EMOTIONAL MODULATION OF EPISODIC MEMORY AND TRANSLATIONAL APPLICATIONS TO AGING AND DEPRESSION-RELATED COGNITIVE IMPAIRMENT

by

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Abstract

Important events in our lives, whether good or bad, “leave a scar upon the cerebral tissue”, as William James stated (James, 1890). While emotional experiences feel better remembered, not all aspects of an emotional experience are well retained. Trade-offs exist in remembering gist and detail information of emotional experiences, where gist information may be more beneficial to hold onto rather than focusing on minute details. A critical component of episodic memory is the ability to overcome interference from overlapping or similar experiences such that they can be stored independently. Understanding the dynamics of how significant memories are stored is crucial to uncovering how our memory system works, how this system may be altered in states of cognitive impairment, and how we might be able to target specific aspects of memory processing to either enhance or impair memory for certain experiences. Using a combination of sensitive cognitive measures, high-resolution functional imaging techniques, and translational applications of these approaches to populations with cognitive impairment (aging, depression, and late-life depression), we investigated amygdala-hippocampal dynamics during performance of an emotional mnemonic discrimination task that is thought to tax hippocampal pattern separation of emotional information. We found unique cognitive and neurobiological profiles in healthy adults as well as in states of aging, depression, and late-life depression.

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Dedicated to

my mom, Jina, my dad, Leo, my sister, Jessica
my grandparents, Hilda & Email Khachatoorian and Narcedalia & Ramon Leal
and to my best friend, Beau Alward, and pup, Dexter

An experience may be so exciting emotionally as almost to leave a scar on the cerebral tissues.

William James (1842-1910)

*The Principles of Psychology (1890)*
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Executive Summary

Important events in our lives, whether good or bad, “leave a scar upon the cerebral tissue”, as William James stated (James, 1890). While emotional experiences feel better remembered, not all aspects of an emotional experience are well retained. Trade-offs exist in remembering gist and detail information of emotional experiences, where gist information may be more beneficial to hold onto rather than focusing on minute details. Understanding the dynamics of how significant memories are stored is crucial to uncovering how our memory system works, how this system may be altered in states of cognitive impairment, and how we might be able to target specific aspects of memory processing to either enhance or impair memory for certain experiences.

Episodic memory, or memory for events in our lives, is an important facet of cognition that allows us to remember our past in order to shape our future. The acquisition and storage of new episodic memories is largely reliant on the medial temporal lobes (MTL), which include the hippocampus, amygdala, and surrounding regions. This network of structures is also known to be particularly vulnerable to neurological and neuropsychiatric diseases. A critical component of episodic memory is the ability to overcome interference from overlapping or similar experiences such that they can be stored independently. Events in our lives tend to share many features (e.g. take place in the same locations or with the same people), which leads to interference that must be resolved. Hippocampal pattern separation, or the process of disambiguating similar representations as distinct from one another, is one mechanism for reducing
interference of similar experiences. The hippocampus is uniquely poised to perform this computation by virtue of its architecture and connectivity. Mnemonic discrimination tasks that are sensitive to hippocampal pattern separation have been designed in order to investigate these computational properties in humans as well as animal models. These tasks assess the ability of the individual to behaviorally discriminate among similar events, which is thought to at least partially index hippocampal pattern separation.

Using a combination of sensitive cognitive measures, high-resolution functional imaging techniques, and translational applications of these approaches to populations with cognitive impairment (aging, depression, and late-life depression), we investigated amygdala-hippocampal dynamics during performance of an emotional mnemonic discrimination task that is thought to tax hippocampal pattern separation of emotional information. There are five major components of this dissertation, designed to achieve the following aims.

**Aim 1:** Develop, optimize, and validate a novel mnemonic discrimination task that assesses discrimination of emotional information and examine emotion related performance. We have recently developed an emotional mnemonic discrimination task, which allows for the assessment of the ability to discriminate highly similar emotional (both negative and positive) and non-emotional stimuli. Participants are shown negative, neutral, and positive scenes during encoding and are then tested on their memory for these scenes either immediately or 24 hours later. Emotion’s influence on memory is
thought to be even greater after time has passed, once consolidation has occurred (Hamann, 2001; McGaugh, 2004). During the memory test, participants are shown the same scenes they saw during encoding (repeats), new scenes (foils), and similar but not identical scenes to ones they saw before (lures) and are asked to determine if the scenes are old (exact same scene they saw before) or new (completely new or slightly different than what they saw before).

We are able to assess two key measures of performance. The first is target recognition (d'; responding “old” to a previously viewed item), which is thought to assess gist knowledge or general familiarity. The second measure is lure discrimination index (LDI; responding “new” to a previously unseen similar lure), which is thought to assess detail knowledge and tax pattern separation. We observed that d’ was enhanced for emotional items but LDI was reduced, an effect that was magnified after waiting 24 hours before test. This suggests a potential mechanism for the emotional modulation of memory, with a bias towards forgetting more emotional detail information and retaining more gist information, possibly indicating an emotion-induced reduction in pattern separation (tested in Aim 2).

**Aim 2:** Utilize high-resolution imaging techniques to query amygdala-hippocampal dynamics during pattern separation of emotional and non-emotional information. To examine emotional modulation of memory at the level of hippocampal subfields, we used high-resolution functional magnetic resonance imaging (fMRI, 1.5 mm isotropic) of the MTL while participants performed the emotional mnemonic discrimination task.
described in Aim 1. Consistent with prior reports, we observed engagement of the hippocampal dentate gyrus (DG) and CA3 during accurate discrimination of highly similar scenes. Furthermore, we observed an emotional modulation of this signal (negative > neutral) specific to trials on which participants accurately discriminated similar emotional scenes. The amygdala was also modulated by emotion, regardless of the accuracy of discrimination. This suggests that hippocampal DG/CA3 processes emotional information markedly different than non-emotional information and that this region’s capability of performing pattern separation may be the underlying mechanism for the gist versus detail trade-offs we see in emotional memory processing.

**Aim 3:** Elucidate alterations in emotional pattern separation associated with depressive symptoms (DS) in young adults. The emotional mnemonic discrimination task in combination with high-resolution imaging may offer a sensitive framework in which to characterize alterations of the amygdala-hippocampal network in states of cognitive impairment such as those associated with depression. We tested young adults with depressive symptoms (DS+) on the emotional mnemonic discrimination task and found that DS+ young adults were impaired in remembering neutral information (both target recognition and lure discrimination), but hyperdiscriminated negative items, which correlated with depressive symptom severity. Additionally, we found aberrant amygdala-hippocampal network activity in DS+ young adults, where amygdala activation was enhanced and DG/CA3 activation was diminished during negative discrimination compared to those without depressive symptoms (DS-). Depressive symptom severity
was negatively correlated with DG/CA3 activity. These findings suggest a novel mechanistic account for how this network may be altered in mood disorders.

**Aim 4:** Elucidate alterations in emotional pattern separation associated with individual differences in age-related memory impairment. We also investigated how this network may be altered in aging, as there have been mixed findings for how emotional information is processed in older adults. We found that older adults were impaired on neutral target recognition but had intact emotional target recognition when tested immediately. We also found that the pattern of results we reported in young adults (reduced emotional compared to neutral discrimination of similar lures) was reversed in older adults. When tested after 24-hours, young adults exhibited less forgetting of emotional targets compared to neutral, while older adults exhibited more forgetting of emotional targets. Finally, discrimination of highly similar positive items was preserved in older adults.

During high-resolution fMRI, we tested cognitively normal older adults that we stratified into aged-unimpaired (AU) and aged-impaired (AI) groups to investigate individual differences in memory performance in aging. We found signals consistent with emotional pattern separation in the DG/CA3 in AU but not in AI individuals, suggesting a loss of emotional pattern separation in subclinical memory impairment. During false recognition (lure false alarms), we found increased DG/CA3 activity in AI compared to AU, regardless of emotional content, consistent with previous findings of dysfunctional hippocampal hyperactivity. The basolateral amygdala (BLA) showed increased activity
for negative versus neutral stimuli during false recognition. Furthermore, we found that the relationship between BLA and DG/CA3 activity during false recognition depended on the level of subclinical memory impairment, such that more impairment predicted high BLA and DG/CA3 activity. To investigate amygdala-hippocampal network connectivity, we measured functional connectivity of indirect (via the lateral entorhinal cortex, LEC) and direct amygdala-hippocampal connectivity and well as intra-hippocampal connectivity. AI individuals showed a selective deficit in the indirect pathway (BLA—LEC—DG/CA3) during negative discrimination, consistent with a loss in emotional pattern separation ability. We found evidence of hyperconnectivity in the AI group across the three networks during negative false recognition, suggesting overgeneralization in the hippocampal and amygdala network.

**Aim 5:** *Elucidate alterations in emotional pattern separation associated with late-life depression.* We also investigated the impact of depressive symptoms on emotional memory in older adults, as both depression and aging influence MTL function. We found that DS+ older adults had reduced DG/CA3 activity during negative discrimination, but increased activity during neutral discrimination compared to healthy older adults. The increase in activity during neutral discrimination was correlated with DS severity. Interestingly, increased BLA activity during neutral discrimination was associated with increased DG/CA3 activity, which predicted greater DS. During positive false recognition, the LEC showed increased activity in the DS+ group, and its interaction with the BLA predicted symptom severity. Finally, the DG/CA3 and BLA’s effect on negative
discrimination depended on DS severity, such that higher DG/CA3 and BLA activity led to better negative discrimination, but only in those with high DS severity.

**N.B. Sources and publications:** The publications listed below were adapted for this dissertation.

**Chapter 1:**

- Book chapter from *The Preservation of Memory* (Psychology Press, Taylor & Francis) (Leal & Yassa, 2015b)
- A review article in Ageing Research Reviews (Leal & Yassa, 2013)
- An invited review article in *Trends in Neurosciences* (Leal & Yassa, 2015a)
- A funded NIMH R01 grant on Emotional Pattern Separation Abnormalities in Depression (R01 MH102392)

**Chapter 2/4:**


**Chapter 3/4:**

- Leal, Tighe, Jones, & Yassa, *Hippocampus*, 2014

**Chapter 5:**

- Leal & Yassa, *Behavioral Neuroscience*, 2014
- Leal, Noche, Murray, & Yassa, *submitted*
- Leal, Noche, Murray, & Yassa, *Learning & Memory (under revision)*

**Chapter 6:**

- Leal, Noche, Murray, & Yassa, *submitted*
A summary table of the major cognitive and neurobiological findings is shown in Table 1.1 below.

Table 1.1. Summary table of major cognitive and neurobiological findings on emotional mnemonic discrimination task.

<table>
<thead>
<tr>
<th>Population</th>
<th>Cognitive Findings</th>
<th>Neurobiological Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy young adults</td>
<td>+ Emotional recognition</td>
<td>+ DG/CA3 emotional pattern separation</td>
</tr>
<tr>
<td></td>
<td>+ Emotional discrimination</td>
<td>+ Amygdala emotional modulation</td>
</tr>
<tr>
<td></td>
<td>- Neutral recognition &amp; discrimination</td>
<td></td>
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<tr>
<td></td>
<td>+ Negative discrimination</td>
<td></td>
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<tr>
<td></td>
<td>DS correlate with negative discrimination</td>
<td></td>
</tr>
<tr>
<td>Depressed young adults</td>
<td></td>
<td>DG/CA3 negative hypoactivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amygdala negative hyperactivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DS correlate with DG/CA3 negative discrimination</td>
</tr>
<tr>
<td>Healthy older adults</td>
<td>+ Emotional recognition</td>
<td>+ DG/CA3 emotional pattern separation</td>
</tr>
<tr>
<td></td>
<td>+ Emotional discrimination</td>
<td>+ Amygdala-hippocampal functional connectivity</td>
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<tr>
<td>Age-impaired older adults</td>
<td>No significant effects</td>
<td></td>
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<tr>
<td></td>
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<tr>
<td>Depressed older adults</td>
<td>- Discrimination</td>
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<td>DS correlate with negative discrimination</td>
<td>DG/CA3 neutral hyperactivity</td>
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<td>DG/CA3 mediates BLA’s effect on DS (neutral discrimination)</td>
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<td>LEC mediates BLA’s effect on DS (positive false recognition)</td>
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<td></td>
<td></td>
<td>Influence of DG/CA3 and BLA on negative discrimination depends on DS</td>
</tr>
</tbody>
</table>
Chapter 1: Background and Significance

Hippocampal Memory and Pattern Separation

Episodic memory

Episodic memory, or memory for events, is an important facet of cognition that allows us to remember our past in order to shape our future. Episodic memory consists of many components including object (what), spatial (where), and temporal (when) information (Tulving, 2002). The hippocampus is thought to be involved in forming, storing, and retrieving episodic memories, but the mechanism by which it accomplishes this has been widely debated.

One proposal is that the role of the hippocampus in memory involves two processes: recollection and familiarity (Wixted, Mickes, & Squire, 2010; Yonelinas, Aly, Wang, & Koen, 2010). The distinction between these two processes can be illustrated by the experience when you recognize and are familiar with a person, but aren’t able to recollect who the person is or where you know them from. Recollection is associated with recall of specific details, such as location, color, feature, or modality of the information (e.g. you remember a person, where you met them, who they are, etc.). Familiarity involves a sense of experience, but lacks any specific details (e.g. you remember the person, but can’t remember where you met them). Recollection is
thought to be represented by a threshold process while familiarity is thought to be represented by a standard signal detection model based on Gaussian distribution of memory strength (Yonelinas, 2002). In many situations, both recollection and familiarity can support effective recognition performance, however, when targets and foils share a high level of perceptual overlap (i.e. high interference), the familiarity system cannot guide accurate performance. Familiarity can be effective when each target is grouped with foils that are perceptual variants of that target (Westerberg et al., 2006). Another approach that attempts to account for situations with high interference is pattern separation.

From a computational perspective, two key computations are involved in forming episodic memories: pattern separation and pattern completion. Pattern separation is the process of disambiguating similar experiences so that there is little to no overlap, and pattern completion is the process of recalling a previously stored experience based on partial or degraded information (Marr, 1971; Yassa & Stark, 2011). The balance between these two computations is thought to give rise to our rich episodic memories. More details on how these computations are supported by the hippocampus to form episodic memories will be discussed over the next few sections.

Anatomy of the medial temporal lobe

The MTL has a well-established role in memory processing (Milner, Squire, & Kandel, 1998; Squire, Stark, & Clark, 2004) and is made up of multiple structures including the
hippocampus (which includes CA1-4 (cornu ammonis) and the DG), subiculum (SUB), presubiculum, parasubiculum, perirhinal cortex (PRC), parahippocampal cortex (PHC), entorhinal cortex (EC), and the amygdala (see Fig 1.1). Each structure has multiple input and output pathways and is part of an extensive neural network with intricate connectivity. There are multiple levels of associative processing in the MTL, with the type of information processed becoming more complex from cortical structures to hippocampus (Lavenex & Amaral, 2000).

**Figure 1.1.** Simplified schematic of the hippocampal network and summary of neurobiological changes occurring in the context of age-related memory impairment. The most upstream age-related vulnerability in this network is hypothesized to occur at the level of the perirhinal cortex (PRC), as well as the lateral entorhinal cortex (LEC), in particular the superficial layers. Downstream, perforant path (PP) input from the EC to the dentate gyrus (DG) and CA3 is blunted with age, while the mossy fiber (MF) and Schaffer collateral (SC) projections appear largely intact. The DG exhibits loss of neurogenesis as well as aberrant ensemble dynamics, while the CA3 appears to be hyperexcitable, thus reinforcing the region’s auto-associative recurrent collateral network. Inhibitory interneuron (Int.) projections to all hippocampal subregions are also degraded with age, perhaps contributing to the shifted excitation/inhibition balance in the hippocampus. Modulatory cholinergic (ACh) input from the medial septum (MS) and diagonal band of Broca (not shown) is diminished. Similarly, dopaminergic (DA) projections from the ventral tegmental area (VTA) as well as noradrenergic (NE) projections from the locus coeruleus (LC) are also diminished with age. Synaptic plasticity as well as place cell firing alterations in the CA1 region also occur with age. Overall, the network exhibits several neurobiological changes that shift the computational bias from learning new information towards emphasizing previously learned information. Lines and boxes in broken red lines are hypothesized to exhibit some age-related deficits. Green lines and black border boxes...
suggest that there are insufficient data in the literature to make informed conclusions regarding age-related impairment (from Leal & Yassa, 2015a).

The hippocampus is implicated in forming new associative memories, storing memories independently of each other, retrieving memories from partial cues, and flexibly applying stored memories to novel situations. It is well positioned to receive information from multiple sensory cortical areas, process that information in combination with other modulatory inputs, and then output this transformed information to the cortex. The hippocampus has a highly distributed three-dimensional organization of intrinsic associational connections, and each region may be acting semi-independently from, as well as in concert with, other hippocampal subfields (Anderson, Morris, Amaral, Bliss, & O’Keefe, 2007).

Briefly, the PRC and PHC receive sensory input from association cortices. This information projects to the EC, in which EC layer II neurons project to the DG and CA3 subfields of the hippocampus (via the perforant path, PP). Granule cells in the DG project to CA3 via the mossy fiber pathway (MF). Pyramidal cells in CA3 project to CA1 via Schaffer collaterals. Additionally, CA3 pyramidal cells heavily project back onto themselves, creating a recurrent collateral network. Pyramidal cells in CA1 project to the subiculum. Both CA1 and subiculum project to the deep layers of the EC (layers IV and V). This is the major output pathway from the hippocampus to cortical structures, including back to PRC and PHC, which project back to neocortical regions (Fig 1.1).
This description of hippocampal connectivity and processing is overly simplified, but encompasses the major studied pathways of the hippocampal “trisynaptic circuit” (EC→DG→CA3→CA1) (Amaral, Scharfman, & Lavenex, 2007; Lavenex & Amaral, 2000; Squire et al., 2004). The hippocampus is also under modulatory control from several sources: 1) The DG, CA1, and CA3 receive cholinergic input from the medial septum and 2) The DG receives noradrenergic input from the locus coeruleus, serotonergic input from the Raphe nuclei, and dopaminergic input from the ventral tegmental area.

**Pattern separation and pattern completion**

Computational models suggest that hippocampal DG and CA3 perform two key computations: pattern separation and pattern completion. Pattern separation is the process of reducing interference among similar inputs by using non-overlapping representations and is thought to rely on hippocampal DG. It is able to accomplish this due to 1) the large number of cells it has (compared to EC layer II or CA3), which allows divergence and convergence of information flow to occur from EC to DG via the PP and from DG to CA3 via the MF pathway and 2) its very sparse activity, which minimizes interference by using unique codes for each event. If only a few units are active per input pattern, then overlap between the hippocampal representations of different items is minimal (Marr, 1971). This process is important for encoding new information; otherwise, without it there would be catastrophic interference (McClelland, McNaughton, & O'Reilly, 1995).
Pattern completion is the process by which representations can be retrieved when given partial or degraded input and is ascribed to hippocampal CA3, where the region’s recurrent collaterals may act as an auto-associative network (Marr, 1971; McClelland et al., 1995; Treves & Rolls, 1994; Yassa & Stark, 2011). To accomplish this, two distinct input synapses must be present: 1) strong synapses (but not associatively modifiable) to force CA3 cells into a pattern of activity relatively independent of any inputs being received from recurrent collaterals (e.g. MF pathway) and 2) a large number of associatively modifiable synapses on each receiving cell in order to relay a signal specific enough to initiate the retrieval process (e.g. PP input). Competition between DG and CA3 is necessary and is one of the powerful computational features of the hippocampus that enables learning (Treves & Rolls, 1994). When the DG is lesioned, input from DG to CA3 is blocked, which impairs encoding but not retrieval, whereas lesioning the PP directly to CA3 impairs retrieval but not encoding (Lee & Kesner, 2004). This suggests the CA3 network utilizes input from the MF pathway and the PP input from EC layer II to facilitate later recall. Thus, the balance between pattern separation and completion is modulated by how strongly the DG projects to CA3. CA1 may function as a comparator between the direct input from EC III and the inputs via Schaffer collaterals from CA3, comparing “reality” from EC to predictions made by CA3 (Lisman & Grace, 2005).

Cross species evidence of pattern separation
Empirical evidence for the subfield specificity of these computations has been recently shown in animals using electrophysiology and immediate early gene (IEG) imaging (Lee, Yoganarasimha, Rao, & Knierim, 2004; Leutgeb, Leutgeb, Moser, & Moser, 2007; Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; Vazdarjanova & Guzowski, 2004) and in humans using high-resolution fMRI (Bakker, Kirwan, Miller, & Stark, 2008; Lacy, Yassa, Stark, Muftuler, & Stark, 2011). Other data from lesion studies and genetic knockout mice has also shown that the DG is necessary for pattern separation, with a potential contribution of newborn granule cells in this region to pattern separation, although the latter remains less understood (see Yassa & Stark, 2011 for review). In a dynamically changing environment, in which familiar landmarks on a behavioral track and along the wall were rotated relative to each other, the population representation of the environment was more coherent between the original and cue-altered environments in CA3 than in CA1 (Lee et al., 2004), consistent with pattern completion in CA3. In another study, hippocampal CA1 and CA3 were examined when rats were placed in rooms with common spatial elements. In CA3, distinct subsets of cells were activated in each room, regardless of the similarity of the testing space. In CA1 however, the activated populations overlapped such that the overlap increased in similar environments. After exposure to a novel room, ensemble activity developed slower in CA3 than in CA1, suggesting that the representations emerged independently and is consistent with CA3 performing pattern separation (Leutgeb et al., 2004). While these two studies seem to yield conflicting evidence, they display the ability of CA3 to perform either pattern separation or pattern completion depending on the similarity of the input.
An IEG study mapped activity-dependent population responses in CA1 and CA3 and found that when environmental changes were small, overlap in CA3 was greater than in CA1 (consistent with pattern completion in CA3) but when the changes were made somewhat larger, overlap was greater in CA1 than CA3 (consistent with pattern separation in CA3) (Vazdarjanova & Guzowski, 2004). This suggests that these processes should not be treated as simple binary distinctions but rather as different aspects of tuning functions that transform the input (Guzowski, Knierim, & Moser, 2004). CA3 is capable of exhibiting pattern completion under circumstances where there are small changes in sensory input and pattern separation when there are larger changes in sensory input. Hippocampal CA1 exhibits a linear transformation indicating that it is neither separating nor completing.

