisms underlying learning.

Another potential avenue for future research is to investigate the role of genetics in age-related differences in implicit associative learning. Given that polymorphisms in the dopamine transporter gene have been found to contribute to individual differences in implicit learning of associative regularities in younger adults (see Chapter III), it is likely that this effect will be amplified with age (Mattay & Goldberg, 2009). Indeed, a recent review paper has suggested that the functional significance of polymorphisms in dopaminergic genes will increase over the lifespan due to age-related decreases in neural resources (Lindenberger et al., 2008). Furthermore, several comprehensive reviews have recently outlined striking relationships between healthy aging, cognition and genetics (Deary, Wright, Harris, Whalley, & Starr, 2004; Greenwood & Parasuraman, 2003; Mattay & Goldberg, 2009;
Payton, 2009; Reinvang et al., 2010), but this growing field has not yet explored genetic contributions to individual differences in age-related changes in implicit forms of learning.

Neuroimaging can also be used to complement cognitive genetics work (termed imaging genomics) by elucidating the underlying neural systems that mediate or moderate genetic variations in cognition (Green, Munafo et al., 2008). Specifically, imaging genomics can reveal genetically-related differences in neural structure or function that provide the obligatory and direct link between genes and behavior (Hariri & Weinberger, 2003). To date, there has been relatively little integration of neuroimaging and genetics in aging, but this method is particularly important since genetic effects have a more nuanced relationship with the brain than with behavior. Future cognitive neuroscience of aging research should aim to integrate brain function, genetics and cognitive performance to further understanding of these complex relationships in implicit associative learning. Such an undertaking would have been invaluable to this dissertation to learn whether dopaminergic involvement can directly explain the relationship between deficits in striatal activity and learning among older adults.

Finally, the ability to ameliorate declines in learning will be critical for independent and successful aging in the graying world. Genetics can be a new promising tool in this regard, helping to reveal which individuals are most likely to benefit from various lifestyle factors, therapeutics or behavioral interventions by
accounting for interindividual differences in training (Nicklas, 2010; Witte, Jansen, Schirmacher, Young, & Floel). A better understanding of genetics, cognition and aging may also create opportunity to develop personalized treatments that could slow or even reverse age-related declines in cognition (Reinvang et al., 2010). For example, studies have shown enhanced skill learning in older adults after administration of striatal dopaminergic agents (Floel et al., 2008; Peretti, Gierski, & Harrois, 2004). But, as described by the ‘inverted-U’ relationship between dopamine levels and behavior, too much dopamine can be as detrimental to learning as too little (Backman et al., 2006), and so different sensitivities to dopamine administration based on genotype and age may be useful to explore in future studies. Although the promise of cognitive neurogenetics to inform future behavioral treatments and interventions is still largely unknown, this approach can at least provide greater insight into the anatomical and neurochemical correlates of implicit forms of learning, and how they vary by genotype, brain function and age.

Limitations

The primary limitation of this dissertation research was the relatively small sample size in each study. When studies are underpowered due to small sample size, the probability that an association in the data is due to a true signal is low relative to the probability that it is due to noise. This problem is particularly evident in behavioral genetics studies (see Chapter III), which often rely on samples that are too small to ensure that there is adequate power to detect true associations (e.g., low effect sizes),
thereby resulting in frequent replication failures in molecular genetics literature (Ioannidis, 2005, 2007). Similarly, underpowered studies in functional neuroimaging can be problematic, particularly in aging research (see Chapter IV). Small samples can exaggerate existing challenges in group comparisons, such as group differences in brain morphology or the hemodynamic response function (Gazzaley & D'Esposito, 2005; Samanez-Larkin & D'Esposito, 2008). Thus, as a result of the modest number of participants studied here, further research is needed to duplicate the present findings using larger samples.