Minimal changes in the shape of an environment in which rats are exploring can dramatically alter correlated activity patterns among place-modulated granule cells in the DG. When the environments are made more different, new cell populations are recruited in CA3 but not DG (Leutgeb et al., 2007). More recently, a study simultaneously recorded activity from CA3 and DG of behaving rats as the testing environment was distorted to varying degrees, with the hypothesis that pattern separation would be identifiable as large changes in the neural representation following small changes in the environment while pattern completion would show minor changes. In the DG, both single-unit and population-level analyses demonstrated a rapid decorrelation of the neural representation following any distortion of the testing enclosure, consistent a pattern separation signal. Furthermore, the rapid decorrelation
of the DG representations was not a simple reflection of the tuning of the representations observed in the EC. In CA3, the same analyses showed that the representation remained relatively coherent over the varying levels of distortion (Neunuebel & Knierim, 2014). Likewise, the observation that the CA3 representation remained coherent with respect to the positions of the local cues, a feature that appeared to be weakly represented in the upstream areas, indicated that CA3 was able to pattern complete a previously learned neural representation given noisy inputs. This is the first direct evidence of pattern separation in the DG and of pattern completion in CA3 and provides additional support for the long-standing hypotheses regarding the functional properties of these areas (Marr, 1971; McNaughton & Morris, 1987; Treves & Rolls, 1994).

The first empirical evidence for pattern separation in the human hippocampus was reported in a high-resolution fMRI study in 2008 (Bakker et al., 2008). The study used a mnemonic discrimination task with pictures of objects that were either presented once or repeated at a later time. On some trials, similar but not identical versions of the objects (lures) were presented during the second time. They were able to exploit the repetition-suppression effect (Krekelberg, Boynton, & van Wezel, 2006) such that if a region treated the lure item as the same as the original presentation (i.e. pattern completion), the response should be adaptation, where activity would be lower compared to the first presentation (repetition-suppression). If a region was treating the lure as a new item (i.e. pattern separation), then activity should show a response similar to an initial response (no repetition-suppression). They found that the DG/CA3 showed
activity consistent with pattern separation (i.e. activity for lures was highly similar to activity for first presentations and not repetitions). This was not true for other hippocampal subfields such as CA1.

In another study, participants were exposed to room layouts with different numbers of changes relative to previously learned rooms. Here, DG/CA3 showed an abrupt, binary response, whereas CA1 showed a linear response that was modulated by the number of changes in the rooms (Duncan, Ketz, Inati, & Davachi, 2012). To show that hippocampal computations were indeed demonstrating different transfer functions (Duncan, Curtis, & Davachi, 2009; Kumaran & Maguire, 2007), the similarity of the stimuli was varied (Lacy et al., 2011), such that the appropriate input/output functions could be mapped. A highly discontinuous response was observed in the DG/CA3 whereas a smooth linear trend was observed in the CA1, demonstrating that both have access to the necessary sensory information, but have different transfer functions in response to changes in input.

In summary, animal studies have shown direct evidence of pattern separation computations performed by the DG, showing dramatic changes in firing pattern with small input changes, and pattern completion computations performed by the CA3, showing consistent firing patterns even when inputs are changed. CA3 can also perform pattern separation under appropriate conditions (e.g. DG signal overrides CA3 signal). High-resolution imaging studies in humans have provided cross-species evidence consistent with the role of the DG/CA3 region in pattern separation, suggesting this
region is sensitive to small changes in input while CA1 shows no evidence of this processing ability (e.g. linear changes).

**Emotional Modulation of Episodic Memory**

**Emotional memory**

Emotions color our memories and can afford them a special status that preserves them from loss and forgetting. This relationship is thought to be adaptive; the association of memories with positive or negative affect allows them to more successfully guide future action. While studies in animals have reliably demonstrated a facilitatory effect of arousal on memory consolidation (McGaugh, 2004), studies in humans suggest a more complicated picture. Flashbulb memories (memories for the circumstances in which one heard about a newsworthy event) initially suggested that memory was better for emotional events and their context compared to neutral events (Brown & Kulik, 1977). These memories were thought to be vivid due to inclusion of many peripheral details. While emotion leads to vivid recollections, these recollections are not completely accurate and may result from post-hoc reconstructions of the emotional event (Heuer & Reisberg, 1990). Thus, the hallmark of an emotional memory may be the subjective vividness with which it is remembered rather than the accuracy with which it is retained (Kensinger, 2009).
More recently, several studies have suggested that emotion’s effects on memory are asymmetrical, such that emotional modulation of memory for the gist is enhanced, while memory for details is impaired (Kensinger, 2009; Loftus, Loftus, & Messo, 1987; Mather & Sutherland, 2011). Such selectivity suggests an emotion-induced memory trade-off, where individuals remember the central emotional content of an experience but often forget the details (Buchanan & Adolphs, 2002). An example of this phenomenon is the “weapon focus” effect, where eye-witnesses often recall the weapon used in a crime with great detail but fail to encode (or perhaps more quickly forget) peripheral details such as the perpetrator’s clothing (Loftus et al., 1987). Typically these effects are most robust after a delay, where emotional arousal can influence the consolidation of information (McGaugh, 2004). However, several studies have shown that memory for emotional stimuli can be enhanced even when tested immediately, which suggests that emotion affects both encoding and consolidation mechanisms (Hamann, 2001). Mather and Sutherland (2011) have put forth the Arousal-Biased Competition (ABC) model in which arousal during an event can either enhance or impair memory for events, depending on bottom-up and top-down factors that bias competition in favor of high priority stimuli.

In a recent study, Segal and colleagues examined the effect of emotional arousal on mnemonic discrimination (Segal, Stark, Kattan, Stark, & Yassa, 2012) and demonstrated that increased emotional arousal (measured using salivary alpha amylase, a biomarker for endogenous peripheral noradrenergic activation) was correlated with enhanced mnemonic discrimination for similar neutral objects. These
findings suggest that emotion may modulate mnemonic discrimination abilities when interference is high (i.e. when test items are similar to study items), which is thought to rely on hippocampal pattern separation (Yassa & Stark, 2011). However, this study only evaluated emotion as a pre-study state effect (i.e. a state of increased arousal could have enhanced attention or increased vigilance) and not on a trial-by-trial basis (thereby losing stimulus specificity).

**Anatomy of the amygdala**

The amygdala contains about 13 nuclei that have widespread connections with many areas in the brain and can interact with information from every sensory modality. The amygdala is involved in processing emotional information (LeDoux, 2007; McGaugh, 2004). There are three major groups of amygdala nuclei: 1) basolateral nuclei (lateral, basal, and accessory basal nucleus) as is typically referred to as the BLA, 2) cortical-like nuclei (nucleus of the lateral olfactory tract, bed nucleus of the accessory olfactory tract, anterior and posterior cortical nuclei, and periamygdaloid cortex), 3) centromedial nuclei (central, medial, and the bed nucleus of the stria terminalis) (Sah, Faber, Lopez De Armentia, & Power, 2003). Nuclei within the amygdala can be distinguished functionally by their connectivity and by the distributions of neurotransmitters within each grouping (Swanson & Petrovich, 1998).

The BLA projects to the hippocampus via: 1) indirect connections through the EC, 2) indirect connections through the hypothalamus and medial septum, and 3) direct
connections to CA3, CA2, CA1, subiculum, and parasubiculum. The LEC is more strongly connected to the BLA and hippocampus while the MEC does not have a strong amygdala connection (Canto, Wouterlood, & Witter, 2008; McDonald & Mascagni, 1997). It is unclear which amygdala-hippocampal connections are driving the emotional modulation of memory or if they all contribute some role to emotional memory processing. There are no known direct connections of the amygdala to DG. The hippocampus and surrounding cortical regions also project back onto the amygdala. The EC, CA1, and SUB project to the amygdala (mostly BLA). There are also reciprocal connections of the PRC and PHC with the amygdala. DG, CA3, CA2, and pre/parasubiculum do not project to the amygdala (Petrovich, Canteras, & Swanson, 2001; Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000). Furthermore, it has been suggested that the dorsal hippocampus performs primarily cognitive functions, while the ventral hippocampus performs functions related to stress, emotion, and affect due to its greater connectivity with the amygdala. Gene expression in the dorsal hippocampus has been shown to correlate with cortical regions involved in information processing, while genes expressed in the ventral hippocampus correlate with regions involved in emotion and stress (amygdala and hypothalamus; Fanselow & Dong, 2010).

**Cross species evidence of emotional modulation of memory**

The effect of emotional arousal on memory is thought to be mediated by the influence of the amygdala on hippocampal processing. Post-training electrical stimulation of the amygdala can either enhance or impair memory for aversive training (inhibitory
avoidance), depending on the stimulation intensity and the training conditions and indicates that the effects are modulatory, not simply memory impairing (McGaugh, 2004). One of the first studies to investigate post-training drug infusions to study the involvement of the amygdala in memory consolidation found that beta-adrenoceptor antagonists into the amygdala impaired rats' retention of inhibitory avoidance, and concurrent infusion of norepinephrine (NE) blocked the memory impairment (Gallagher, Kapp, Musty, & Driscoll, 1977; Gallagher & Kapp, 1981).

The adrenal gland releases epinephrine, which stimulates the vagus nerve, which then stimulates the nucleus of the solitary tract. This allows permeation of the blood-brain barrier and stimulation of the BLA. It is here that NE is released, which then allows the amygdala to exert its influences on hippocampal function (McGaugh, 2004). The BLA has been implicated in assigning affective value to stimuli and is well positioned for associative learning. Lesions of the BLA block acquisition and expression of fear conditioning (Sah et al., 2003). The adjacent central nucleus of the amygdala (CEA) does not appear to play a significant role in modulating memory consolidation, however, it has been implicated in fear conditioning. Lesions of the CEA block the expression of fear conditioned response using visual or auditory conditioned stimulus (Sah et al., 2003). The CEA also appears to regulate the processing of cues when predictive relationships between events are first noticed or altered (i.e. an unexpected or surprising event) (Gallagher & Holland, 1994). Furthermore, BLA lesions suppress adult DG neurogenesis and prevent selective activation of immature newborn neurons in response to a fear-conditioning task, while lesions of the CEA do not (Kirby et al., 2012).
The amygdala is promiscuous in influencing the consolidation of memory for many kinds of motivationally arousing experiences, either appetitive (i.e. positive) or aversive (i.e. negative) (McGaugh, 2002, 2004). It has been suggested that arousal and valence is represented separately, with amygdala representing arousal and the orbitofrontal cortex (OFC) representing valence. However, these distinctions don't fully capture patterns of brain activity, as there is some interaction between arousal and valence (Hamann, 2012). Human studies suggest that the amygdala modulates the strength of episodic memory according to emotional importance, regardless of whether the emotion is pleasant or aversive (as these types of stimuli are more important than neutral stimuli for reproductive success) (Hamann, Ely, Grafton, & Kilts, 1999).

In rodents, stimulating the amygdala after training on hippocampus-dependent tasks facilitates learning (Packard, Cahill, & McGaugh, 1994). Damage to the amygdala results in a profound impairment in learning, especially in tasks that require the formation of emotional associations (Richter-Levin & Akirav, 2001). Lesions, NMDA antagonists, or local anesthetics infused into BLA decrease long-term potentiation (LTP) in the DG. Conversely, high-frequency stimulation of the amygdala facilitates induction of LTP in the DG (Davis & Whalen, 2001). Emotionally arousing encoding tasks result in memory enhancement that is positively correlated with endogenous NE (McIntyre, Hatfield, & McGaugh, 2002; Segal et al., 2012). Human fMRI studies have shown that activity in the amygdala and hippocampus is correlated during encoding (Kensinger & Corkin, 2004) and retrieval (Kensinger & Schacter, 2007) of emotional information.
Furthermore, amygdala-hippocampal functional connectivity predicts enhanced later recall of emotional memories (St Jacques, Dolcos, & Cabeza, 2009). According to the modulation hypothesis, the difference in remembering emotional versus non-emotional events is due to the modulatory effect of the amygdala on the hippocampus during memory encoding and consolidation. Both the amygdala and hippocampus were more activated for remembered emotional than for remembered neutral pictures (Dolcos, LaBar, & Cabeza, 2004; McGaugh, 2002). Another study found that subjects’ memory for scenes tested three weeks after brain scanning correlated with amygdala activity induced by viewing the scenes, a powerful subsequent memory effect. The relationship between amygdala activity during encoding and memory was greatest for the scenes rated as most emotionally intense (Canli, Zhao, Brewer, Gabrieli, & Cahill, 2000).

In humans with selective bilateral lesions of the amygdala, memory for emotionally arousing material is not enhanced, as in normal subjects. In a classic fear-conditioning paradigm, during which a neutral blue square is paired with an aversive shock to the wrist, patients with amygdala damage fail to show a normal physiological fear response to the blue square, even though they are able to report that the blue square predicted the shock. Patients with damage to the hippocampus show the opposite pattern, in which they demonstrate a physiological arousal response to the blue square, but are not able to consciously recollect that it is paired with the shock (Phelps, 2004). This double dissociation highlights the independent but complementary functions of these two memory systems. Emotional modulation of memory requires the amygdala-hippocampal system to remain intact.
Memory Impairments in Depression

Cognitive view

Major depressive disorder (MDD) is one of the most pressing public health challenges our society faces today. Elucidating the neurobiology of MDD is of paramount importance to accurate diagnosis and effective intervention. Although significant strides have been made in understanding the neurochemical and circuit changes that occur in MDD, the etiology and pathophysiology of the disorder remain largely unknown. Limitations in our understanding of neural mechanisms as well as the lack of detailed means to assess circuit-level structure and function in humans have hindered progress.

Both emotional and neutral memories are abnormal in MDD, implicating the amygdala and hippocampal systems described earlier. Several studies have reported episodic memory deficits in MDD (Airaksinen, Wahlin, Forsell, & Larsson, 2007; Airaksinen, Wahlin, Larsson, & Forsell, 2006; Bierman, Comijs, Jonker, & Beekman, 2005; Dere, Pause, & Pietrowsky, 2010), as well as a mood-congruent negative emotional memory bias where negative information tends to be better remembered than positive information (i.e. negativity bias: Gordon, Barnett, Cooper, Tran, & Williams, 2008; Haas & Canli, 2008; Hasler, Drevets, Manji, & Charney, 2004; Watkins, Vache, Verney, Muller, & Mathews, 1996; Watkins, Martin, & Stern, 2000). It has also been reported that patients suffering from MDD are unable to exclude irrelevant negative information
from working memory and complain about negative autobiographical memories (Dere et al., 2010). Furthermore, recent work suggests that individuals with depressive symptoms have a diminished capacity for discrimination of highly similar object stimuli (Shelton & Kirwan, 2013).

**Neurobiological view**

Converging evidence from human and rodent studies of depression have found structural and functional changes in the hippocampus. In humans, structural imaging studies have generally reported reductions in hippocampal volume in depression (Bremner et al., 2000; Gerritsen et al., 2011; MacMaster & Kusumakar, 2004; but see Lloyd et al., 2004; Vakili et al., 2000). Typically, time spent depressed, total number of recurrent episodes, and earlier age of onset are associated with hippocampal volume loss (Sheline, 2003, 2011). These features have been linked to cognitive deficits in processing emotional memories (Dere et al., 2010). Functional imaging studies have shown decreased hippocampal activity during positive memory encoding (van Tol et al., 2012). Post-mortem human studies of depression have generally failed to support massive cell loss in the hippocampus but are consistent with a deficit in neuropil mostly in DG and CA3 (Stockmeier et al., 2004).

Animal models of depression offer a way by which invasive techniques can be used to probe the altered neural circuitry underlying depression in greater detail than afforded by neuroimaging studies in humans. Although it is difficult to reproduce and evaluate
symptoms of depression in an animal model, chronic stress manipulations have been used with some success to model many of the core endophenotypes of depression such as anhedonia, despair, appetite changes, and anxious behavior (Hasler et al., 2004). Studies in which animals are exposed to chronic stress have been used to study the hippocampal shrinkage observed in depression. Multiple factors have been attributed to this volume loss: dendritic retraction, suppressed adult neurogenesis, and neuronal death. More support that hippocampal volume reduction is not due to morphological cell loss but rather subtle synaptic, dendritic, and axonal changes has been found in rodents (Czéh & Lucassen, 2007). These more subtle changes in hippocampal neuropil are more likely to contribute to the volume loss, specifically dendritic retraction of CA3 apical dendrites (Watanabe, Gould, & McEwen, 1992). Several studies have found that this dendritic retraction within the hippocampus in rats under chronic stress is reversible ten days after termination of the stressor and is described as “remodeling” of the hippocampus during chronic stress (Conrad, 2006).

Recent studies have shown that DG newborn granule cells may play a role in pattern separation (Aimone, Deng, & Gage, 2011; Deng, Aimone, & Gage, 2010). Ablation of DG neurogenesis impairs behavioral discrimination (Clelland et al., 2009), whereas enhancing neurogenesis improves it (Sahay et al., 2011). Inhibiting immature granule cells results in pattern separation deficits whereas inhibition of old granule cells does not (Nakashiba et al., 2012). DG neurogenesis is also important for emotional memory formation (Hernández-Rabaza et al., 2009; Kitamura et al., 2009; Saxe et al., 2006). BLA lesions suppress DG neurogenesis as well as activation of immature cells in a fear-
conditioning task (Kirby et al., 2012). Importantly, DG neurogenesis is also implicated in depression and the action of anti-depressants (Dranovsky & Hen, 2006; Sahay & Hen, 2007; Samuels & Hen, 2011). Reduction in the rate of neurogenesis in the adult DG is another hypothesis of hippocampal volume loss in depression (Dranovsky & Hen, 2006). It was reported that adult-born granule cells in the DG buffer the ability of this region to counteract stress. A recent study showed that inhibiting adult neurogenesis in the DG resulted in HPA axis dysregulation whereby recovery from glucocorticoids was slower and depressive symptoms were readily apparent (e.g. increased food avoidance in a novel environment after acute stress, increased behavioral despair in the forced swim test, and decreased sucrose preference, a measure of anhedonia) (Snyder, Soumier, Brewer, Pickel, & Cameron, 2011). Anti-depressants suppress the toxic effects on the hippocampus and increase hippocampal neurogenesis and synaptic plasticity, however, there are neurogenesis-independent mechanisms of anti-depressant action (MacQueen & Frodl, 2011). While neurogenesis is an attractive mechanism by which to alter memory and mood, a reduction in neurogenesis does not seem to be the major cause of the volume reduction. Rates of neurogenesis in adults is very low, making it unlikely that such minute alterations can significantly contribute to the 10-15% reduction in entire hippocampal volume (Czéh & Lucassen, 2007).

In addition to structural and functional changes in the hippocampus, the amygdala also undergoes significant alterations in depression. Increases in amygdala volume have been reported (Dere et al., 2010; Drevets, 2001; Drevets, Price, & Furey, 2008; Weniger, Lange, & Irle, 2006), however, some studies have also reported decreases
(Hasler et al., 2004). Functional imaging studies have demonstrated increased amygdala activity during negative memory encoding (Drevets, 2001; Sheline et al., 2001). Increased amygdala involvement during negative word encoding may underlie heightened experience of, and an inability to disengage from, negative emotions in depressive disorders (van Tol et al., 2012).

Although repeated stress produces dendritic remodeling in the CA3 region and impairs hippocampal-dependent learning (Conrad, Galea, Kuroda, & McEwen, 1996), the BLA has been shown to be essential for stress-induced facilitation of aversive learning (Liang, Hon, & Davis, 1994). Vyas and colleagues (2002) examined whether chronic stress induced morphological changes in the amygdala and found that chronic stress induced opposite patterns of dendritic remodeling in hippocampal and amygdala neurons. Chronic immobilization stress elicited significant dendritic atrophy in hippocampal CA3 pyramidal neurons, but caused dendritic hypertrophy in BLA neurons. The amygdala may mediate stress-related enhancement of hippocampal memory processes and LTP (McGaugh, 2002; Richter-Levin & Akirav, 2001). The BLA plays an important role in both the impairing and enhancing effects of stress on hippocampal functioning (Liang et al., 1994; Richter-Levin & Akirav, 2001). Stress effects on the BLA are not uniform, but seem to depend on stress characteristics such as intensity, valence, duration, and controllability. Rats trained under “high-stress” conditions (cold water) learned to find the hidden platform in the Morris water maze faster than rats trained under “low-stress” conditions (warm water) (Akirav, Sandi, & Richter-Levin, 2001).
Elucidating the neurobiology of depression is important for accurate diagnosis and effective intervention. Although we have an understanding of the neurochemical and circuit changes that occur in depression, the etiology and pathophysiology of the disorder remain fairly elusive. Understanding the neural mechanisms that underlie the emotional modulation of memory is crucial to alleviating dysfunctions in these neural processes can lead to changes in memory function.

**Memory Impairments in Aging and Alzheimer’s disease**

**Cognitive view**

Memory impairments are one of the most common dysfunctions reported by older adults as they age (Newson & Kemps, 2006). Episodic memory has been shown to decline with age (Craik & Simon, 1980; Glisky, 2007; Hedden & Gabrieli, 2005, 2004; Jennings & Jacoby, 1997; Newman & Kaszniak, 2000; Small, Stern, Tang, & Mayeux, 1999). Spatial memory (i.e., memory for spatial configurations and ability to navigate in an environment) is one component of episodic memory that declines with age across species (Barnes, 1979; Gallagher, Burwell, & Burchinal, 1993; Gallagher & Pelleymounter, 1988; Gaylord & Marsh, 1975; Perlmutter, Metzger, Nezworski, & Miller, 1981; Salthouse, Mitchell, & Palmon, 1989). However, not all aged individuals show memory deficits as a function of chronological age. When examining learning index scores on the Morris water maze, Gallagher and colleagues have consistently shown
individual differences in aged rats such that some rats performed on par with the young rats and some manifest age-related spatial learning and memory deficits (Gallagher et al., 1993) (Fig 1.2). It is not yet known why this occurs, although many hypotheses have been proposed. For example, some suggest that older adults who do not show age-related decline may have higher “cognitive reserve,” possibly enabling them to be more resilient to brain changes than others (Stern, 2012).

![Figure 1.2. Individual differences in spatial learning in young and aged Long Evans rats. (a) Amount of time it takes (search error, cm) for rats placed in the Morris water maze to find their way to a hidden platform. Aged rats are slower at finding the platform. (b) Learning index measure based on performance across the training trials suggests that not all aged animals show impairments. A subset of aged animals (Aged Unimpaired) perform just as well as young animals, while a subset of aged animals is impaired (Aged Impaired) (from Leal & Yassa, 2015).](image_url)

Animal and human data suggest that recollection is selectively impaired by the aging process, while familiarity is relatively spared (Bastin & Van der Linden, 2003; Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2006; Duverne, Habibi, & Rugg, 2008; Howard, Bessette-Symons, Zhang, & Hoyer, 2006; Prull, Dawes, Martin, Rosenberg, & Light, 2006; Robitsek, Fortin, Koh, Gallagher, & Eichenbaum, 2008). This suggests a trade-off in remembering specific information versus more general information in aging. Recent
data in aged rodents and humans have also suggested that mnemonic discrimination (disambiguating similar experiences) is also compromised with aging and may explain many of the phenotypes associated with age-related memory decline (e.g. reduced recollection, spatial memory, contextual memory, etc.). The ability of older adults to discriminate highly similar experiences has been shown to decrease across many domains including object (Stark, Yassa, Lacy, & Stark, 2013; Toner, Pirogovsky, Kirwan, & Gilbert, 2009; Yassa, Lacy, et al., 2011), spatial (Holden & Gilbert, 2012; Reagh et al., 2014; Stark, Yassa, & Stark, 2010), and temporal (Roberts, Ly, Murray, & Yassa, 2014; Tolentino, Pirogovsky, Luu, Toner, & Gilbert, 2012) information (Fig 1.3).

**Figure 1.3.** Performance on mnemonic discrimination tasks across object, spatial, and temporal domains in young and older adults. (a) In the Mnemonic Discrimination Task – Objects Version (MDT-O), participants are shown objects (2500ms, 500ms inter-stimulus interval (ISI)) and asked to respond whether they are indoor or outdoor (encoding phase). After a 5 minute delay, participants are tested on their memory for the items they saw. They are shown repeated items (targets), similar but not identical items (lures), and completely new items (foils) and are asked to perform yes/no recognition; (b) In the Mnemonic Discrimination Task – Spatial Version (MDT-S), participants are shown objects in different locations on the screen (2500ms, 500ms ISI) and are asked to respond whether objects are on the left or right of the screen (encoding phase). After a 5 minute delay, participants are tested on their memory for item locations. They are shown items in the same locations and items that changed locations (lures), which varied in metric distance from the original locations; (c) In the Mnemonic Discrimination Task –
Temporal Version (MDT-T), participants are shown objects (2500ms, 500ms ISI) and are asked to indicate whether the current object is bigger or smaller than the previous object (encoding phase); this type of instruction was intended to foster sequential encoding. During the test phase, participants were pairs of stimuli on the screen and asked which one came first in the study sequence (i.e. a temporal order judgment). Test item pairs varied in the temporal lag between them during the study phase (d) Performance measured by lure discrimination (ability to correctly reject similar lures) shows that with increasing object dissimilarity, performance improves across age groups. Older adults were split into aged impaired (AI) and aged unimpaired (AU) groups according to whether or not they were within the young norms on the RAVLT. Both AU and AI adults performed worse than young adults, however impairment was larger in AI adults; (e) Performance measured by proportion correct shows that with increasing metric movement, performance improves across age groups. AU adults perform on par with young adults in the easiest and hardest conditions, but are impaired in the middle conditions. AI adults perform more poorly than both AU and young adults across all conditions except for the most difficult discrimination, where all three groups approached floor performance; (f) Performance measured by proportion correct shows that with increasing number of intervening items (i.e. temporal lag), performance improves in all age groups. In this case, older adults were split into AI and AU groups based on their performance on a primacy measure (see Roberts et al. 2014 for details). AU adults perform on par with young adults in the easiest and hardest conditions, but are impaired in the middle conditions. AI adults perform more poorly than both AU and young adults across all conditions except for the most difficult discrimination, where all three groups approached floor performance (from Leal & Yassa, 2015).

Object domain

The ability of older adults to discriminate among similar object stimuli was tested by Toner and colleagues (2009) as well as Yassa and colleagues (2010) using a continuous mnemonic discrimination task. Both studies used an identical task design in which young and older adults were shown pictures of everyday objects and asked to indicate for each object whether it was new, repeated, or similar to the items preceding it. Participants were shown a mixture of new items, repeated items, and items that were similar but not identical to ones previously shown (lures). Both studies reported an increase in false alarms for similar lures in older adults compared to young adults. Yassa et al. (2010) further demonstrated that the discrimination/generalization functions for these stimuli categorized by mnemonic similarity level clearly dissociated young and
older adults, but performance on novel and repeated items was indistinguishable between groups.

On a similar version of the object mnemonic discrimination task (Fig 1.3a), older adults were classified into AU and AI groups based on delayed word recall performance (a standard neuropsychological measure for long-term memory drawn from the Rey Auditory Verbal Learning Task (RAVLT)). There were greater impairments in object mnemonic discrimination in the AI group, but no overall impairment in recognition performance across both groups. In contrast, those individuals diagnosed with mild cognitive impairment (MCI) exhibited significantly worse performance on both object discrimination and recognition memory performance (Stark et al., 2013).

Similar studies have been conducted in rodents, where young and aged rats performed a spontaneous object recognition task. When the test objects contained overlapping features (similar to lure items as discussed in the human studies), only the young rats showed an exploratory preference for the lure item (Burke et al., 2011). An analogous procedure was used in non-human primates, and when object pairs contained overlapping features, the aged monkeys took significantly longer than the young animals to learn to discriminate between the rewarded and the unrewarded object (Burke et al., 2011). Together, these results suggest that discrimination of similar object information is reduced in aging, in a manner that is consistent across species.
**Spatial domain**

Studies evaluating mnemonic discrimination of spatial information were based on early work in hippocampal-lesioned rodents (Gilbert, Kesner, & DeCoteau, 1998), which assessed the ability to judge whether stimuli had moved from locations in which they were originally presented. The difficulty of discrimination is typically tested by systematically varying the metric distance between the study and test phases. Recent studies have used spatial mnemonic discrimination tasks and have found that a subset of older adults who were impaired (based on the RAVLT) were significantly impaired on the spatial discrimination task compared to AU and young adult groups (Reagh et al., 2014; Stark, Yassa, & Stark, 2010, Fig 1.3b). Another study (Holden & Gilbert, 2012) using a delayed-match-to-sample discrimination task also showed impairments in older adults ability to discriminate spatial information.

**Temporal domain**

Studies evaluating mnemonic discrimination in the temporal domain are based on the notion that experiences that are presented in close temporal proximity will be more difficult to disambiguate than experiences that are presented farther apart in time. A recent investigation (Tolentino et al., 2012), which was modeled after temporal discrimination tasks in young rodents (Gilbert, Kesner, & Lee, 2001), tested temporal memory of a sequence (i.e. when a dot appeared in a specific location in an eight-arm radial maze) and found that older adults were impaired relative to young adults.
recent study examined temporal discrimination in young and older adults using study-test blocks that were composed of sequences of 30 objects and were asked to indicate which one came first in the sequence. Older adults were impaired relative to young adults at moderate and high temporal lags (Roberts, Ly, Murray, & Yassa, 2014, Fig 1.3c), but not at low lags (where performance approached floor).

In general these deficits can be characterized as memory rigidity where there is an age-related requirement for higher levels of dissimilarity across experiences for discrimination to be successful. Data are consistent with a conceptual model in which the relationship between interference and performance is largely linear in young adults and more exponential in older adults (Fig 1.4, right).

**Fig 1.4. Schematic representation of cross-species computational changes occurring in the aging hippocampus and cognitive consequences.** Neurobiological (left) and cognitive (right) alterations are described as a function of interference level (x-axis), which is quantified on a continuum from exact repetitions (left) to novel experiences (right). Given this framework, changes occurring in the DG/CA3 region of the hippocampus can be characterized as a form of representational rigidity. Across species, DG/CA3 coding properties are consistent with pattern separation in young subjects, and reduced pattern separation (a bias towards pattern completion) in older subjects. The consequence of these neurocomputational alterations manifests as an impairment in discrimination performance on tasks designed to assess hippocampal pattern separation. In other words, older subjects tend to overgeneralize.
at the expense of discrimination. This appears in the absence of global memory deficits in conditions where there is minimal interference such as exact repetitions, or brand new items.

_Emotional memory in aging_

While episodic memory deficits are a hallmark characteristic of aging, emotion’s modulatory influence on memory remains less well characterized in aging. While some studies show no age-related differences, others have reported alterations with aging, most often those using tasks involving low intensity shock and few training sessions (Foster, DeFazio, & Bizon, 2012). This suggests that the level of arousal is an important component in determining whether there are age-related changes with respect to the impact of emotion on memory retention (McGaugh, 2006).

Several human studies have shown that memory for emotional experiences is preserved with age (Denburg, Buchanan, Tranel, & Adolphs, 2003; Kensinger, Brierley, Medford, Growdon, & Corkin, 2002). Some studies have suggested that there is a “positivity effect,” where older adults may be more likely to attend to positive information in the environment (Mather & Carstensen, 2005). In contrast, some have suggested that memory for detailed information in older adults reveals no such positivity effect and may actually be biased towards remembering negative details (Kensinger, Garoff-Eaton, & Schacter, 2007). This suggests that the relationship between aging and emotional modulation of memory is complex and requires a more thorough investigation.
Neurobiological view

In 2006, Wilson and colleagues proposed a model of neurocognitive aging, suggesting that small concurrent changes during aging strengthen the auto-associative network of the CA3 subfield of the hippocampus at the cost of processing new information coming in from the EC (Wilson, Gallagher, Eichenbaum, & Tanila, 2006). This reorganization of the system causes information that is already stored to become the dominant pattern of activity at the expense of encoding new information. In other words, the model hypothesizes that the hippocampal network is biased away from pattern separation and towards pattern completion in aging. A number of studies (Wilson et al., 2003; Wilson, Ikonen, Gallagher, Eichenbaum, & Tanila, 2005; Wilson et al., 2004; Wilson, Ikonen, Gurevicius, et al., 2005) point to a specific age-related impairment in pattern separation that manifests as “rigidity” in spatial representations in CA3 neurons while navigating similar environments (i.e. bias towards pattern completion, producing the same representation despite changes in the input). Mechanistically, it is proposed that this shift away from pattern separation in the CA3 is the result of the degradation of the perforant path associated with aging, both via degraded input to DG and directly to CA3. It is hypothesized that, consequently, CA3 activity is driven more by the recurrent auto-associative fibers and less by the pattern-separated signal it would normally receive from the DG. In addition, the model proposes that hyperactivity in CA3’s auto-associative network is the result of attenuated cholinergic modulation of the hippocampus and concomitant reduction in inhibitory interneuron activity.
Reductions in hippocampal volume have been reported in aging, the extent of which predicts declining memory performance (Golomb et al., 1996). High-resolution MRI studies have suggested that this volume loss is specific to CA1 and the DG/CA3 subfields (Mueller & Weiner, 2009). While earlier rodent studies suggested that the volume reduction seen in the hippocampus of aged animals was due to neuronal cell loss (Scheibel, Lindsay, Tomiyasu, & Scheibel, 1976), more recent studies using unbiased stereological techniques (Rapp & Gallagher, 1996), reliably demonstrated that this was not the case. The primary cause underlying volume reductions in the hippocampus appears to synapse loss (Burke & Barnes, 2006). This synapse loss appears to be highly specific to the cortical inputs into the hippocampus (e.g. the PP). In aged rats, the DG receives approximately one-third fewer synaptic connections from EC layer II than in young rats (Geinisman, deToledo-Morrell, Morrell, Persina, & Rossi, 1992; Smith, Adams, Gallagher, Morrison, & Rapp, 2000). The amount of synaptic reduction of the PP correlates with the degree of spatial memory impairment in aged rats (Smith et al., 2000). Electrophysiological studies have shown that stimulating the PP generates less excitation of the DG in aged rats compared to young rats (Burke & Barnes, 2006).

The above model was extended to older humans using high-resolution fMRI, capable of resolving hippocampal subfields. During an object mnemonic discrimination task, older adults were found to exhibit “representational rigidity” in the DG/CA3 region, which manifested as a diminished novelty response to similar but not identical items (Yassa, Lacy, et al., 2011). Compared to young adults, older adults’ performance on the object
mnemonic discrimination task was impaired, and the extent of the impairment correlated with the extent of their fMRI activity rigidity. Using ultrahigh-resolution diffusion tensor imaging (DTI), which measures microstructural features of white matter, the study also quantified both the integrity of the PP as well as DG/CA3 dendritic integrity and found that both were correlated with DG/CA3 representational rigidity (Yassa, Mattfeld, Stark, & Stark, 2011; Yassa, Muftuler, & Stark, 2010). The extent of PP reduction in older adults correlated with performance on the object mnemonic discrimination task and the RAVLT, which confirms that PP integrity is related to memory impairment (Yassa, Muftuler, et al., 2010; Yassa, Mattfeld, et al., 2011). Overall, these results provide strong cross-species support for the model proposed by Wilson and colleagues (2006).

There is also evidence for excitation/inhibition imbalances in the aging hippocampus. Data from Wilson and colleagues demonstrate that place cells in aged rodents’ CA3 region exhibit abnormally elevated firing rates (Wilson, Ikonen, Gallagher, et al., 2005). This is perhaps due to the loss of inhibition that occurs with age, which may reinforce the CA3 recurrent collateral network, simultaneously leading to hyperactivity and to a shift in the balance between excitation and inhibition (Hasselmo, 2006; Vela, Gutierrez, Vitorica, & Ruano, 2003), which may then lead to a shift in computational processing from pattern separation to pattern completion (Wilson et al., 2006). One important aspect of the hippocampal network are the numerous interneurons that are involved in inhibition, which is important for keeping the system from becoming over excited. Interneurons decline as a function of age in the hippocampus (Cadiacio, Milner,
Consistent with animal studies, human fMRI studies have also demonstrated similar evidence of hippocampal hyperactivity. Using a face-name association task, elevated activity in the hippocampus was found in low-performing older adults (Miller et al., 2008). More recently, high-resolution fMRI was used to specifically test whether the DG/CA3 selectively expressed such hyperactivity. Using the object mnemonic discrimination task, Yassa and colleagues found that older adults exhibited elevated activation levels selectively in the DG/CA3 region during task performance, the extent of which predicted memory impairment (Yassa, Lacy, et al., 2011). Evidence of a more dramatic version of this hyperactivity was also reported in individuals with MCI, a prodrome for Alzheimer’s disease (AD) (Yassa, Stark, et al., 2010). Across species, the data suggest that hyperactivity is an index of network dysfunction and disinhibition and not evidence of adaptive compensation. A recent clinical trial used a low-dose of levetiracetam (LEV), an anti-epileptic drug, in individuals with MCI to try and reduce the hyperactivity occurring in the DG/CA3 subfield. LEV successfully reduced DG/CA3 hyperactivity and reversed deficits on the object mnemonic discrimination task (Bakker et al., 2012). Hippocampal hyperexcitability in aging and age-related disorders is a generally reversible condition in both animals and humans (Bakker et al., 2012; Bakker, Albert, Krauss, Speck, & Gallagher, 2015; Koh et al., 2014; Sanchez et al., 2012), which has immense translational potential for age-related memory loss and AD.
There are also many other changes to the hippocampal system in aging. A reduction in DG neurogenesis in aging has been reported (Jessberger & Gage, 2008; Warner-Schmidt & Duman, 2006). While neurogenesis may contribute to DG function, neurogenesis alone is not sufficient to preserve function during normal aging (Galvan & Jin, 2007). Furthermore, while neurogenesis clearly declines in aged animals, the extent to which it is linked to age-related memory deficits is unclear (e.g. Bizon & Gallagher, 2003). There is also reduced neurotrophic support in the aging hippocampus (Smith, 1996), where decreases in brain-derived neurotrophic factor (BDNF) in the hippocampus have been hypothesized to contribute to memory impairment in aging (Silhol, Bonnichon, Rage, & Tapia-Arancibia, 2005; Tapia-Arancibia, Aliaga, Silhol, & Arancibia, 2008). There are often changes in glucocorticoid levels in the aging hippocampus, in which an excess of glucocorticoids may be at least a partial cause of hippocampal alterations seen in aging (McEwen, 1999; Nichols, Zieba, & Bye, 2001; Porter & Landfield, 1998; Sapolsky, Krey, & McEwen, 1986). The threshold for LTP induction is increased in aged rats, potentially making it more difficult for aged rats to encode a memory, while the threshold for long-term depression (LTD) induction is decreased, potentially making it easier for aged rats to erase a memory (Burke & Barnes, 2006; Foster, 1999). Aging is associated with decreases in modulatory inputs to the hippocampus including acetylcholine (Drachman & Leavitt, 1974; Gallagher & Colombo, 1995), dopamine (Bäckman et al., 2006; Chowdhury, Guitart-Masip, Bunzeck, Dolan, & Duzel, 2012; Fearnley & Lees, 1991), and norepinephrine (Kubanis & Zornetzer, 1981; Sternberg, Martinez, Gold, & McGaugh, 1985), which could alter the balance between excitation and inhibition of hippocampal subfields. IEG expression
such as Arc is also altered in the hippocampus with age and can interfere with memory processing (Fletcher et al., 2014; Marrone, Satvat, Shaner, Worley, & Barnes, 2012).

Beyond the hippocampus, there are also age-related changes in the regions surrounding the hippocampus. The PRC is among the brain structures vulnerable to the process of normal aging (Burke, Hartzell, Lister, Hoang, & Barnes, 2012; Liu, Jing, & Zhang, 2009; Liu, Smith, & Darlington, 2008; Moyer, Furtak, McGann, & Brown, 2011). Studies in humans using DTI, which measures white matter integrity, have found changes in the PHC in normal aging (Jahng et al., 2011). A reduction of reelin expression in the LEC was found in aged rats with memory impairment, which was not the case for the medial entorhinal (MEC) (Stranahan, Haberman, & Gallagher, 2011). In humans, EC volumes were measured in healthy older adults using structural MRI acquired twice, 5 years apart, and it was found that longitudinal shrinkage of the EC was associated with reduced memory performance (Rodrique & Raz, 2004). The EC has also been implicated in the early stages of AD and is one of the first sites of degeneration (Gómez-Isla et al., 1996). A recent study used high-resolution fMRI to map metabolic deficits in patients and mouse models of AD in the EC. Findings suggest that it is the LEC rather than MEC that is most susceptible to tau pathology early in AD, where aberrations begin in the LEC and spread to other cortical sites (Khan et al., 2014).

In general these neurobiological alterations contribute to a condition in the aging hippocampus that alters its computational balance such that there is neural rigidity.
where there is an age-related requirement for higher levels of dissimilarity across experiences for pattern separation to be successful. Data are consistent with a conceptual model in which the relationship between interference and performance is largely logarithmic in young adults and more linear in older adults (Fig 1.4, left).

The amygdala and its relationship with the hippocampus is also altered in aging. In normal aging, there is a reduction in amygdala volume of about 4% compared to young adults, however, loss may be specific to the central nucleus of the amygdala/cortical nuclei of the amygdala (CEA/CORT) and not to the BLA (Whalen & Phelps, 2009). A reduction of noradrenergic input to the hippocampus (Kubanis & Zornetzer, 1981) as well as changes in peripheral epinephrine levels (Sternberg et al., 1985) have been reported in animal models of aging. Age-related alterations in synaptic plasticity in the amygdala-hippocampal network have also been reported (Almaguer, Estupiñán, Uwe Frey, & Bergado, 2002). St Jacques and colleagues (2009) suggested that an age-related reduction in the contribution of amygdala-hippocampal mechanisms may be compensated by enhanced contribution of amygdala-prefrontal mechanisms to the formation of emotional memories (Murty et al., 2009; St Jacques, Dolcos, et al., 2009). Furthermore, evidence of the positivity bias in older adults is also evident in amygdala activity, where older adults show greater activity in the amygdala when viewing positive images compared to young adults (Mather et al., 2004).

*Increased variability in age-related memory impairment*
Increased variability in age-related memory impairment has been shown in both rats and humans (Gallagher et al., 2006; Hilborn, Strauss, Hultsch, & Hunter, 2009; Robitsek et al., 2008). Gallagher and colleagues have examined this increased variability in memory performance by dichotomizing older rats into two groups (Gallagher et al., 1993). One group of older rats performs on par with young rats on the Morris Water Maze (AU), while another group performs below young rat performance (AI). They have found that these two groups show differences in medial temporal lobe functioning, where AI rats show synaptic loss in the perforant path (Smith et al., 2000), loss of inhibitory tone (Spiegel, Koh, Vogt, Rapp, & Gallagher, 2013), hyperexcitability in the CA3 subregion of the hippocampus (Wilson, Ikonen, Gallagher, et al., 2005; Wilson et al., 2004), as well as loss of reelin and increased phosphorylated tau expression in the lateral entorhinal cortex (Stranahan et al., 2011). In humans, similar approaches have been applied. When dividing aged participants into AU and AI groups based on the RAVLT, AI individuals show deficits in the ability to discriminate highly similar experiences (Holden, Toner, Pirogovsky, Kirwan, & Gilbert, 2013; Reagh et al., 2014; Stark et al., 2010). Individual differences in age-related memory impairment has been associated with perforant path loss (Yassa, Muftuler, et al., 2010), hyperactivation in the dentate/CA3 network (Yassa, Lacy, et al., 2011), loss of entorhinal-hippocampal functional connectivity, and reduced dendritic integrity in the dentate/CA3 region (Yassa, Mattfeld, et al., 2011).