In addition, Chapters II and IV were limited in that they only included high functioning (e.g., high education and high cognitive status) older adults, who may not be reflective of the aging population at large. In other words, the performance and underlying neural activity of the older adults studied here may not be characteristic of “normal” or lower functioning older individuals. In fact, Cherry and Stadler (1995) reported that older adults with lower socioeconomic status and verbal abilities show less evidence of implicit learning than their higher ability counterparts, which suggests that age-related deficits in implicit associative learning may even be larger in the general population than are reported here. Then again, older adults tested in these studies were not followed since the original testing date, so it is possible that some of these older individuals had preclinical levels of dementia or other aging-related diseases that may have negatively influenced the present findings. Future comparisons
between high and low functioning older adults would help to clarify whether or not observed results reflect a ubiquitous change in aging.

Another limitation is that the studies described in Chapters II and IV used an extreme-groups cross-sectional model, focusing exclusively on young adulthood (mean age: 19 in both studies) and young seniors (mean age: 68 and 71, respectively). Though this approach is useful for exhibiting age-related differences in implicit associative learning, nothing can be said about the trajectory of learning over the lifespan and how it changes with age. Moreover, this approach limits the extent to which conclusions can be made about other developmental periods, such as adolescence, middle age, or very old age. Accordingly, any claims regarding impaired or spared cognitive and neural functions must be restricted to the developmental periods under investigation here. Nonetheless, these cross-sectional studies kept an 8-year span for both age groups, with the exception of one 87 year old senior in Chapter II. The analysis of narrow-age cohorts (i.e. samples of nearly the same chronological age) is the most useful approach for understanding aging-related changes outside of a longitudinal study because it limits population-level effects and mean-induced covariation (Hofer & Sliwinski, 2001). Though a longitudinal approach was not within the scope of this dissertation, it marks an important future direction for studies examining the status of implicit associative learning in adulthood, particularly in light of the fact that implicit learning is not often included as a measure in longitudinal studies of aging.
Finally, this dissertation specifically reflects learning about regularities in sequential events over time, and so other kinds of implicit associative learning might be different from what has been observed here. For example, learning about spatial contexts can be both implicit and associative, but this type of learning involves different forms of regularities and underlying neural substrates than sequential learning (see Chapter III). In fact, implicit spatial context learning has previously been dissociated from implicit sequence learning in a variety of studies, ranging from aging to genetics to patient groups (for a review, see Howard & Howard, In press). Thus, individuals may differ as to the kinds of environmental regularities to which they are most sensitive and the results of this dissertation cannot be generalized to all forms of implicit associative learning.

Implications

Age-related deficits in learning complex probabilistic associations, especially after extended exposure, may make it difficult for older adults to learn new skills or become sensitive to subtle regularities in their environments. In fact, second language acquisition in immigrants is compromised by the age at which they enter a new country (Birdsong, 2006), demonstrating at least one example in which this age-related implicit learning decline might hurt everyday learning. The ability to remain sensitive to probabilistic associative events is necessary throughout the lifespan, as it can support cognitive flexibility as well as help older adults to anticipate and adapt to an ever-changing world (i.e. new settings, people, and technologies). Moreover, acquiring new
habits that promote cognitive health and successful aging, such as exercise routines or a balanced diet, are especially important later in adulthood. Thus, it is critical to the research effort and to society to establish a behavioral assay that is sensitive enough to detect deficits in implicit associative learning, particularly in older adults. The Triplets Learning Task may be one such measure, given that its shortened testing sessions are ideal for situations in which testing time is at a premium (e.g., functional neuroimaging studies or behavioral studies of patients or older adults). In addition, the Triplets Learning Task may be a useful tool to define normal age-related alterations in striatal brain function during implicit associative learning, which may ultimately help in detecting common aging-related diseases in their early pre-clinical stages. With a more coherent picture of the cognitive and neural bases of implicit associative learning, educational and rehabilitation programs can be developed that take advantage of the older person’s spared capacities and compensate for those that have declined. Such advances in our understanding of implicit associative learning might eventually help to extend independent living and improve the quality of life of older adults and their caretakers, enabling the growing aging population to use their talents to benefit society.
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