**MCI and AD**
Based on a survey of the neuroimaging literature in aging, MCI, and AD, we proposed a preliminary model to understanding MTL structural and functional changes along the continuum from aging to MCI to AD. Many of the features in this model are on a quantitative, rather than a qualitative scale, suggesting that there are key features that change very early in the course of the disease and which can be targeted for diagnosis and intervention. The hypothetical model is shown in Figure 1.5, and extends several models recently hypothesized in the literature (e.g. Ewers et al. 2011; Sperling et al. 2011) with more specific emphasis on neurobiological features in the MTL network.

We suggest that during the preclinical stage, the biomarker signals undergoing the most change are hippocampal hyperactivity and perforant path loss, with the structural signals in the EC the hippocampus being much more difficult to detect as frank neurodegeneration has not yet occurred. During the MCI stage, hippocampal hyperactivity reaches its peak, coupled with a considerable decline in the PP. Decline in this pathway is hypothesized to have retrograde effects (loss of entorhinal cortical neurons leading to cortical thinning) and anterograde effects (loss of neurons in the hippocampus leading to volume decline). Given converging reports from autopsy and MRI, we further suggest that the retrograde effect on the entorhinal cortex precedes and predicts the later change to the hippocampus. Since AD pathology tends to accumulate first in the transentorhinal region (Braak stage I), we suggest that the impact of synaptic loss in the perforant path may manifest first in the entorhinal cortex by interacting with hyperphosphorylated tau pathology in this region.
In MCI, the most sensitive biomarkers (those exhibiting the largest change) will vary as a function of how far along individuals are in the disease process. If individuals are earlier in that spectrum, then hippocampal hyperactivity and perforant path loss will be the most salient (with detectable but smaller changes in EC thickness and hippocampal volume). If individuals are farther along in that spectrum, hippocampal hyperactivity will have diminished, thus making this biomarker less useful. Using structural features such as perforant path integrity, entorhinal cortical thickness, and hippocampal volume as longitudinal markers of decline may be more robust against individual variations in progression. In AD, the PP is completely deteriorated, thus biomarker change here may not be salient; however, structural assays of neurodegeneration, i.e. hippocampal volume and EC thickness, will likely be the most salient. It is worthy of note that although hippocampal activity may also be a salient feature here (lower magnitude in AD), functional markers are most helpful in the absence of structural change (i.e. in the preclinical and early MCI phase).

Figure 1.5 represents our current understanding of how different regions of the MTL change from healthy aging to MCI to AD, but it is important to note that it is a work in progress and much still remains to be understood. For example, the individual contributions of each of the hippocampal subfields is not entirely understood, although we suggest that the DG and CA3 are especially vulnerable earlier on, with the vulnerability extending to the CA1 and SUB later on in the disease. Furthermore, other subregions of the MTL such as the PRC and the PHC have not received as much attention in the literature on neurocognitive aging and the pathophysiology of AD,
although they are undoubtedly implicated by virtue of pathology accumulation and network connectivity. Finally, the relationship between these biomarkers and other indices of brain dysfunction is not well understood. Future studies focused on investigating connectivity changes within and across brain networks with focus on the medial temporal lobe and posterior parietal networks will significantly inform this understanding.

Figure 1.5. Summary model for hypothesized changes in the MTL in the preclinical phase (asymptomatic), MCI, and AD. Functional activity in the hippocampus increases during the preclinical stage and continues to do so during the MCI stage, then declines towards the end of the MCI stage and more so in AD. The earliest structural decline occurs in the perforant path (measured using DTI), followed by entorhinal cortical thinning, followed by hippocampal volume loss, with each of these features independently predicting episodic memory deficits and disease decline. See text for discussion (from Leal & Yassa, 2013).

Late-life depression

Depression later in life has serious consequences, including patient and caregiver distress and increased mortality due to high suicide rates in the elderly (Reynolds & Kupfer, 1999). Distinctions have also been made in comparing early- versus late-onset depression, such that early-onset depression is associated with greater sensitivity of
depressive symptoms while late-onset depression is associated with more severe
cognitive and neurological changes (Sachs-Ericsson et al., 2013). Depression in late life
is frequently comorbid with other physical and psychiatric conditions, especially in the
oldest old (Blazer, 2003). Reduced hippocampal volumes have been found in older
adults with depression, which was associated with deficits in visual and verbal memory
performance (Hickie et al., 2005). There is also reduced amygdala volume in depressed
older adults compared to non-depressed older adults (Burke et al., 2011). Elderly
depressed patients with cognitive impairment may experience improvement in some
domains following anti-depressant treatment but may not necessarily reach normal
levels of performance, particularly in memory and executive functions (Butters et al.,
2000). Understanding the interaction between aging and depression and their
influences on MTL function together is important determining proper treatment in late-
life depression. Older adults may experience memory deficits due to age-related
memory impairment, due to depression, or both. Determining the source of memory
impairment will allow for more directed treatment options.

Chapter 2: Asymmetric effects of emotion on mnemonic
discrimination

Introduction
Remembering significant experiences such as those with emotional content is important for future behavior. Gist versus detail trade-offs reported in the literature suggest that emotion’s influence on memory is asymmetrical such that memory for emotional gist is enhanced while memory for emotional detail is impaired, however, the mechanism by which this occurs is unknown. The current investigation offers an alternative conceptual framework of the gist versus detail trade-off reported in human emotional memory studies. We examined the effect of emotion on mnemonic discrimination by systematically manipulating both emotional content (negative, neutral, positive) and stimulus similarity (high and low). Memory tests were conducted both immediately and after a 24-hour delay to examine effects of consolidation. Based on the gist versus detail trade-offs reported previously, we hypothesized that emotional target recognition would be enhanced while emotional lure discrimination would be impaired relative to neutral memory performance. Stimulus similarity was manipulated to create highly interfering test stimuli, with the high and low similarity conditions expected to alter performance on the task, where highly similar items should be more difficult to discriminate than low similarity items. We did not have any strong hypotheses for whether these similarity conditions would vary with emotion.

**Materials and Methods**

**Participants.** Participants were recruited from Johns Hopkins University and received either course credit or monetary remuneration for their participation for the primary experiments. In the immediate condition, 24 participants were tested (mean age ± SD,
21 ± 3, 16 female). In the 24-hour delayed condition, 14 participants were tested (20 ± 2, 6 female). Supplementary experiments required additional participants (demographics listed in respective sections). Informed consent was obtained from all participants, with all procedures approved by the Johns Hopkins University Institutional Review Board.

Inclusion/exclusion criteria. All participants were screened against self-reported major medical, psychiatric, and substance use comorbidity. All participants had normal or corrected to normal vision.

Valence, arousal, and similarity measures. An independent sample (N=50, all means ± SD, 22 ± 5, 32 female) rated the images for emotional valence on a scale 1-9 (1 being the most negative, 9 being the most positive, and 5 being neutral). Images rated 1-3.5 were called Negative, images rated 3.6-6 were called Neutral, and images rated 6.1-9 were called Positive [all Tukey HSD $P < .001$; Fig. S2.1a]. Another sample (N=16, 23 ± 5, 4 female) rated the images for emotional arousal on a scale 1-9 (1 being the least arousing, 9 being the most arousing). Negative and positive images received higher arousal ratings than neutral, although negative images were more arousing than positive images [all Tukey HSD $P < .001$; Fig. S2.1b]. A third sample (N=17, 20 ± 1, 11 female) was used to examine relative similarity of negative, neutral, and positive images as measured by number of false alarms/total responses in a separate sample. Here, we found a significant difference between the two similarity bins ($t(98) = -4.68$, $P < .001$) when stimuli were collapsed across all emotional categories as well as within each
emotional category [Negative $t(31) = -2.74$, $P = .01$; Neutral $t(30) = -2.77$, $P = .01$; Positive, $t(33) = -2.51$, $P = .02$; Fig. S2.1c]. See Appendix 1 for all Supplementary Information.

While we had assigned a priori similarity classes to these items (high similarity vs. low similarity) we determined that our assignment was valid by collecting subjective similarity ratings on pairs of stimuli presented side-by-side in a fourth sample (N= 31, 19 ± 1, 21 female). We compared subjective ratings on the two classes of stimuli using a two-sample t-test and found a significant difference between the two similarity bins ($t(97) = -4.95$, $P < .001$) when stimuli were collapsed across all emotional categories as well as within each emotional category (Negative $t(31) = -3.88$, $P = .001$; Neutral $t(30) = -2.10$, $P = .04$; Positive, $t(32) = -3.01$, $P = .01$). Together, these experiments showed that high and low similarity stimuli were both perceived subjectively by participants as such and influenced responses in the predicted manner. The stimulus set is available to use and download by request.

**Emotional mnemonic discrimination task.** The stimulus set was comprised of novel scenes freely available online, sized to a width of 600 pixels. As described above, images were rated for emotional valence, arousal, and similarity in orthogonal experiments with separate samples. The experimental paradigm consisted of 149 images during the encoding phase (divided roughly equally between negative, neutral, and positive) and 291 images during the retrieval phase (divided roughly equally between negative, neutral, and positive stimuli). Targets, lures, and foils were roughly
evenly distributed across emotion and similarity level during retrieval. Lures were divided into 50% high similarity and 50% low similarity.

An Apple iMac equipped with MATLAB (Version R2010a, Natick, MA) software and PsychToolbox version 3.0 was used to present the stimuli and record keyboard responses. Each trial consisted of 2 displays: an image display and a fixation display. During both encoding and retrieval phases, images were presented on the center of the screen with a black background for 2500 ms. The fixation display consisted of a white fixation cross on the center of the screen with a black background for 500 ms.

Participants underwent an incidental encoding phase where they were shown emotional and non-emotional images, presented in randomized order, and were asked to rate the images for emotional valence using a 1-9 scale (1 being most negative, 9 being most positive, and 5 being neutral). Participants were told to spread their responses across the scale. Participants were given a subsequent surprise memory test either immediately after encoding or 24 hours later, in which they saw another series of stimuli, some of which were seen once before in the incidental task (targets), some were similar to ones seen in the incidental task but not identical (lures), and some were new (foils) (Fig. 2.1). Some of the lures were very similar (high similarity) to the original images and some were less similar (low similarity) classified as such based on supplementary studies in a separate sample (Fig. S2.1c). Participants were asked to indicate whether items were “old” or “new” by button responses on the keyboard.
Participants were explicitly told that in order for an image to be called “old,” it had to be the exact same image they saw before.

Figure 2.1. Emotional mnemonic discrimination task. During encoding, participants rated images according to their emotional valence from 1 (most negative) to 9 (most positive). Each image was presented for 2500 ms with a 500 ms inter-stimulus-interval (ISI). Either immediately after study or after a 24-hour delay, participants underwent a surprise recognition test where they viewed negative, neutral, and positive targets, foils, and lures varying in similarity and were asked to indicate whether items were “old” or “new”.

Our two key outcome measures of interest were target recognition (d’) and lure discrimination index (LDI). Target recognition was measured by a discriminability index, which was calculated as $z($Hits$) – z($False Alarms$). Hits and false alarms refer to correct recognition of old items and false recognition of new items, respectively. D’ was calculated as the difference of z-transformed values. In order to measure how well participants discriminated similar items (lures), we examined performance using a bias-
corrected LDI operationalized as $p(\text{New}|\text{Lure}) - p(\text{New}|\text{Target})$. This corrected for the general tendency to reject (i.e. call an item ‘New’) and is similar to other metrics we used in prior work (Yassa, Lacy, et al., 2011; Yassa, Stark, et al., 2010).

**Match-to-sample control task.** A separate sample of 37 participants was used for this control task. Two conditions were tested: 2500 ms presentation time and 1000 ms presentation time (2500 ms: $N = 18$, 22 ± 3, 12 female, 1000 ms: $N = 19$, 21 ± 3, 13 female). The match-to-sample task consisted of trials that were composed of 4 sequential displays: first image, a pixelated noise mask display, a second image, and an inter-trial fixation display. Images were identical to those used in the discrimination experiments. Participants were told to determine whether the two images were exactly the same or different. Yoked images were either identical (repetitions) or similar (lures).

Each participant was tested in a single testing room in which the experimenter familiarized all participants with the task by providing oral and written instructions. Each trial began with the presentation of an image for 2500 ms or 1000 ms followed by the pixelated screen for 1000 ms (same across both presentation times), and then the second image for the same time as presented initially. This was followed by the fixation display (500 ms). Participants were told to respond while the second image was presented. Responses were recorded by keyboard press.

**Results**
Reduced discrimination of similar emotional items immediately after encoding

To assess the effect of emotion on target recognition, we used a repeated-measures one-way ANOVA (negative, neutral, and positive), which revealed that d’ differed significantly among positive, negative, and neutral stimuli \(F(2,46) = 6.35, P < .01\). Post-hoc contrasts showed that d’ was enhanced in negative and neutral compared to positive stimuli \(F(1,46) = 22.52, P < .01\), critical Scheffé=6.40; Fig. 2.2a]. To assess the effect of emotion on overall discrimination of similar items (i.e. lures collapsed across high and low similarity), we conducted another repeated measures ANOVA, which revealed that LDI differed significantly among negative, neutral, and positive stimuli \(F(2,46) = 9.65, P < .001\]. Post-hoc contrasts revealed that LDI was diminished for emotional stimuli (both positive and negative) compared to neutral stimuli \(F(1,46) = 18.20, P < .001\), critical Scheffé =6.40] (Fig. 2.2b]. In order to assess the potential interaction between emotion and interference (i.e. lure similarity), we conducted a 2x2 ANOVA, which revealed a significant effect of emotion \(F(2,46) = 9.81, P < .001\] as well as a significant effect of similarity level in which low similarity lures were easier to discriminate compared to high similarity lures \(F(1,23) = 70.8, P < .001\] (Fig. S2.2a). Since there were no interactions with similarity, we decided to collapse across high and low similarity for all analyses (Fig. S2.2).
Figure 2.2. Performance in immediate and 24-hour delayed testing (a) Target recognition (d’) was significantly better in negative and neutral compared to positive images in the immediate condition (N=24). d’ overall was worse after 24 hours, however, the difference was larger in the neutral images than in the emotional images (N=14); (b) Lure Discrimination Index (LDI) was significantly worse in negative and positive compared to neutral images in the immediate condition. LDI overall was also worse after 24 hours, however, the magnitude of the difference was larger in the emotional images than the neutral images. See statistics in corresponding tables.

Emotional modulation not due to attention or perceptual effects

A potential interpretation of the data above is that the emotional effect is secondary to a shift in attentional focus and not necessarily due to a mnemonic process. For example, participants may not have perceptually encoded all of the details of the emotional images during the encoding phase and this lack of attention to detail may have affected subsequent memory performance. Consistent with this idea, Mather and Sutherland (Mather & Sutherland, 2011) recently proposed that arousal during an event can either enhance or impair memory for events, depending on attentional factors that bias competition in favor of high priority stimuli.
To examine this possibility, we tested 37 new participants on a match-to-sample (MTS) task using the same stimuli. We measured target hit rate and lure rejection rate and found no significant differences across negative, neutral, and positive items for 2500 ms (Fig. S2.3a) and 1000 ms (Fig. S2.3b). This suggests that while attention may play a role in emotional processing, it did not significantly contribute to the effects observed here. A related possibility is that encoding and consolidation mechanisms interact so that emotionally arousing items are differentially processed during encoding, in such a manner that their long-term consolidation is also altered (Hamann, 2001).

**Preserved emotional target recognition after 24 hours**

Previous studies have shown that the enhancement of emotional memories tends to be greater after a delay (Eysenck, 1976; Heuer & Reisberg, 1990; Kleinsmith & Kaplan, 1963; LaBar & Phelps, 1998; Sharot & Phelps, 2004). This delayed enhancement may be due to endogenous norepinephrine release during a narrow time window after encoding (i.e. during consolidation) that may enhance the strength of memories (Buchanan & Lovallo, 2001; McGaugh, 2002; McIntyre et al., 2002; Segal et al., 2012). We tested this hypothesis in a separate sample (N=14) that completed the same emotional discrimination task, but performed the memory test after a 24-hour delay. We measured target recognition and LDI in these participants and compared their performance to the group tested immediately using an emotion * time of testing ANOVA. After a delay, overall target recognition was worse compared to immediate testing \[ F(1,36) = 13.61, P = .001 \], as expected. There was a main effect of emotion \[ F(2,72) = \]
8.30, \( P = .001 \) as well as an interaction between emotion and time of testing \( [F(2,72) = 3.20, \ P = .047] \) such that memory for emotional stimuli was preserved over time (i.e. less forgetting), whereas target recognition for neutral items was impaired (i.e. more forgetting) \( [F(1,72) = 6.39, \ P < .05, \ \text{critical Scheffé} = 6.24; \ \text{Fig. 2.2a}] \).

**Impaired emotional lure discrimination after 24 hours**

Similar to \( d' \), LDI was worse after a 24-hour delay compared to immediate testing \( [F(1,36) = 46.78, \ P < .001] \), consistent with forgetting. There was a main effect of emotion \( [F(2,72) = 23.43, \ P < .001] \) as well as an interaction between emotion and time of testing \( [F(2,72) = 3.45, \ P = .04] \). However, the nature of the interaction was opposite of that observed with \( d' \). Lure discrimination differences were greater for emotional stimuli compared to neutral stimuli over time \( [F(1,72) = 6.28, \ P < .05, \ \text{critical Scheffé} = 6.24; \ \text{Fig. 2.2b}] \) such that there was more forgetting of emotional details after 24 hours compared to neutral details.

We also analyzed the effect of similarity level across immediate and delay tested groups using a emotion * similarity * time of testing ANOVA and found a main effect of similarity (as found in the immediate study) \( [F(1,36) = 125.61, \ P = <.001; \ \text{Fig. S2.2a,b}] \). There was no significant interaction between similarity and time of testing and no significant three-way interaction between emotion, similarity, and time of testing.
Gender differences were examined in detail in all experiments and we did not find any significant gender differences or interactions with gender in any of our analyses (SI Text and Fig. S2.4). It is important to note that since our studies were not powered to detect gender differences, these results should not be taken as evidence for the absence of such differences. Prior studies have indeed noted gender differences on emotional memory tasks (Cahill, 2006). Complete analyses of reaction time (RT) data are also shown in supplementary materials (SI Text and Fig. S2.5).

Discussion

Emotions have long been known to play a role in the persistence of memories (James, 1884; McGaugh, 2013). The goal of this study was to examine the relationship between emotion and the minimization of interference that is necessary for encoding unique conjunctive representations, a putative function of the hippocampal circuit. The emotional mnemonic discrimination task allowed us to investigate this interaction at a cognitive level and revealed a potential mechanistic basis for emotion’s asymmetric effects on memory. We suggest that emotion results in a preservation of gist information (thought to rely on pattern completion) and a loss of detail information (thought to rely on both pattern completion and pattern separation, i.e. “recall to reject”).

Overall, our results suggest that emotion’s effects on memory are magnified after a 24-hour delay, consistent with a role in consolidation (McGaugh, 2004; Payne, Stickgold, Swanberg, & Kensinger, 2008). For target recognition, emotional items were preserved
from forgetting whereas neutral items were more likely to be forgotten, consistent with prior work (Kensinger, 2009; LaBar & Phelps, 1998). However, lure discrimination showed the opposite pattern after a 24-hour delay, where emotional stimuli (in which performance was impaired in the immediate condition) were even more likely to be forgotten after 24 hours. It appears that emotion plays at least two distinct roles in modulating memory strength: 1) an impairment of detail-based discrimination (taxing pattern separation) when tested immediately, and 2) a selective retention of gist information and forgetting of detail information over a 24-hour period (presumably due to an effect on consolidation). Interestingly, we did not see selective retention of gist information for positive information when tested immediately. Our positive stimuli were not rated as arousing as the negative stimuli (Fig. S2.1b), which may be playing a role in the reduced memory for positive targets. Valence-based effects on memory specificity have been shown in the past (Kensinger, Garoff-Eaton, & Schacter, 2006). In addition, while we saw better performance for low similarity lures compared to high similarity lures, we did not see any interactions between similarity and emotion.

The emotional modulation effect reported here may be an adaptive mechanism, in which only the central and/or salient features of events (i.e. the gist) are strengthened while the peripheral and/or non-salient features (i.e. the details) are weakened. The latter weakening allows for the flexible generalization of gist information to novel situations, which may be required for survival behaviors (e.g. fight or flight). While emotion-induced gist versus detail trade-offs have been studied in the past (Kensinger & Schacter, 2007; Kensinger, 2009; Mather & Sutherland, 2011), the mechanisms
underlying these behavioral effects have remained elusive. Viewing this trade-off as a shift in the balance of computational functions of the hippocampus (perhaps via modulation by the amygdala) provides a potential neurobiological context.

The BLA in particular is thought to be a major modulator of the hippocampus. The BLA projects to the hippocampus via multiple routes, including indirect connections through the EC as well as direct connections to CA3, CA1, and subiculum (Petrovich et al., 2001; Pitkänen et al., 2000). These connections are thought to modulate the strength of emotional memories (LeDoux, 2007; McGaugh, 2004). More specifically, stress hormones influence memory consolidation via neuromodulatory interactions with the BLA (i.e. norepinephrine or glucocorticoids) modulating the strength of memory for aversive or appetitive events (McGaugh, 2002). Also, it is worth noting that prior work in animals has suggested that the amygdala is sensitive to interference based on reward value (Gilbert & Kesner, 2002). We suggest that discriminating highly interfering emotional information may be supported by amygdala-hippocampal interactions such that the amygdala input to the hippocampus may bias the system away from pattern separation and towards pattern completion.

It is possible that the effect of norepinephrine is two-fold: a state-wide effect that enhances arousal and vigilance in stressful situations, as well as a transient effect that allocates resources to processing individual stimuli and their respective emotional value. We suspect that the enhanced vigilance state induced by norepinephrine would result in better overall encoding, thus explaining why in our prior work emotional arousal was
associated with enhanced subsequent discrimination performance on neutral items (Segal et al., 2012). In the current experiment, however, brief stimuli were used to trigger arousal and thus the current manipulation may have been more sensitive to norepinephrine’s transient effects. The latter may explain the apparent difference in the results between the two studies. Also, the prior study used object stimuli and not rich scene stimuli, which allow for detailed investigations of gist and detail information, thus subtle mnemonic effects could have been obscured.

Paradigms that test mnemonic discrimination offer a robust empirical framework by which hippocampal function can be assessed (Hunsaker & Kesner, 2013). Indeed, much work has already been done using this framework including the assessment of changes in neurocognitive aging (Stark et al., 2010; Toner et al., 2009; Yassa, Lacy, et al., 2011; Yassa & Stark, 2011), mild cognitive impairment (Yassa, Stark, et al., 2010), perforant path degradation (Yassa, Muftuler, et al., 2010; Yassa, Mattfeld, et al., 2011), and neurogenesis loss of function (Clelland et al., 2009) as well as gain of function (Sahay et al., 2011). In human high-resolution fMRI studies, behavior on discrimination tasks has been specifically associated with pattern separation signals in the hippocampal DG and CA3 (Yassa, Lacy, et al., 2011) as well as the integrity of the perforant path input to the hippocampus from the entorhinal cortex (Yassa, Muftuler, et al., 2010). Here, we extended the pattern separation framework to investigate the impact of emotional modulation on hippocampal memory. Manipulating of the similarity of lure stimuli allows us to examine a potential cognitive correlate of hippocampal pattern separation (Yassa & Stark, 2011).
Limitations of the study include sample sizes that were too small to thoroughly investigate gender differences, which have been demonstrated in prior studies of emotional memory (Larry Cahill, Gorski, Belcher, & Huynh, 2004; Nielsen, Ahmed, & Cahill, 2013). This absence of evidence should not be taken as evidence of absence and we realize that there are likely gender differences here that need to be considered in future experiments. Also, we used naturalistic stimuli and not computer-generated, controlled morphs, thus specific features (e.g. orientation, color, etc.) were quite variable. It is possible that future studies with more controlled stimuli can be used to examine mnemonic asymmetry for emotional items in more detail by directly manipulating individual aspects of the images.

In conclusion, our study suggests a novel mechanistic account by which emotional stimuli can have asymmetrical effects on long-term memory and may alter hippocampal pattern separation. Although a large body of research has investigated the role of emotion on making memories stronger, none have attempted to use a mnemonic discrimination task sensitive to hippocampal pattern separation to investigate the specific role of the amygdala on the computations of subfields of the hippocampus. The emotional mnemonic discrimination task may offer a window into how emotional arousal may alter pattern separation computations in service of episodic memory ultimately to promote survival. Equipped with a better understanding of hippocampal dynamics and a more detailed assessment of the cognitive effects of emotion on memory, future studies
can investigate the specific relationship between amygdala-hippocampal connectivity and pattern separation in emotional contexts.

Chapter 3: Pattern separation of emotional information in hippocampal dentate and CA3

Introduction

We have developed a task that is sensitive to emotional modulation of memories with high interference and gives us the power to investigate the influence of emotion on hippocampal pattern separation. Thus far, mnemonic discrimination tasks have been used to investigate hippocampal pattern separation of neutral objects. In the current study, we utilized high-resolution fMRI during performance of the emotional mnemonic discrimination task to test hypotheses about specific subfields of the hippocampus and their involvement in the pattern separation of emotional stimuli. We hypothesized that the DG/CA3 subfield would show pattern separation signals during both negative and neutral item discrimination, but would be more strongly modulated by negative items. We also hypothesized that the amygdala would be modulated by emotion, as seen in previous studies. The current investigation offers an alternative conceptual framework by which to examine the impact of emotion on hippocampal computations.

Materials and Methods
Participants. Eighteen healthy participants (N=18) were recruited from Johns Hopkins University and received $40 for their participation. Informed consent was obtained from all participants, with all procedures approved by the Johns Hopkins University Institutional Review Board. Demographic data and neuropsychological results are shown in Table 3.1.

Table 3.1. Participant Demographics and Neuropsychological Test Results

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Healthy young adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>18</td>
</tr>
<tr>
<td>M:F</td>
<td>9:9</td>
</tr>
<tr>
<td>Variables</td>
<td>Mean, SEM</td>
</tr>
<tr>
<td>Age</td>
<td>21.2, 0.9</td>
</tr>
<tr>
<td>Beck Depression Inventory-II</td>
<td>3.2, 0.6</td>
</tr>
<tr>
<td>RAVLT Immediate Recall</td>
<td>12.4, 0.5</td>
</tr>
<tr>
<td>RAVLT Delayed Recall</td>
<td>11.8, 0.7</td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>11.9, 0.5</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>8.3, 0.4</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>29.1, 0.3</td>
</tr>
<tr>
<td>Trail Making Test A</td>
<td>19.4, 1.3</td>
</tr>
<tr>
<td>Trail Making Test B</td>
<td>46.9, 2.3</td>
</tr>
</tbody>
</table>

Inclusion/exclusion criteria. All participants were screened against major medical or psychiatric morbidities, substance abuse history, as well with the additional exclusion criteria of MRI contraindications such as the presence of metal in the body. All participants were right-handed with normal or corrected to normal vision.

Imaging data collection. Functional MRI data were collected using a 3-Tesla Philips scanner equipped with a SENSE head coil using both higher-order shims and SENSE imaging techniques. Functional images were collected using a high-speed EPI single-
shot pulse sequence (1.5 mm isotropic resolution, 19 oblique axial slices parallel to the principal axis of the hippocampus, field of view = 96 x 96 mm, flip angle = 70°, SENSE parallel reduction factor = 2, TR/TE = 1500/30 ms, matrix size = 64 x 64).

We additionally collected a novel ultrahigh-resolution structural MPRAGE scan that we developed for accurate delineation of hippocampal subfields and high-resolution diffeomorphic alignment (0.55 mm isotropic resolution; 273 sagittal slices, field of view = 240 x 240 mm, flip angle = 9°, TR/TE = 13/5.9 ms, matrix size = 448 x 448, inversion pulse TI=1110 ms). SENSE parallel imaging was used in two directions (2x1.5). The SAR (<10%) and PNS (<75%) were within required limits based on the scanner-calculated values. Total scan time for the volume was 7m 51s. These scans were also used to create a group template, which was used as the standardized space for alignment of participant data before group analyses.

**Neuropsychological battery.** The battery was designed to examine memory function, as well as other aspects of general cognition. The assessment included the following: (1) Mini-Mental State Exam (MMSE) to assess global cognitive status, (2) RAVLT to assess verbal learning, immediate and delayed recall, and recognition, (3) Digit Span backwards and forwards to assess working memory, (4) Trail Making Tests A and B to assess attention, visual search, and mental processing speed, and (5) Beck Depression Inventory-II (BDI-II) to assess depressive symptoms. This battery was given on the day of the scan, 30 minutes prior to the scanning session. Results are shown in Table 3.1.
**Emotional mnemonic discrimination task.** Please refer to the Materials and Methods in Chapter 2 for details on the task. One minor change to the task was the number of response options during encoding. The imaging version of the task limited emotional valence ratings to a three-response option of negative, neutral, or positive.

**Image analysis.** All data analyses were conducted using Analysis of Functional NeuroImages (AFNI – RRID:nif-0000-00259) (Cox, 1996). Images were corrected for slice timing and subject motion. Time points in which significant motion events occurred (movement exceeded 3 degrees of rotation or 2 mm of translation in any direction relative to prior acquisition ± 1 time point) were censored from further analyses. Functional images were then co-registered to the structural scans acquired in the same session using AFNI’s 3dAllineate algorithm. Structural scans were aligned to a common template based on the entire sample using Advanced Normalization Tools (ANTs – RRID:nlx_75959) (Avants et al., 2011) which uses a powerful diffeomorphic algorithm (SyN) (Klein et al., 2009) to warp individual participants into the template space. The transformation parameters were then applied to the co-planar functional data.

Behavioral vectors based on trial type (classified according to emotion, similarity, and behavioral decision) were used to model the data using a deconvolution approach based on multiple linear regression. The resultant fit coefficients (betas) estimated activity versus an implicit baseline (novel foils) for a given time point and trial type in a voxel. The sum of the fit coefficients over the expected hemodynamic response (3-12 s
after trial onset) was taken as the model’s estimate of the response to each trial type (relative to baseline).

**Extracting region of interest (ROI) voxels.** We selected voxels for subsequent analyses based on combining the voxels that changed with any of the task conditions in the healthy sample with anatomical ROIs. Active voxels were selected based on the overall F, agnostic to specific condition or contrast so as not to bias subsequent analyses and remove concerns regarding circularity and double-dipping (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009). This served to remove voxels that did not respond to any of the task conditions so that the analyses can be more sensitive to subtle changes across conditions.

This voxel mask was then combined with anatomical ROI masks that were based on manual delineations of the subfields and regions of interest on the common template. Amygdala segmentation was based on our prior published protocol (Yassa, Hazlett, Stark, & Hoehn-Saric, 2012). Briefly, we segmented the amygdala on axial slices using the hippocampal uncus and temporal horn of the lateral ventricle as the posterior boundary in superior slices and using the hippocampus itself as the posterior boundary in inferior slices. The lateral boundary was defined by an arbitrary line drawn from the most medial white matter to the lateral fissure excluding gray matter medial to this line. The medial boundary was set by the hippocampal uncus in anterior slices and by white matter in the posterior slices.
Hippocampal subfield segmentation was also based on prior work (Yassa, Lacy, et al., 2011), which is defined according to the atlas of Duvernoy (Duvernoy, 2005). The subfields are defined on eight coronal slices along the anterior–posterior axis of the hippocampus. Representative slices in each hippocampus that best (closest) resembled the slices described were chosen and segmented according to the atlas description. The segmentation then proceeded from these slices in both directions slice by slice to ensure a smooth transition across slices. The ROI masks for both the amygdala and hippocampal subfields are shown in Fig 3.1a,b for reference. Voxel betas from the resulting hybrid functional/structural ROIs were averaged and all subsequent statistical analyses were conducted on these averages.

**Statistical analyses.** All statistical analyses of behavioral variables and ROI activation means were conducted in SPSS v. 20.0 (IBM Corp., released 2011, Armonk, NY). Planned comparisons were conducted using additional F-tests or t-tests. Post hoc statistical tests were corrected for multiple comparisons using Scheffé’s correction, with critical F values indicated in the text corresponding to the degrees of freedom (df) of the F-test (mentioned only once for each pair of df’s). All tests used the General Linear Model (ANOVA and correlations). Normality assumptions were investigated using Kolmogorov-Smirnov tests and all distributions investigated did not significantly deviate from the normal distribution. Repeated measures tests were corrected for error nonsphericity using Greenhouse-Geisser correction where appropriate. Statistical values were considered significant at a final corrected alpha level of .05, which appropriately controlled for Type I error.
Results

High-resolution fMRI of emotional pattern separation

We analyzed cognitive performance in healthy participants and found a significant effect of emotion \( [F(2,34) = 4.30, P = .024] \) during correct rejections of highly similar lures. There were fewer correct rejections of negative and positive lures compared to neutral \( [F(1,34) = 10.45, P < .01, \text{ critical Scheffé} = 6.54] \). This replicates our prior work (Leal, Tighe, & Yassa, 2014). Raw performance data (accuracy and reaction times) are shown in Table 3.2 and Table S3.1, respectively. There were no gender differences in behavioral performance or fMRI activation. See Appendix 2 for all Supplementary Information.

Next, we utilized high-resolution fMRI to test hypotheses about specific subfields of the hippocampus and their involvement in the pattern separation of emotional stimuli. We focused our analysis on the specific conditions where we could test hypotheses about emotional pattern separation. Thus, our analyses compared the following conditions: 1) retrieval trials where highly similar lures were presented, since these trials were hypothesized to maximize interference and 2) comparison of negative and neutral items (excluding positive items, as these items were not matched to negative items for arousal (Leal, Tighe, & Yassa, 2014). We analyzed ROI activity in hippocampal DG/CA3, CA1,
subiculum, and the amygdala (Fig 3.1a). All data across all ROI's and conditions are in Fig S3.1.

Table 3.2. Accuracy (Proportion Correct) on Emotional Mnemonic Discrimination Task

<table>
<thead>
<tr>
<th>Measures</th>
<th>Mean, SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative lure CR</td>
<td>0.60, 0.05</td>
</tr>
<tr>
<td>Neutral lure CR</td>
<td>0.73, 0.03</td>
</tr>
<tr>
<td>Positive lure CR</td>
<td>0.58, 0.05</td>
</tr>
<tr>
<td>Negative target hits</td>
<td>0.93, 0.02</td>
</tr>
<tr>
<td>Neutral target hits</td>
<td>0.91, 0.02</td>
</tr>
<tr>
<td>Positive target hits</td>
<td>0.93, 0.02</td>
</tr>
<tr>
<td>Negative foil CR</td>
<td>0.97, 0.02</td>
</tr>
<tr>
<td>Neutral foil CR</td>
<td>0.98, 0.01</td>
</tr>
<tr>
<td>Positive foil CR</td>
<td>0.97, 0.01</td>
</tr>
</tbody>
</table>

We operationally define “emotional pattern separation” signals in the context of this experiment as meeting two directional criteria: (1) activity in a particular ROI was higher during lure correct rejections (CR) than lure false alarms (FA), i.e. showing a pattern separation signal (Yassa, Lacy, et al., 2011; Yassa, Stark, et al., 2010); and (2) activity during lure CR’s was higher for negative compared to neutral stimuli (i.e. showing an emotional modulation signal). Thus, in order to determine if specific ROI’s met these criteria, we ran a 2 x 2 ANOVA with memory (lure CR and lure FA) and emotion (negative and neutral) as factors in each ROI. If the region showed a pattern separation signal, we then performed paired comparisons within memory condition to determine if an emotional modulation signal exists (second criterion of emotional modulation signals). The baseline condition consisted of all novel items (i.e. foils).
Pattern separation of emotional information in DG/CA3

In hippocampal DG/CA3 we observed a significant main effect of memory (lure CR > lure FA) \[F(1,17) = 5.93, P = .026\] (Fig 3.1c), which met our criterion for a pattern separation signal. There was greater activity when correctly rejecting a lure compared to falsely recognizing a lure. Furthermore, on trials where lures were correctly rejected, we observed a trend towards an emotional modulation signal, where there was greater activity when negative items were correctly rejected compared to neutral items \[t(17) = 1.76, P = .09\]. While we focused our analysis on highly similar items here, low similarity lures showed the same pattern (Fig S3.1). Thus, we ran a second ANOVA including all trials (collapsing across similarity bins) to increase power and found a significant main effect of memory \[F(1,17) = 12.33, P = .003\] as well as a marginal effect of emotion \[F(1,17) = 4.03, P = .06\]. The planned post-hoc comparison within memory condition revealed an emotional modulation effect specific to lure CR’s \[t(17) = 2.62, P = .018\]. Hippocampal DG/CA3 showed a pattern separation signal (lure CR > lure FA) and an emotional modulation effect specific to lure CR’s across both high and low similarity items. Together, these data suggest that DG/CA3 is involved in pattern separation of emotional stimuli and is involved in resolving emotional interference irrespective of stimulus similarity level. This is consistent with prior work (Lacy et al., 2011) showing that DG/CA3 activity is similar across low and high similarity bins suggesting that it is sensitive to even minor distortions in input (i.e. high interference).
Figure 3.1. Region of interest activity profiles during highly similar lure trials. a) Region of interest (ROI’s) segmentations of the amygdala, DG/CA3, CA1, and subiculum on a custom group template, (b) ROI locations shown along the longitudinal axis of the medial temporal lobe, c) Mean activity beta weights during correct rejection (CR) and false alarms (FA) of highly similar lures in hippocampal DG/CA3, which shows an emotional pattern separation signal (CR>FA and Negative>Neutral in CR), d) Mean activity beta weights during CR and FA of highly similar lures in the amygdala, which show only an emotional modulation signal (Negative>Neutral), e) Mean activity beta weights during CR and FA of highly similar lures in hippocampal CA1, which shows only a marginal pattern separation signal. Error bars are ± S.E.M.

Emotional modulation in amygdala regardless of memory performance

We performed the same analysis in the amygdala, where we observed a significant main effect of emotion (negative > neutral) [$F(1,17) = 5.08, P = .038$] (Fig 3.1d), thus meeting our criterion for an emotional modulation signal. There was greater activity for negative items compared to neutral. There was no main effect of memory and no interaction between emotion and memory ($P > .05$). This suggests that the amygdala is involved in emotional modulation, regardless of memory performance.
In the CA1 subfield of the hippocampus, we observed a marginal effect of memory (lure CR > lure FA) \([F(1,17) = 4.35, P = .052]\) (Fig 3.1e), suggesting the CA1 subfield may also show pattern separation signals. There was no main effect of emotion and no interaction between emotion and memory (\(P > .05\)). Thus, CA1 does not seem to be modulated by emotion but may reflect a downstream pattern separation signal from DG/CA3.

**Discussion**

Past fMRI studies in humans have shown that activity in the amygdala and the hippocampus is correlated during encoding (Kensinger & Corkin, 2004) and retrieval (Kensinger & Schacter, 2007) of emotional information. Furthermore, amygdala-hippocampal functional connectivity predicts enhanced later recall of emotional memories (St Jacques, Bessette-Symons, & Cabeza, 2009). The amygdala is promiscuous in influencing the consolidation of memory for many different kinds of motivationally arousing training experiences (McGaugh, 2002, 2004). This is thought to occur through the amygdala’s ability to modulate hippocampal representations. The BLA projects both directly and indirectly to different subfields of the hippocampus (Petrovich et al., 2001; Pitkänen et al., 2000), which may allow specific influences of the amygdala on particular hippocampal subfields.
Neuroimaging studies on amygdala-hippocampal dynamics have been unable to determine differential contributions of the hippocampal subfields in processing emotional information. More recent studies have used high-resolution fMRI and found similar pattern separation signals using neutral objects (Bakker et al., 2008). The current study is the first to show that the DG/CA3 regions of the hippocampus are involved in pattern separation of emotional stimuli. Using an emotional mnemonic discrimination task, we were able to manipulate emotional valence and similarity of stimuli to investigate the amygdala-hippocampal network's involvement in the pattern separation of emotional information at high-resolution. We observed engagement of hippocampal DG/CA3 during accurate discrimination of similar items (i.e. pattern separation signal). Furthermore, we observed an emotional modulation of this signal (negative > neutral) specific to trials on which participants accurately discriminated similar emotional items. Although the data from high similarity items alone showed a trend towards an emotional modulation effect, increasing our power by analyzing all similar items (both high and low) resulted in a significant emotional modulation signal specific to lure correct rejections. This suggests that the DG/CA3 is using emotional information in order to discriminate similar experiences and is capable of resolving emotional interference irrespective of stimulus similarity level. The amygdala was also modulated by emotion; however, this signal manifested regardless of the accuracy of discrimination. While the amygdala generally responds to highly similar emotional stimuli, the DG/CA3 selectively exhibits emotional modulation when attempting to differentiate interfering information. Absent the accurate discrimination response, the DG/CA3 does not reflect such emotional modulation, suggesting that the amygdala
modulation may be an essential signal when faced with remembering highly interfering emotional experiences.

The underlying mechanism for these effects is still unknown. We do not know how emotional information is processed preferentially by one region versus another in addition to why we see emotion-induced memory trade-offs where emotional gist information is enhanced and detail information is impaired. This finding replicates what we recently reported in Leal et al. (2014) using the same task in an orthogonal sample. Discrimination memory for emotional details is impaired relative to neutral information. This may be specific to a discrimination design, which specifically taxes memory for details. We have shown recently that discrimination and generalization are not synonymous with recollection and familiarity (Kim & Yassa, 2013) and that the two dimensions can be orthogonalized. In addition, the current study does not speak to how emotional memories change over time, in which studies have shown the largest effects of emotion’s influence on memory after a longer delay (Kensinger, 2009). Consolidation of emotional memories after sleep has been shown to increase the likelihood that certain pieces of an experience are stabilized in memory (Payne & Kensinger, 2010), which may play a role in remembering emotional versus neutral items.

Future studies investigating the functional connectivity between the amygdala and hippocampal subfields will be important to determine if connectivity between these regions changes as a function of the task. The computational descriptions of hippocampal function offer a potential mechanistic account by which information storage
may be modulated (i.e. either by enhancing pattern separation or pattern completion).
Paradigms that tax pattern separation offer a robust empirical framework by which
hippocampal function can be assessed.

This study had several limitations that should be noted. While the amygdala responds to
both positive and negative valence, arousal is a major component of amygdala
processing. Since our positive stimuli were not as arousing as negative stimuli, we
opted not to include positive trials in our fMRI analyses. Emotional processing of
positive stimuli is an important facet in the modulation of memory (Hamann et al., 1999)
that, if better understood, may be helpful in reversing the negativity bias found in patient
populations with mood disorders. We did not examine this in detail in our study, but
future experiments should address the role of positive emotions in more detail. In
addition, we limited our analyses to high similarity lure items since these are expected
to rely on pattern separation. Analysis of low similarity stimuli could be interesting as
well, although different neural mechanisms may be at play when interference is not
maximized. One other potential limitation is the correlational nature of fMRI studies,
which limits our interpretations in terms of pattern separation computations. Our
approach in this study was to come up with a set of criteria for an operational definition
for what we termed “emotional pattern separation” but we should caution against strong
interpretations of this concept. Convergent evidence in animal studies using
neurophysiological recordings from hippocampal cells in DG and CA3 during tasks that
manipulate valence will be necessary.
Although we found no significant gender differences in either behavioral performance or fMRI activation, our sample sizes were too small to fully appreciate gender differences. This absence of evidence should not be taken as evidence of absence and we realize that there are likely gender differences here that need to be considered in future experiments. Emotional arousal influences the fidelity by which memories are stored. The experiments reported here provide a novel account of how emotional modulation of memory may occur in the context of resolving interference. We propose that the amygdala and hippocampal DG/CA3 are key players in this process, and that these experiments highlight the complexities of emotional modulation of memory and help tease apart the nuances underlying the neural mechanisms of amygdala-hippocampal interactions.

Chapter 4: Emotional pattern separation in depression

Effect of emotion on mnemonic discrimination in individuals with depressive symptoms

Introduction Part I

Depression is a neuropsychiatric phenotype involving a disturbance in emotional memory processing. Many studies of depression have documented general deficits in episodic memory (Airaksinen et al., 2007; Airaksinen et al., 2006; Dere et al., 2010). In addition, depressed individuals tend to better remember negative items compared to
neutral or positive items (Gordon et al., 2008; Haas & Canli, 2008; Hasler et al., 2004; Watkins et al., 1996; Watkins et al., 2000). Furthermore, a recent study has suggested that individuals with depressive symptoms show impairment on an object mnemonic discrimination task (Shelton & Kirwan, 2013). We tested the utility of the emotional mnemonic discrimination task in picking up on subtle cognitive changes in emotional memory processing in individuals with depressive symptoms. We hypothesized that individuals with depressive symptoms would 1) show general episodic memory deficits for neutral scenes and 2) show a bias towards discriminating negative scenes.

Materials and Methods Part I

Participants. All participants performed the emotional mnemonic discrimination task described in Chapter 2. Data from individuals with depressive symptoms (N = 15, mean age 22 ± 4SD, 11 female, DS+) was compared to the healthy young adults with no depressive symptoms (DS-) from Chapter 2. Participants with DS were recruited through local campus announcements and posted flyers. See additional Materials and Methods in Chapter 2.

Inclusion/exclusion criteria. The BDI-II was given to all participants. Assignment to DS+ versus DS- group was based on BDI-II cutoff (BDI-II < 7 = DS- group, BDI-II > 15 = DS+ group). These cutoff criteria were based on the BDI-II symptom severity scale in which 16 is the cutoff for a mild mood disturbance (scores above 16 are suggestive of clinical depression; the higher the score indicates greater severity of DS) (Watkins et al., 2000). Participants did not receive a diagnostic psychiatric evaluation as part of this
study and were medication-free. All participants had normal or corrected to normal vision.

Results Part I

Impaired neutral recognition in depression

We conducted an emotion x group ANOVA of target recognition performance, which revealed a main effect of emotion \( F(2,74) = 6.92, P = .002 \) and a significant interaction between emotion and group \( F(2,74) = 4.19, P = .02 \). Participants with depressive symptoms displayed worse target recognition for neutral items compared to healthy controls \( F(1,74) = 9.21, P < .05 \), critical Scheffé = 6.24; Fig. 4.1a]. Recognition of emotional items was not significantly different among groups.

Fig. 4.1. Performance in young adults with (DS+) and without depressive symptoms (DS-) (a) \( d' \) was significantly impaired in the DS+ group compared to DS- only in the neutral condition but not in the emotional conditions (N=15); (b) LDI was significantly impaired in the neutral condition and enhanced in the negative condition in the DS+ group relative to the DS- group; (c) Positive correlation between depressive symptoms quantified by the BDI-II and LDI specifically on negative items (N=31).
Impaired neutral discrimination but enhanced negative discrimination in depression

Next, we conducted an ANOVA of emotion x group with LDI as the dependent measure. While there were no main effects of either factor, we found a significant interaction [F(2,74) = 5.99, \( P = .004 \)]. Compared to the DS- group, the DS+ group showed an impairment in discrimination of neutral lures [F(1,74) = 10.65, \( P < .05 \), critical Scheffé = 6.24] and an enhancement in discrimination of negative lures [F(1,74) = 12.55, \( P < .05 \), critical Scheffé = 6.24; Fig. 4.1b]. There was no significant difference between groups in lure discrimination of positive items. We also repeated the same analyses but included similarity as a factor and found no interactions with between group and similarity, although the main effect of similarity was present, as in previous experiments [F(1,37) = 106.48, \( P < .001 \)] (Fig. S2.2c). Performance measures in participants with DS stratified by gender are shown in Fig. S2.4c. RT data for participants with DS are shown in Fig. S2.5c. See Appendix 1 for Supplementary Information.

Finally, we tested whether severity of DS (measured by the BDI-II) was associated with negative lure discrimination performance. We expanded our sample to include individuals showing any DS (BDI-II > 0), which increased our final sample size to 31 participants for this analysis. We found a robust positive correlation between BDI-II symptom severity and negative lure discrimination index [Pearson’s \( r = .50, P = .005 \); Fig. 4.1c], consistent with the notion that enhanced memory for negative experiences is a core endophenotype of depression that becomes more exaggerated as DS increase.
We also tested individuals with DS after a 24-hour delay. Please refer to Appendix 3 for results.

**Discussion Part I**

While past studies have observed a negativity bias in depression, the interpretation has traditionally been that this is due to an overgeneralization of negative information (Fulford, Rosen, Johnson, & Carver, 2011). However, results from our paradigm offer an alternative account. In individuals with DS, discrimination of similar neutral items was impaired, consistent with recently reported results using an object discrimination task (Shelton & Kirwan, 2013). At the same time, discrimination of similar negative items was enhanced, and the degree of such enhancement was correlated with symptom severity. It is possible that this emphasis on negative details is associated with the mood dysregulation that is characteristic of depression. Overemphasizing negative details can come at the cost of processing neutral or positive information, and thus may affect processing stimuli across a wide range of experiences. The aberration in negative discrimination sheds new light on the negativity bias phenomenon and highlights the value of using this paradigm in the future to examine amygdala-hippocampal interactions in major depression to fully understand the nature of emotional memory abnormalities in the disorder.

Another potential mechanism by which individuals with depression show abnormalities in remembering emotional stimuli is attention. The capture of attention by stimuli previously associated with reward has been demonstrated across a wide range of
However, individuals with depressive symptomology do not show the same attentional capture to previously rewarded items. Individuals experiencing depressive symptoms largely ignore previously high-value stimuli, suggesting that such stimuli are less attention-grabbing in depression. In addition, the severity of depressive symptoms (as measured using the BDI-II) was negatively correlated with the magnitude of value-driven attentional capture (Anderson, Leal, Hall, Yassa, & Yantis, 2014). This sharp contrast in performance indicates that value-based attentional biases depend on the normal functioning of the brain's reward system and suggests that a failure to preferentially attend to reward-related information may play a role in the experience of depression. These data suggest individuals with depression may attend to emotional stimuli differently than healthy young adults, which may explain some of the biases we see towards remembering more details of negative stimuli. If you are not distracted by the emotionality of the negative items and spend more time processing the details, you could potentially remember more details later on.

A limitation of the study is that participants with DS did not receive a formal psychiatric evaluation or a diagnosis of depression. Thus, it is unknown whether the depressive symptoms reported are due to MDD or perhaps another etiology such as anxiety, which is highly comorbid with depression. Future work should attempt to extend the use of this task to a group of depressed participants with a confirmed diagnosis of major depression according to the Diagnostic and Statistical Manual. In conclusion, this paradigm may also offer a novel tool to assess aberrations in emotional memory,
perhaps offering a deeper understanding of abnormal emotional mnemonic processing associated with disorders with an abnormal mood component.

Hippocampal pattern separation of emotional information in individuals with depressive symptoms

Introduction Part II

Cross-species studies have shown amygdala-hippocampal alterations in depression and in chronically stressed rodents. Reduced hippocampal volume, possibility due to subtle MTL changes such as retraction of CA3 dendrites and reduced DG neurogenesis, as well as increases in amygdala structure and function have been found (Sheline et al., 2001; Sheline, 2011; Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000). We sought to investigate how depressive symptoms impact amygdala and hippocampal function by using the emotional mnemonic discrimination task. We utilized high-resolution fMRI to test hypotheses about hippocampal pattern separation of emotional stimuli in individuals with DS. We hypothesized that the hippocampal-amygdala network would be altered in depression such that the amygdala may be overactive and exert more of an influence on hippocampal functioning, yielding better negative discrimination performance. Furthermore, we hypothesized that the DG/CA3 subregion of the hippocampus may be selectively affected, as we see an enhancement in negative discrimination.

Materials and Methods Part II
**Participants.** Ten participants experiencing symptoms of depression (DS+ group: N=10) were recruited *(please refer to Chapter 3 for further details)*. The DS+ group showed moderate to severe depressive symptoms according to the BDI-II (score >10; average BDI-II = 23 ± 8.8 SD). We used participants from Chapter 3 as controls with little to no depressive symptoms (DS- group: average BDI-II = 3.22 ± 2.56 SD). Participant demographics and neuropsychological testing results are shown in Table 4.1. All data across all ROI’s and conditions are in Fig S4.1 *(Appendix 4)*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DS-</th>
<th>DS+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>M:F</td>
<td>9:9</td>
<td>3:7</td>
</tr>
</tbody>
</table>

Table 4.1. Participant Demographics and Neuropsychological Test Results

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean, SEM</th>
<th>Mean, SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21.2, 0.9</td>
<td>20.4, 0.6</td>
</tr>
<tr>
<td>Beck Depression Inventory-II</td>
<td>3.2, 0.6</td>
<td>23.2, 2.8</td>
</tr>
<tr>
<td>RAVLT Immediate Recall</td>
<td>12.4, 0.5</td>
<td>13.4, 0.6</td>
</tr>
<tr>
<td>RAVLT Delayed Recall</td>
<td>11.8, 0.7</td>
<td>13.0, 0.6</td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>11.9, 0.5</td>
<td>12.3, 0.6</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>8.3, 0.4</td>
<td>8.7, 0.8</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>29.1, 0.3</td>
<td>29.1, 0.4</td>
</tr>
<tr>
<td>Trail Making Test A</td>
<td>19.4, 1.3</td>
<td>19.3, 1.6</td>
</tr>
<tr>
<td>Trail Making Test B</td>
<td>46.9, 2.3</td>
<td>40.1, 4.5</td>
</tr>
</tbody>
</table>

**Inclusion/exclusion criteria.** All participants were screened against major medical or psychiatric morbidities, substance abuse history, as well with the additional exclusion criteria of MRI contraindications such as the presence of metal in the body. For participants in the DS+ group, a depression diagnosis was an inclusion criterion but lack of such a diagnosis was not used to exclude participants. All healthy participants had a
BDI-II score of 10 or below, while anyone in the DS+ group received a BDI-II score above 11. These cutoff criteria were based on the BDI-II symptom severity scale in which 1-10 is considered within normal limits. All participants included in the DS+ group were medication-free. All participants were right-handed with normal or corrected to normal vision.

**Neuroimaging analysis.** Image analysis, extraction of ROI voxels, and statistical analyses were identical to the procedure described in *Chapter 3*.

**Results Part II**

**Emotional pattern separation alterations in individuals with depressive symptoms**

We used a 2 x 3 ANOVA with group (DS+, DS-) and emotion (negative, positive, and neutral) to assess behavioral differences. Consistent with our prior work (Leal, Tighe, & Yassa, 2014), there was a significant interaction \[F(2,52) = 3.28, P = .046\] between emotion and group. A post-hoc contrast showed that the DS+ group was better than DS- group at discriminating emotional lures \[F(1,52) = 5.36, P < .05, \text{critical Scheffé} = 4.03\]. Raw performance data are shown in Table 4.2 and Table S4.1. *See Appendix 4 for Supplementary Information.*
Next, we analyzed negative and neutral retrieval trials for highly similar lure CR’s for each ROI. We conducted an ANOVA of DG/CA3 regional activation during lure CR’s and found an emotion x group interaction [F(1,26) = 6.35, P = .018; Fig 4.2a] but no significant main effect of emotion or group (P > .05). Post-hoc contrasts confirmed the interaction was driven by a reversal of the emotional modulation signal in DG/CA3, where DS- individuals showed increased activity on negative versus neutral lure discrimination trials while DS+ individuals showed increased activity on neutral compared to negative lure discrimination trials [F(1,26) = 8.10, P < .05; critical Scheffé = 4.23]. We conducted an ANOVA of amygdala regional activation during lure CR’s, which revealed a significant main effect of emotion [F(1,26) = 6.16, P = .02; Fig 4.2b], but no main effect of group or interaction between emotion and group (P > .05). Thus, both DS+ and DS- groups showed an emotional modulation signal in the amygdala. We conducted an ANOVA on CA1 regional activation during lure CR’s and found no significant effects (all P’s > .05; Fig S3.1 and S4.1).

Table 4.2. Accuracy (Proportion Correct) on Emotional Mnemonic Discrimination Task

<table>
<thead>
<tr>
<th>Measures</th>
<th>DS- Mean, SEM</th>
<th>DS+ Mean, SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative lure CR</td>
<td>0.60, 0.05</td>
<td>0.70, 0.06</td>
</tr>
<tr>
<td>Neutral lure CR</td>
<td>0.73, 0.03</td>
<td>0.68, 0.04</td>
</tr>
<tr>
<td>Positive lure CR</td>
<td>0.58, 0.05</td>
<td>0.75, 0.05</td>
</tr>
<tr>
<td>Negative target hits</td>
<td>0.93, 0.02</td>
<td>0.96, 0.02</td>
</tr>
<tr>
<td>Neutral target hits</td>
<td>0.91, 0.02</td>
<td>0.95, 0.02</td>
</tr>
<tr>
<td>Positive target hits</td>
<td>0.93, 0.02</td>
<td>0.96, 0.02</td>
</tr>
<tr>
<td>Negative foil CR</td>
<td>0.97, 0.02</td>
<td>0.98, 0.01</td>
</tr>
<tr>
<td>Neutral foil CR</td>
<td>0.98, 0.01</td>
<td>0.97, 0.02</td>
</tr>
<tr>
<td>Positive foil CR</td>
<td>0.97, 0.01</td>
<td>0.98, 0.01</td>
</tr>
</tbody>
</table>
To examine the relationship between the amygdala and hippocampal DG/CA3 during correct lure discrimination, we conducted a 3-way ANOVA with emotion (negative, neutral), group (DS+, DS-), and region (amygdala and DG/CA3) during lure CR's. Here, we observed a significant emotion x region interaction \([F(1,26) = 4.59, P = .04]\) as well as significant emotion x region x group interaction \([F(1,26) = 7.87, P = .009]\) (Fig 4.2a,b). The interaction between emotion and region was driven by the emotional modulation effect in the amygdala, where negative activity was greater than neutral activity across groups \([F(1,26) = 6.7, P < .05]\). A post-hoc contrast showed that the three-way interaction was driven by the reversal in DG/CA3 where DS- participants showed greater activity for negative compared to neutral items and DS+ participants showed greater activity for neutral compared to negative items, while the amygdala

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**Figure 4.2. Amygdala-hippocampal network alterations with depressive symptoms.** a) Mean activity beta weights during correct rejection (CR) of highly similar lures in hippocampal DG/CA3, which shows decreased DG/CA3 activity in DS+ compared to DS- during negative lures, b) Mean activity beta weights during CR of highly similar lures in the amygdala, which shows increased activity in the DS+ group during negative lure CR’s, c) Negative correlation between depressive symptom severity and DG/CA3 beta weight during negative lure CR’s. See corresponding statistics.
showed greater activity for negative compared to neutral items across groups \[F(1,26) = 12.27, P < .05].

Furthermore, to investigate the relationship between the amygdala and DG/CA3 specifically during correct negative lure discrimination, we conducted an ANOVA with group (DS+, DS-) and region (DG/CA3, amygdala) and found a significant group x region interaction \[F(1,26) = 7.49, P = .011\], where a post hoc contrast showed that DS-individuals had increased DG/CA3 activity relative to DS+, while DS+ individuals had increased amygdala activity compared to DS- \[F(1,26) = 8.08, P < .05\]. We did not observe any significant effects for neutral lure discrimination (all P’s > .05).

We next examined whether activity patterns in the DG/CA3 were related to DS severity. We included all individuals with BDI-II scores of above zero (N = 25). We observed a significant negative correlation between activity in the DG/CA3 region during negative lure correct rejections and BDI-II scores \[Pearson r = -0.44, P = .026; Fig 4.2c\], suggesting that DS severity was linearly associated with decreased activity in this hippocampal subfield. There was no correlation between amygdala activity and DS.

**Discussion Part II**

Clinical disorders such as depression, anxiety, and AD all display alterations in mood and memory, in which a subset of their cognitive and behavioral symptoms are produced by alterations in the medial temporal lobes. To determine if our emotional mnemonic discrimination task may provide a sensitive measure of MTL changes in
disorders of mood and memory, we tested a small sample of individuals experiencing depressive symptoms on the emotional discrimination task. We have previously found cognitive alterations in the emotional discrimination task in an orthogonal sample of individuals with depressive symptoms (Leal, Tighe, & Yassa, 2014).

We found the emotional mnemonic discrimination task to be sensitive to subtle neurobiological changes occurring in the human MTL, where amygdala and hippocampal DG/CA3 activity tends to decouple in individuals experiencing depressive symptoms. The magnitude of DG/CA3 activity during negative discrimination was inversely correlated with depressive symptoms. This suggests that enhanced processing of negative information may be due to a network imbalance between the amygdala and the DG/CA3 where the influence of the amygdala is increased and the influence of the DG/CA3 is decreased. Given the behavioral results, the most parsimonious explanation of the DG/CA3 diminished signal and the heightened amygdala activity is that the amygdala’s activity may have enhanced processing of negative information that facilitated discrimination in the absence of the normal DG/CA3 response. Thus, we surmise that a discrimination behavioral response can be the result of either effective pattern separation in the DG/CA3 or enhanced processing by the amygdala. Whether this effect is driven by impairment in the amygdala, DG, CA3, or all of the regions is still unknown, although data from animal studies suggest that structural changes in the CA3 may be critical (Conrad et al., 1996, 1999; Vyas et al., 2002; Watanabe et al., 1992). Reductions in DG neurogenesis may also contribute to this effect (Dranovsky & Hen, 2006). Also, we should note that our analyses used
diffeomorphically-aligned images, which control for any structural differences between groups, thus our results are unlikely to arise from volumetric differences. Our results demonstrate the power of the emotional pattern separation framework and its utility for examining memory and mood disturbances.

Additionally, we should note that while our sample of individuals with depressive symptoms was small, we were clearly powered to detect effects both in the DG/CA3 and the amygdala, further demonstrating the capabilities of our high-resolution imaging approach coupled with targeted task design. In addition, using a continuous measure of depressive symptoms resulted in an increased sample size for our correlational analyses. While our data in those with depressive symptoms are suggestive, future studies with larger samples including depression diagnosis, information regarding the history of depression, number of depressive episodes, and treatment status are required to bridge this work to translational applications. We propose that the amygdala and hippocampal DG/CA3 are key players in this process and that aberrations in this network might manifest in the context of mood disorders. The pattern separation framework is a powerful approach to investigate the impact of emotional modulation on hippocampal memory in disorders of mood and memory, such as depression.

Chapter 5: Emotional pattern separation in normal aging and subclinical memory impairment
Effect of aging on mnemonic discrimination of emotional information

Introduction Part I

Episodic memory loss is one of the hallmarks of age-related cognitive decline and a major symptom of AD. However, whether emotional memories are preserved with age or if these memories are just as susceptible to loss and forgetting is not well understood. In aging, some studies have suggested that there is a “positivity effect,” where older adults may be more likely to attend to positive information in the environment (Mather & Carstensen, 2005; Wong et al., 2012). In contrast, some have suggested that memory for detailed information in older adults reveals no such positivity effect and may actually be biased toward remembering negative details (Kensinger, Garoff-Eaton, & Schacter, 2007). This suggests that the relationship between aging and emotional modulation of memory is complex and requires a more thorough investigation. We have shown that emotion alters how highly interfering memories are stored using an emotional mnemonic discrimination task. Here, we extend this work to testing older adults at two time points (immediately after encoding and 24-hours later) to examine differences in emotional mnemonic discrimination in aging.

Materials and Methods Part I

Participants. Participants were recruited from Johns Hopkins University as well as the local Baltimore community via local campus announcements, flyers, and ads in local newspapers. Participants were between the ages of 18-35 for the young adult groups
(same participants as in Chapter 2, please refer to Chapter 2 for Methods and Demographics) and 60-85 for the older adult groups. Older adults received monetary remuneration for their participation. The older adult group consisted of 22 participants that were tested immediately (mean age 67 SD 4, 14 female) and 16 participants that were tested 24 hours later (mean age 71 SD 8, 12 female). Informed consent was obtained from all participants, with all procedures approved by the Johns Hopkins University Institutional Review Board.

**Inclusion/exclusion criteria.** All participants were screened against major medical or psychiatric morbidities as well as substance abuse history. Older participants received a neuropsychological evaluation during their visit (*please refer to Chapter 3 for details*). We added the Geriatric Depression Scale (GDS) to assess depressive symptoms more specific to older adults (see Table 5.1 for results). There were no significant differences between the older adult groups in age, education, and all other neuropsychological measures. All participants had normal or corrected to normal vision.
Table 5.1. Older Participant Demographics and Neuropsychological Test Results

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean, SEM</th>
<th>Mean, SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>66.7, 0.9</td>
<td>70.6, 1.9</td>
</tr>
<tr>
<td>Education</td>
<td>16.0, 0.5</td>
<td>15.4, 0.7</td>
</tr>
<tr>
<td>Beck Depression Inventory-II</td>
<td>4.2, 0.5</td>
<td>5.1, 1.1</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>1.3, 0.2</td>
<td>1.4, 0.4</td>
</tr>
<tr>
<td>RAVLT Immediate Recall</td>
<td>10.3, 0.5</td>
<td>9.7, 0.8</td>
</tr>
<tr>
<td>RAVLT Delayed Recall</td>
<td>10.0, 0.6</td>
<td>9.4, 0.8</td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>12.5, 0.5</td>
<td>11.0, 0.6</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>8.3, 0.5</td>
<td>7.1, 0.5</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>28.6, 0.4</td>
<td>34.7, 3.7</td>
</tr>
<tr>
<td>Trail Making Test A</td>
<td>28.6, 2.1</td>
<td>34.7, 3.7</td>
</tr>
<tr>
<td>Trail Making Test B</td>
<td>69.8, 2.3</td>
<td>89.1, 10.4</td>
</tr>
</tbody>
</table>

**Emotional discrimination task.** Please refer to the task description in Chapter 2 for details. One difference is that for older adults we used a three-response option of negative, neutral, or positive so older adults wouldn’t have to keep track of 9 button responses during encoding. Raw response proportions and RT data for all experimental groups are in Supplementary Information (Appendix 5, Table S5.1 and Fig. S5.1).

**Match-to-sample task.** Please refer to task description in Chapter 2. Older adults were tested on this task to control for attention or perceptual effects (N = 13, 65 ± 6, 7 female). We compared performance to the young adult group from Chapter 2.

**Statistical analysis.** All statistical analyses were conducted in SPSS v. 20.0 (IBM Corp., released 2011, Armonk, NY). For immediate testing, 2-way repeated-measures
ANOVAs (age and emotion) were performed for target recognition and lure discrimination index (high and low similarity ANOVAs conducted separately). For 24-hour delay testing, 3-way repeated-measures ANOVAs (age, emotion, time of testing) were performed for target recognition and lure discrimination index (again, high and low similarity ANOVAs conducted separately). Forgetting rates were calculated post-hoc for visualization purposes only (i.e. no statistics were run on the forgetting rates). This was a between-subjects design, thus, forgetting rates were calculated on the group means (e.g. mean D’ (immediate) – mean D’ (delay) and not on individual subject scores). Repeated measures tests were corrected for error non-sphericity using Greenhouse-Geisser correction where appropriate. Post hoc statistical tests were corrected for multiple comparisons using Scheffé’s correction, with critical F values indicated in the text corresponding to the degrees of freedom (df) of the F-test (mentioned only once for each pair of df’s). Statistical values were considered significant at a final corrected alpha level of .05, which appropriately controlled for Type I error.

Results Part I

Impaired neutral but preserved emotional recognition in aging

We investigated the effect of emotion (negative, neutral, and positive) on target recognition when tested immediately across age groups (young and old) using a two-way ANOVA, which revealed a significant effect of emotion [F(2,88) = 5.3, P = .01], where negative targets were better remembered compared to neutral and positive
targets \(F(1,88) = 9.7\), critical Scheffé = 6.2\]. There was a significant effect of age where young adults performed better than older adults \(F(1,44) = 7.7, P = .01\). In addition, there was a significant interaction between emotion and age \(F(2,88) = 3.7, P = .04\). We parsed this interaction using a post-hoc contrast examining the effect of emotional valence (positive and negative vs. neutral) across groups (young vs. old). We found that older adults were impaired relative to young adults on neutral target recognition but were preserved on emotional (positive and negative) target recognition, on par with young adults \(F(1,88) = 6.7\), critical Scheffé = 6.2; Fig. 5.1a). We will refer to “emotional target recognition or lure discrimination” as comprised of both negative and positive stimuli unless otherwise noted.
Fig. 5.1. Performance in young and older adults. (a) d’ in the immediate condition; (b) Low similarity LDI in the immediate testing condition; (c) High similarity LDI in the immediate testing condition; (d) d’ in the 24-hour delay condition; (e) Low similarity LDI in the 24-hour delay condition; (f) High similarity LDI in the 24-hour delay condition; (g) d’ forgetting rate plotted as the difference between immediate target recognition [(d’(im)) and 24-hour delay target recognition [d’(24)]; (h) Low similarity LDI forgetting rate plotted as the difference between immediate lure discrimination [(LDI(im))] and 24-hour delay lure discrimination [LDI(24)]; (i) High similarity LDI forgetting rate plotted as the difference between LDI(im) and LDI(24). See main text for data interpretation. See corresponding statistics below the graphs.

Reversed emotional modulation of discrimination in aging

Next, we assessed the effect of emotion on discrimination of low similarity lures (LS-LDI) across age groups using a two-way ANOVA, which revealed a significant effect of
age, in which young adults performed better than old adults [F(1,44) = 47.6, P < .001].

We also found a significant interaction between emotion and age [F(2,88) = 4.5, P = .02]. We parsed this interaction using a post-hoc contrast examining the effect of emotional valence (positive and negative vs. neutral) across groups (young vs. old). We found that older adults show increased emotional versus neutral lure discrimination, while young adults show reduced emotional versus neutral lure discrimination [F(1,88) = 8.2, critical Scheffé = 6.2; Fig. 5.1b). While discrimination of emotional stimuli was better compared to neutral stimuli in older adults, it is important to note that even with a boost in memory performance for emotional items, older adults do not overcome the overall memory deficit.

We evaluated the effect of emotion on high similarity LDI (HS-LDI) across age groups using a two-way ANOVA, which revealed a significant effect of age [F(1,44) = 47.6, P < .001], where young adults performed better than older adults. There were no significant differences between age groups for emotion or an interaction between age and emotion. While young adults show a similar pattern of reduced emotional versus neutral lure discrimination, older adults show no differences in discrimination across highly similar emotional and non-emotional items (Fig. 5.1c).

**Emotional modulation not secondary to attention or perceptual effects**

A potential interpretation of the emotional modulation effects we report is that age-related changes in attentional focus or working memory capacity could influence
behavioral performance. For example, participants may not have perceptually encoded all of the details of the emotional images during the encoding phase and this lack of attention to detail may have affected subsequent memory performance. Consistent with this idea, Mather and Sutherland (2011) proposed that arousal during an event can either enhance or impair memory for events, depending on attentional factors that bias competition in favor of high priority stimuli (Mather & Sutherland, 2011).

To examine the possibility of extra-mnemonic influences on task performance, we tested 31 new participants (13 older adults) on a MTS task using the same stimuli that were used in the emotional mnemonic discrimination task. Participants were shown yoked pairs of similar or repeated images with a static noise mask in between and asked to determine if the images were the same or different. We measured target hit rate and lure rejection rate and found a main effect of age for target hit rate \( [F(1,29) = 6.88, P = .014; \text{Fig. S5.2a}] \), where older adults performed slightly better than young adults. There were no significant differences across age groups for lure rejection rate (all \( P \)'s > .05; Fig. S5.2b). This suggests that while attention may play a role in emotional processing, it did not significantly contribute to the effects observed here. A related possibility is that encoding and consolidation mechanisms interact so that emotionally arousing items are differentially processed during encoding, in such a manner that their long-term consolidation is also altered (Hamann, 2001).

**Increased forgetting of emotional targets after 24 hours in aging**
Next, we had separate groups of young and older adults perform the same task, but perform the surprise memory test 24 hours later. We investigated the effect of emotion, age, and time of testing (immediate vs. 24-hour delay) on target recognition using a three-way ANOVA, which revealed a significant effect of emotion \( [F(2,144) = 8.8, P < .001] \), where negative targets were better remembered compared to neutral and positive targets \( [F(1,144) = 14.3, \text{critical Scheffé} = 6.12] \). There was a significant effect of age where young adults performed better than older adults \( [F(1,72) = 49.8, P < .001] \). There was a significant effect of time of testing where immediate performance was better than after 24 hours \( [F(1,72) = 49.1, P < .001] \). In addition, there was a significant interaction between age and time of testing \( [F(1,72) = 10.8, P = .002] \). We parsed this interaction using a post-hoc contrast examining the effect of time of testing (immediate vs. delay) across groups (young vs. old). We found that old and young adults performed similarly on target recognition when tested immediately, while older adults had worse target recognition (i.e. more forgetting) after a 24-hour delay. Interestingly, we found a three-way interaction between time of testing, emotion, and group \( [F(2,144) = 3.5, P = .037] \). We performed a post-hoc contrast examining the effect of time of testing (immediate vs. delay) and emotional valence (negative and positive vs. neutral) across groups (young vs. old). We found that young adults exhibited less forgetting of emotional targets compared to neutral, while older adults exhibited more forgetting of emotional targets compared to neutral \( [F(1,144) = 6.8, P = .011; \text{Fig. 5.1d,g}] \).

**Increased false recognition of emotional lures after 24 hours across age groups**
We also assessed the effect of emotion, age, and time of testing for low similarity lure discrimination index using a three-way ANOVA, which revealed a significant effect of emotion \(F(2,144) = 3.2, P = .048\), where positive and negative lures were more poorly discriminated compared to neutral lures \(F(1,144) = 3.5, P < .05\). There was a significant effect of age, where young adults performed better than older adults \(F(1,72) = 69.9, P < .001\). There was a significant effect of time of testing, where performance was better when tested immediately versus at a 24 hour delay \(F(1,72) = 61.1, P < .001\). We also found a significant interaction between emotion and time of testing. We performed a post-hoc contrast examining the interaction between emotional valence (negative and positive vs. neutral) and time of testing (immediate vs. delay), where emotional lures were more falsely recognized over time compared to neutral lures across age groups \(F(1,144) = 6.0, P = .017\); Fig. 5.1e,h]. We also found a significant interaction between emotion and age \(F(2,144) = 9.3, P < .001\], where a post-hoc contrast of emotional valence across age groups showed that older adults have increased emotional versus neutral lure discrimination, while young adults have decreased emotional versus neutral lure discrimination \(F(1,144) = 18.0, P < .001\], suggesting that the aforementioned reversal of the emotional modulation of lure discrimination in older adults continues at a delay of 24 hours.

**Preserved discrimination of highly similar positive lures in aging**

We assessed the effect of emotion, age, and time of testing on high similarity LDI using a three-way ANOVA, which revealed a significant effect of emotion \(F(2,144) = 10.5, P\)
<.001], where discrimination of highly similar emotional lures was worse than neutral lure discrimination \[F(1,144) = 12.2, \ P = .001\]. There was a significant effect of age \[F(1,72) = 34.6, \ P < .001\], where young adults performed better than older adults. There was a significant effect of time of testing \[F(1,72) = 35.5, \ P < .001\], where performance was better when tested immediately versus after a 24 hour delay. Additionally, we found an emotion by age interaction \[F(2,144) = 3.1, \ P = .048\]. A post-hoc contrast of emotional valence across age groups revealed that young adults had worse discrimination on both positive and negative items relative to neutral. Older adults, on the other hand had worse discrimination only on negative items relative to neutral, but were preserved on positive items \[F(1,144) = 3.5, \ P < .05; \text{Fig} \ 5.1f,i\].

In order to determine if older adults show greater impairment when discriminating high similarity lures compared to low similarity lures, we conducted an additional ANOVA in older adults with emotion and similarity as within-subject factors and time as a between-subject factor and found a significant effect of similarity, where older adults were more impaired when discriminating high similarity lures compared to low similarity lures \[F(1,36) = 109.11, \ P < .001\].

Discussion Part I

While general impairment of episodic memory in age-related cognitive decline has been well established, it is not clear whether there are existing mechanisms that may allow older adults to compensate for this memory loss by altering modulatory systems in the brain. The current study examined whether there were age-related changes in the
emotional modulation of memory, specifically in the context of interference and pattern separation changes associated with aging. Since emotion’s influence on memory can occur during encoding and furthermore during consolidation, we tested separate groups of participants immediately after encoding and 24 hours later.

**Emotional modulation of memory encoding in aging**

When testing participants immediately after encoding, we found an overall enhancement in young adult performance compared to older adults in general recognition and discrimination. Older adults showed a preservation of emotional target recognition compared to young adults, but showed an impairment of neutral target recognition compared to young adults. This finding is consistent with previous findings that emotional memory may be preserved across the lifespan (Denburg et al., 2003; Kensinger, Krendl, & Corkin, 2006; Waring & Kensinger, 2009). While older adults show a general decline in episodic memory, memories tied to an emotional context are remembered with more fidelity. Although forgetting increases with age, these results and others suggest that there is a selective remembering of emotional experiences, serving to create lasting memories of our more important experiences.

Whereas older adults were impaired in discriminating similar items relative to young adults overall, the effect of emotion was reversed with age. Young adults were more likely to falsely recognize similar emotional lures than older adults. This effect was evident at least for the low similarity lure items. The shift in emotional modulation could
be due to at least two possible explanations: 1) a compensatory effect such that emotional arousal can boost discrimination performance on similar items and help increase memory for more important emotional events or 2) an aberration of emotional-mnemonic processing in older adults such that the boost in emotional discrimination is actually maladaptive, since it would be better to remember the gist for emotional events rather than the details. Young adults’ discrimination (ability to suppress false recognition) was enhanced on neutral items compared to emotional items presumably due to a trade-off between gist and detail. Thus, it may be more adaptive to forget minute details of emotional experiences in favor of retaining the bigger picture (Adolphs et al., 2001; Kensinger, 2009; Loftus et al., 1987). Older adults, on the other hand, appear to suppress false alarms better for emotional items, suggesting that they may be engaging in a more costly mnemonic operation without clear adaptive value.

**Emotional modulation of memory consolidation in aging**

We then conducted the study with a 24-hour delay between the study and test phase and compared immediate and delayed performance across groups. Young adults exhibited less forgetting of emotional targets compared to neutral after 24 hours, while older adults exhibited more forgetting of emotional targets after 24 hours. Thus, with the passage of time, emotional gist memory seems to be preserved in young adults and reduced in older adults. Initially, memories tied to an emotional context seem to be better remembered in older adults, but these memories may not undergo consolidation
to the same extent as in young adults. Over time, emotional arousal does not seem to provide an additional boost for remembering the same image seen before.

For stimuli that were somewhat similar but not identical to previously viewed stimuli (i.e. low similarity lures), false recognition was higher for emotional compared to neutral items across both age groups. This suggests that both young and older adults discriminate emotional and neutral information similarly after consolidation has occurred (i.e. both age groups falsely recognize more emotional versus neutral information over time). For stimuli that were very similar but not identical to previously viewed stimuli (i.e. high similarity lures), false recognition of negative items (but not neutral or positive items) was higher in older adults. The ability of older adults to recall the details of positive stimuli to correctly discriminate them from highly similar lures seems to be unaffected by age. This is consistent with the positivity bias reported in past literature (Mather & Carstensen, 2005). In addition, the effects we find on memory consolidation may be associated with changes in the sleep-wake cycle, where typical findings have shown that older adults have irregular sleep patterns (Buckley & Schatzberg, 2005), which may affect their ability to consolidate emotional memories (Payne & Kensinger, 2010).

**Potential mechanisms for shifts in emotional modulation of memory with age**

Here, we extended the mnemonic discrimination framework to investigate the impact of emotional modulation on hippocampal memory in aging. Although pattern separation
was not directly assessed here, our manipulation of the similarity of lure stimuli allows us to examine a potential behavioral correlate of hippocampal pattern separation (Yassa & Stark, 2011). We observed a shift in the balance of processing similar emotional information with age, which may be due to a shift in amygdala-hippocampal interactions. St Jacques and colleagues (2009) suggested that an age-related reduction in the contribution of amygdala-hippocampal mechanisms may be compensated by enhanced contribution of amygdala-prefrontal mechanisms to the formation of emotional memories (Murty et al., 2009; St Jacques, Dolcos, et al., 2009). In normal aging, the DG/CA3 regions seem to be affected such that there is hyperactivity in the CA3 region (likely driven by the region’s excitatory recurrent collaterals that are disinhibited with age) concurrent with a decrease in input from the EC (Gallagher & Koh, 2011; Yassa, Muftuler, et al., 2010; Yassa, Stark, et al., 2010). The amygdala is relatively well preserved, but still shows age-related change, especially in the BLA (Dere et al., 2010; Herzog & Kemper, 1980; Vereecken, Vogels, & Nieuwenhuys, 1994).

It is hypothesized that emotional arousal, via NE release in the BLA, strengthens hippocampal memory representations (Gallagher, Kapp, Musty, & Driscoll, 1977; McGaugh, 2004). Decreases in peripheral epinephrine levels (Sternberg, Martinez, Gold, & McGaugh, 1985) and noradrenergic involvement in age-related memory dysfunction (Kubanis & Zornetzer, 1981) have been reported in animal models of aging. Alterations in synaptic plasticity in the amygdala-hippocampal network with age have also been reported. Young rodents display early-LTP when stimulating the perforant path, which is prolonged into late-LTP when the BLA is stimulated 15 minutes later.
However, aged rodents show no enhancement of PP–DG LTP after BLA stimulation (Almaguer, Estupiñán, Uwe Frey, & Bergado, 2002). A combination of deficient synaptic plasticity and alterations in the noradrenergic system may therefore impair amygdala-hippocampal interactions with aging. Additionally, studies have shown that there is no upregulation of phosphorylated CREB in the BLA and hippocampus in low and moderate intensity shock, although aged rodents do show behavioral enhancements in the moderate shock condition (Morris & Gold, 2012). Findings such as this suggest that increasing the level of arousal may allow for compensation in aged rodents. It is not clear whether these deficits are due to hippocampal alterations or an age-related decrease in activity in modulatory regions such as the amygdala.

There are some limitations of the current study. It is difficult to rule out the possibility that our older adult group includes individuals with preclinical AD, which may present with different behavioral effects on emotional memory. While we attempted to minimize this possibility by excluding any individuals who presented with deficits in neuropsychological test performance, we cannot be certain that some of the behavioral effects observed are not driven at least in part by incipient AD pathology.

Furthermore, while we screened all of our young adults against major cognitive disorders, we did not perform detailed neuropsychological testing, thus investigating correlations between task performance and neuropsychological test performance was not feasible. Additionally, sample sizes were too small to thoroughly investigate gender differences, which have been demonstrated in some prior studies of emotional memory.
(Cahill, 2006). Finally, we used naturalistic stimuli and not computer-generated, controlled morphs, thus specific features (e.g. orientation, color, etc.) were quite variable. It is possible that future studies with more controlled stimuli can be used to examine mnemonic asymmetry for emotional items in more detail by directly manipulating individual aspects of the images. In addition, as mentioned in the Methods section, young and older adults used different scales for rating the valence of the images seen during the study phase. Young adults rated images on a 1-9 point scale while older adults used a similar scale, but without the large range of responses (limited to 3 button responses versus 9). We chose to limit the response options for older adults to make the decision process easier and remove this difficulty, however, this could potentially underlie differences seen in task performance between young and older adults. This possibility can be examined in future studies by changing the young adults’ rating scale to the same 3 button response as older adults to match across groups.

In conclusion, our data suggest that there may be age-related changes to how emotional memories are processed, such that emotional details of the experience may be remembered with higher fidelity in older adults. These results highlight an interesting behavioral phenomenon with age and a novel neuropsychological paradigm that can be used in conjunction with high-resolution neuroimaging to test the neural mechanisms of pattern separation of emotional information and how they change with age and disease.

**Individual differences in age-related memory impairment in emotional pattern separation**
Introduction Part II

While episodic memory deficits are a hallmark characteristic of aging and have been associated with alterations in the MTL, emotion’s modulatory influence on memory remains less well characterized in aging in addition to the amygdala’s role in influencing hippocampal memory. Additionally, there is increased variability in older adults memory performance such that not all older adults show age-related memory impairment. Examining episodic memory performance for significant events through the lens of an individual differences approach may reveal memory deficits selective to older adults with memory impairment. The current study utilized the emotional mnemonic discrimination task to investigate emotional pattern separation in aging. We hypothesized that healthy older adults (AU) would show evidence for emotional pattern separation, however, those with subclinical memory impairment (AI) may 1) also show an emotional pattern separation signal, as significant events may be preserved in subclinical memory impairment or 2) show an impairment in emotional pattern separation, which suggests more generalized deficits in pattern separation, regardless of its importance to remember. Furthermore, if emotional pattern separation deficits exist, we expect there to be network differences between AU and AI groups, such that the AI group may show impaired amygdala-hippocampal connectivity. To determine the specificity of network connectivity, we planned to investigate three sub-networks within the MTL: 1) BLA – CA1 connectivity (direct) 2) BLA – LEC – DG/CA3 connectivity (indirect), and 3) DG/CA3 – CA1 connectivity (intra-hippocampal).
Materials and Methods Part II

Participants. Twenty-seven participants (N=27, 18 female; mean age 72.2 + 7.6SD, age range = 60-91) were recruited from the local Orange County community via local campus announcements, flyers, and ads in local newspapers. Participants received monetary remuneration for their participation. Informed consent was obtained from all participants, with all procedures approved by the University of California, Irvine Institutional Review Board.

Inclusion/exclusion criteria. All participants were screened against major medical or psychiatric morbidities as well as substance abuse history. Participants received a neuropsychological evaluation during their visit. The battery was designed to examine memory function, as well as other aspects of general cognitive ability. The assessment included the MMSE, RAVLT, Digit Span backwards and forwards, Trail Making Tests A and B, BDI-II, GDS, Letter-Number Sequencing (LNS) to assess executive function, Stroop Color and Word Test to assess executive function, Beck Anxiety Inventory (BAI) to assess symptoms of anxiety, and a modified version of the Pittsburgh Sleep Quality Index (PSQI) to assess sleep quality and recent stress (see Table 5.2 for results). Participants included were healthy older adults with no diagnosed disorders associated with memory impairments such as MCI or AD. We split participants into two groups: AU (N=15, 11 female; mean age 70.9 + 6.6SD) and AI (N=12, 7 female; mean age 73.9 + 8.6SD), based on a median split of performance on the RAVLT-delayed test (≤ 10 in the AI group). There were no significant differences between the older adult groups in age,
education, and cognitive status (MMSE) (p’s > .05). All participants had normal or corrected-to-normal vision.

**Table 5.2. Older Participant Demographics and Neuropsychological Test Results**

<table>
<thead>
<tr>
<th>Groups</th>
<th>AU</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
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<tr>
<td>M:F</td>
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<td>5.7</td>
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<tr>
<td>Age</td>
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<td>73.9</td>
</tr>
<tr>
<td>Education</td>
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<td>16.8</td>
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<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean, SEM</th>
<th>Mean, SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit Span Forward</td>
<td>10.2, 0.5</td>
<td>10.0, 0.5</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>6.8, 0.5</td>
<td>6.0, 0.6</td>
</tr>
<tr>
<td>Letter-Number Sequencing</td>
<td>18.9, 0.7</td>
<td>19.0, 0.5</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>0.7, 0.3</td>
<td>0.7, 0.4</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>28.9, 0.3</td>
<td>28.9, 0.3</td>
</tr>
<tr>
<td>*RAVLT Immediate Recall</td>
<td>12.9, 0.5</td>
<td>8.7, 0.9</td>
</tr>
<tr>
<td>*RAVLT Delayed Recall</td>
<td>13.1, 0.4</td>
<td>7.5, 0.7</td>
</tr>
<tr>
<td>*RAVLT Recognition Recall</td>
<td>14.5, 0.3</td>
<td>13.1, 0.6</td>
</tr>
<tr>
<td>Trail Making Test A</td>
<td>28.4, 1.5</td>
<td>31.4, 1.8</td>
</tr>
<tr>
<td>*Trail Making Test B</td>
<td>81.6, 4.2</td>
<td>89.1, 10.3</td>
</tr>
<tr>
<td>*Stroop (Word-Color)</td>
<td>36.5, 2.1</td>
<td>28.3, 2.3</td>
</tr>
<tr>
<td>Beck Anxiety Inventory</td>
<td>3.8, 0.8</td>
<td>4.1, 1.2</td>
</tr>
<tr>
<td>Beck Depression Inventory-I</td>
<td>2.9, 0.9</td>
<td>3.1, 1.2</td>
</tr>
<tr>
<td>Hours of Sleep (night before)</td>
<td>8.0, 0.4</td>
<td>7.9, 0.3</td>
</tr>
<tr>
<td>Level of stress (past month,1-7)</td>
<td>2.7, 0.3</td>
<td>1.9, 0.3</td>
</tr>
</tbody>
</table>

* = significantly different between groups: RAVLT immediate recall (t(25) = -4.2, p < .001), RAVLT delayed recall (t(25) = -7.7, p < .001), RAVLT recognition (t(25) = -2.2, p = .04), Trail Making Test B (t(25) = 2.7, p = .01), Stroop Test (Word-Color) (t(25) = -2.6, p = .02).

**Imaging data collection.** Functional MRI data were collected using a 3-Tesla Philips scanner equipped with a SENSE head coil using both higher-order shims and SENSE imaging techniques. Functional images were collected using a high-speed EPI single-shot pulse sequence (1.5 mm isotropic resolution, 19 oblique axial slices parallel to the principal axis of the hippocampus, field of view = 180 x 28.3 mm x 180 mm, flip angle =
70°, SENSE parallel reduction factor = 2, TR/TE = 2200/26 ms, matrix size = 128 x 128).

We collected a high-resolution structural MPRAGE scan that we developed for accurate delineation of hippocampal subfields (Leal, Tighe, Jones, & Yassa, 2014) and high-resolution diffeomorphic alignment (0.65 mm isotropic resolution; 231 sagittal slices, field of view = 232 mm x 240 mm x 150 mm, flip angle = 18°, TR/TE = 11/5.03 ms, matrix size = 448 x 448). SENSE parallel imaging was used in two directions (2x1.5). The SAR (<10%) and PNS (<75%) were within required limits based on the scanner-calculated values. Total scan time for the volume was 5m 44s.

**Emotional mnemonic discrimination task.** Please refer to task description in Chapter 2 with the modification to valence rating response options in Chapter 3.

**Image analysis.** Image analysis and extraction of ROI voxels was identical to the procedure described in Chapter 2. However, we also included manual tracings of the amygdala nuclei and surrounding cortical regions including LEC and MEC in addition to hippocampal subfields. Amygdala segmentation procedures were based on (Entis, Doerga, Barrett, & Dickerson, 2012), but were modified to define three regions: BLA, CEA, and CORT. To label these subregions of the amygdala, three key points were identified: 1) the medial tip of the alveus (up to where optic tract is), 2) the most lateral point of the endorhinal sulcus, and 3) bottom of the circular sulcus. These three points are easily observable and provide a reliable landmarking system for segmenting the
amygdala subregions. After identifying the three points, lines are drawn to connect the
three points to each other, creating four quadrants (basomedial and basolateral were
combined to form the basolateral complex). We collapsed across CEA and CORT
regions, as these were quite small. ROIs included in the analysis are shown in Figure
5.2a and the full set of ROIs are shown in Figure 5.2b.
CA1, SUB, LEC, medial entorhinal cortex, central (CEA) and cortical nuclei (CORT) of the amygdala. Also shown on this template but not analyzed in the current study are perirhinal cortex (PrC) and parahippocampal cortex (PHC).

**Statistical analyses.** All statistical analyses of behavioral variables and ROI activation means were conducted in SPSS v. 21 (IBM Corp., released 2012, Armonk, NY). Planned comparisons were conducted using repeated-measure ANOVAs. Post-hoc contrasts were conducted where appropriate and critical Scheffe’s were reported during the first instance of reporting. All tests used the General Linear Model (ANOVA and correlations). Normality assumptions were investigated using Kolmogorov-Smirnov tests and all distributions investigated did not significantly deviate from the normal distribution. Repeated measures tests were corrected for error nonsphericity using Greenhouse-Geisser correction where appropriate. Moderation analysis was conducted in SPSS using PROCESS (Hayes, 2013), which applies regression-based path analytic framework for estimating moderator models. Statistical values were considered significant at a final corrected alpha level of .05, which appropriately controls for Type I error.

Correlation matrices (correlograms) were generated in MATLAB across the networks of interest (DG/CA3, LEC, BLA, CA1) using pairwise $r^2$ values (two-tailed) for all conditions and groups (Negative and Neutral, Correct Rejections and False Alarms, AU and AI). *Pearson’s r* values were then used to define three MTL networks: 1) BLA – CA1 connectivity 2) BLA – LEC – DG/CA3 connectivity, which was calculated by averaging the pairwise BLA – LEC and LEC – DG/CA3 correlations, and 3) DG/CA3 – CA1 connectivity. The differences between pairwise dependent correlation coefficients was
then calculated using z-scores (two-tailed) within groups (Lee & Preacher, 2013) as well as the differences between correlation coefficients between groups (VassarStats.net).

Results Part II

Emotional modulation of lure discrimination behavior

To examine lure discrimination behavior, we conducted a repeated measures ANOVA with three levels, and found a marginally significant effect of emotion \( F(2,52) = 3.26, p = .052 \). Our a priori contrast of interest comparing neutral against emotional discrimination showed a significant difference (emotional < neutral) \( F(1,26) = 5.28, p = .03 \). Raw performance and RT data are shown in Supplementary Tables S5.2 and S5.3, respectively (Appendix 5). Consistent with our prior results using this task in young adults, we found no significant effect of sex \( F(1,25) = .436, p = .515 \) or sex by emotion interaction \( F(2,50) = 2.42, p = .1 \), although the it is likely that sample sizes were not large enough to fully examine sex differences.

Emotional discrimination in DG/CA3 vs. false recognition in LEC and BLA

We focused our fMRI analysis on hippocampal subfields DG/CA3 and CA1 and inputs to the hippocampus from the BLA, either directly or through the LEC (Fig 1b). The CEA/CORT and MEC were used as control regions to ensure specificity of the amygdala-hippocampal network subject to analysis. To examine emotional pattern
separation, our analyses focused on the following comparisons: 1) retrieval trials where similar lures were presented and either correctly rejected or falsely recognized, and 2) comparison of negative and neutral items (we excluded positive items from analysis, as these items were not matched to negative items for arousal) (Leal, Tighe, & Yassa, 2014). We defined “emotional pattern separation” signals as increases in BOLD activity during correct discrimination of lures that was additionally modulated by emotion (Leal, Tighe, Jones, et al., 2014). We collapsed across left and right hemispheres, as the patterns were largely similar.

We conducted region-based ANOVAs with emotion (negative vs. neutral) and memory (CRs vs. FAs) as within-subject factors. In DG/CA3, we found a significant emotion x memory interaction \[ F(1,26) = 6.03, p = .021; \text{Fig 5.3a} \], which was driven by greater activity during negative CRs compared to neutral CRs \[ t(26) = 2.19, p = .038 \], with no difference during the FAs. This interaction is consistent with an emotional pattern separation signal in the DG/CA3.

![Fig. 5.3. Older adult medial temporal lobe activity during emotional discrimination.](image)

(a) Mean beta weight in the dentate gyrus (DG)/CA3 for negative and neutral stimuli during correct rejections (CR) and false alarms (FA); (b) Mean beta weight in the lateral entorhinal cortex (LEC) for negative and neutral stimuli during CRs and FAs; (c) Mean beta weight in the basolateral amygdala (BLA) for negative and neutral stimuli during CRs and FAs; (d) Mean beta weight in the CA1 for negative and neutral stimuli during CRs and FAs.
In the LEC, we found a significant emotion x memory interaction, where activity was
greater for negative versus neutral correct rejections, but the pattern was reversed
during false alarms. \[F(1,26) = 4.91, p = .036; \text{Fig 5.3b}\]. In the BLA, we found a
significant emotion x memory interaction \[F(1,26) = 5.23, p = .031; \text{Fig 5.3c}\], where
activity was the opposite of the DG/CA3 pattern with greater activity during negative
FAs compared to neutral FAs \[t(26) = 2.49, p = .020\] with no difference during the CR's.
In CA1, we found no significant effects (all p's > .05) (Fig 5.3d). We did not find any
significant emotion x memory interactions in either the CEA/CORT or MEC (all p's >
.05). Data across all ROI's and conditions are in Supplementary Fig S5.3.

Based on the entire sample of older adults, the data suggest that the emotional pattern
separation signal is selective to the DG/CA3 subregion of the hippocampus, with the
BLA expressing an emotional false recognition signal. This is consistent with a role for
the DG/CA3 in driving discrimination responses that may be dependent on pattern
separation and a role for the BLA in driving overgeneralized responses that may be
dependent on pattern completion.

Interestingly, the LEC expressed a false recognition signal that was also modulated by
emotion but was in the opposite direction from the BLA (increased activity during neutral
compared to negative stimuli). Furthermore, the LEC also expressed a discrimination
signal that was modulated by emotion similarly to DG/CA3. Increases and decreases in
BOLD activity should not be evidence of increased and decreased involvement per se
(e.g. decreased activity could be due to increased precision). The role of the LEC has
not been previously examined in detail in emotional tasks, however, it is an anatomical gateway by which input from the BLA reaches the DG/CA3, thus its involvement in this task showing a mixture of signals similar to BLA and DG/CA3 is not surprising.

Importantly, the effects noted above tended to manifest only in the regions of interest and not in our a priori control regions (MEC and CEA/CORT). Thus, based on these results, we conclude that in older adults, the DG/CA3 tends to be involved in discrimination of emotional information, while false recognition of emotional information tends to involve the BLA and LEC.

**Reduced DG/CA3 emotional pattern separation and enhanced false recognition signals in AI adults**

In order to examine individual variability in emotional mnemonic discrimination, we stratified participants into AU and AI groups based on the RAVLT (see Methods for more details). Importantly, the AI group did not present with memory complaints, nor did they present with memory deficits sufficient for a diagnosis of clinical impairment. Neuropsychological testing revealed significant group differences on the RAVLT (immediate, delayed, and recognition), Trail Making Test B, and the Stroop Test (Word-Color) (see Table 5.2 for statistics). We examined LDI performance across the groups and conducted a repeated measures ANOVA and found no significant differences in LDI for emotion $[F(2,50) = 3.02, p = .064]$, group $[F(1,25) = .2, p = .662]$, or emotion x group $[F(2,50) = .1, p = .889]$. We hypothesized that while behavioral performance was
matched across groups, the underlying neural signals during correct discrimination and false recognition may be different across groups.

We conducted separate repeated measures ANOVAs for CRs and FAs, and then performed a three-way ANOVA to directly compare the effects of memory in each ROI. For DG/CA3 CRs, we found a significant effect of emotion \([F(1,25) = 4.375, p = .047; \text{Fig 5.4a}]\) where there was greater activity for negative compared to neutral trials. We also found a significant emotion x group interaction \([F(1,25) = 5.31, p = .030]\), where the AU group showed greater activity during negative compared to neutral correct rejections \([F(1,25) = 10.87, \text{critical Scheffé} = 8.48, p < .05]\) while the AI group showed no difference between negative and neutral activity during correct rejections \([F(1,25) = .02, p > .05]\). No significant effects or interactions were observed for CA1 (Fig 5.4d) and there were no significant group differences or interactions with group in the LEC and BLA (Fig 5.4b,c). Data across all ROI’s, conditions, and groups are in Supplementary Fig S5.4.

These results suggest that the DG/CA3 emotional discrimination signal identified previously was limited to healthy young adults (Leal, Tighe, Jones, et al., 2014) and AU adults. DG/CA3 activity in AI adults did not appear to discriminate between negative and neutral items (i.e. showed a reduced emotional discrimination signal).
Fig. 5.4. Age-unimpaired (AU) vs. age-impaired (AI) activity during emotional discrimination. (a) Mean beta weight in the dentate gyrus (DG)/CA3 for negative and neutral stimuli during correct rejections (CR) and false alarms (FA); (b) Mean beta weight in the lateral entorhinal cortex (LEC) for negative and neutral stimuli during CRs and FAs; (c) Mean beta weight in the basolateral amygdala (BLA) for negative and neutral stimuli during CRs and FAs; (d) Mean beta weight in the CA1 for negative and neutral stimuli during CRs and FAs; (e-h) Correlation matrices across pairwise ROIs in AU and AI individuals (upper panel) across different conditions. We then compared three sub-network functional connectivity scores based on known anatomical connectivity of the amygdala to the hippocampus and intra-hippocampal connectivity: 1) BLA—CA1 connectivity (direct), 2) BLA—LEC—DG/CA3 connectivity (indirect – via the perforant path), and 3) DG/CA3—CA1 connectivity (intra-hippocampal – Schaffer collaterals). We calculated a sub-network functional connectivity score for three pathways (lower panel). For the indirect pathway, this was done by averaging the pairwise BLA-LEC and LEC-DG/CA3 pairwise correlations. All correlations were normalized using a Fisher's r to z transform and then compared using a z(difference) test.

During FA's, we observed a significant group effect \[ F(1,25) = 14.37, p < .001; \text{Fig 5.4a} \], where the AI group showed increased activity in DG/CA3 during false alarms compared to the AU group regardless of emotion. When directly comparing CRs and FAs in the DG/CA3, we found a significant memory x group interaction \[ F(1,25) = 6.25, p = .019 \], where there was increased activity during FAs vs. CR's in the AI group compared to the AU group.
Previous studies in rodents found elevated firing rates in hippocampal CA3 (Wilson, Ikonen, Gallagher, et al., 2005) and human studies have also found evidence of hyperactivity in hippocampal DG/CA3 which predicted memory impairment (Yassa, Lacy, et al., 2011). Furthermore, interventions reducing the hyperactivity in older rats (Koh, Haberman, Foti, McCown, & Gallagher, 2010), Alzheimer’s transgenic mice (Sanchez et al., 2012), as well as humans with amnestic MCI (Bakker et al., 2012) have all shown evidence of cognitive rescue, suggesting that such hyperactivity is dysfunctional rather than compensatory. In the human clinical work, the reduced hyperactivity was associated with reduced rate of false recognition of similar items on a pattern separation task (Bakker et al., 2015). Here, we see evidence of hyperactivity in DG/CA3 that is selective to the FA condition (mediating false recognition) and limited only to the older adults with subclinical memory impairment and is consistent with the notion that hyperactivity may be a biomarker for impairment and an indicator of age-related cognitive decline. Moreover, the hyperactivity here was not specific to negative or neutral FAs, suggesting a broad overgeneralization of information during false recognition.

Selective deficit of BLA-LEC-DG/CA3 connectivity during negative discrimination in AI adults

To investigate context-dependent amygdala-hippocampal connectivity, we generated correlation matrices (correlograms) using pairwise $r^2$ values across all ROI pairs for
each condition (Negative and Neutral FAs and CRs; Fig 5.4e-h, upper panel). We then compared three sub-network functional connectivity scores based on known anatomical connectivity of the amygdala to the hippocampus and intra-hippocampal connectivity: 1) BLA—CA1 connectivity (direct), 2) BLA—LEC—DG/CA3 connectivity (indirect – via the perforant path), and 3) DG/CA3—CA1 connectivity (intra-hippocampal – Schaffer collaterals). We calculated a sub-network functional connectivity score for each of the three pathways (Fig 5.4e-h, lower panel). For the indirect pathway, this was done by averaging the pairwise BLA-LEC and LEC-DG/CA3 correlations. All correlations were normalized using a Fisher’s r to z transform and then compared using a z(difference) test.

We hypothesized that the AI group would exhibit selective deficits in the indirect pathway but not in the direct or intra-hippocampal pathways. Consistent with this hypothesis, during negative CRs, the AI group showed a selective impairment in connectivity within the indirect sub-network (BLA—LEC—DG/CA3) compared to the direct sub-network (BLA—CA1) \[z = 4.92, p < .001; \text{Fig 5.4e}\] and compared to the intra-hippocampal network (DG/CA3—CA1) \[z = -4.8, p < .001\]. This was not the case in the AU group (p’s > .05). These results suggest that those with subclinical memory impairment show deficits in functional communication through the indirect amygdala-hippocampal network, which is consistent with the impaired emotional pattern separation noted earlier.
The hippocampus receives input from the entorhinal cortex via the perforant path, which is degraded in aging (Barnes, Rao, & Houston; Smith et al., 2000; Yassa, Muftuler, et al., 2010). Evidence from electrophysiological studies suggests that plasticity in the lateral perforant path to the dentate gyrus is compromised with age (Froc et al., 2003). The LEC also expresses increases in phosphorylated tau, decreased reelin expression, and decreased synaptophysin expression in aged impaired outbred rats (Stranahan et al., 2011). Taken together, these data are consistent with the apparent deficits in BLA-LEC-DG/CA3 connectivity observed here. Interestingly, deficits in this pathway occur in the absence of deficits in the other two pathways we examined, the direct BLA-CA1 pathway as well as the intra-hippocampal pathway from DG/CA3 to CA1. It is possible that the intact communications among those pathways accounts for the absence of behavioral performance differences between the AI and AU individuals.

**Enhanced connectivity during negative false recognition across the MTL in AI adults**

During negative FAs, there was increased connectivity across two sub-networks in the AI group compared to the AU group [BLA—CA1 z = -2.53, p = .01, DG/CA3—CA1 z = -2.27, p = .023]. This difference was only marginal in the BLA-LEC-DG/CA3 network but trended in the same direction [z = -1.49, p = .136]. This suggests that the AI group is overgeneralizing across the entire hippocampal-amygdala network during negative false recognition. This is in line with our finding of hippocampal hyperactivity during false alarms, but appears to be selective to negative false alarms. While the BLA did not
show any group differences in mean beta weight activity, there are clear differences in network connectivity between BLA and the hippocampus in the AU and AI groups. There were no significant group differences during neutral FAs and CRs (p’s > .05; Fig 5.4g,h), signifying the specificity of these effects to processing significant emotional stimuli.

The influence of BLA on DG/CA3 activity during false recognition depends on the level of subclinical memory impairment

To investigate the relationship between subclinical memory impairment and amygdala-hippocampal connectivity further, we correlated activity in the DG/CA3 and BLA during CRs and FAs with RAVLT. Since the AI group showed increased DG/CA3 activity during both negative and neutral FAs, we decided to collapse across emotion to gain power. We found a marginal correlation between RAVLT and DG/CA3 FA activity [Pearson’s r = -.375, p = .054; Fig 5.5a], where higher RAVLT activity was correlated with less DG/CA3 activity during false recognition. We expected to see a correlation with BLA as well, however, RAVLT was not significantly correlated with BLA activity during false recognition and only trended towards significance [Pearson’s r = .322, p = .102]. We hypothesized that there might be a more complex interaction between the variables, such that the level of subclinical memory impairment may modify the effect of BLA on DG/CA3 activity during false recognition. To test this hypothesis, we conducted a moderation analyses to determine if the level of subclinical memory impairment moderated the influence of BLA activity on DG/CA3 activity during false recognition.
DG/CA3 and BLA activity during false alarms was entered into the first step on the regression analysis. In the second step of the regression analysis, the interaction term between DG/CA3 and BLA activity during false alarms was entered, and it explained a significant increase in variance in DG/CA3 activity $[\Delta R^2 = .178, F(1,23) = 6.26, p = .02$; Fig 5.5b]. Thus, the influence of BLA activity on DG/CA3 activity during false recognition depends on the level of subclinical memory impairment.

**Fig. 5.5.** Relationship between BLA and DG/CA3 activity during false recognition is moderated by subclinical memory impairment. (a) Negative correlation between the delayed Rey Auditory Verbal Learning Test (RAVLT) and mean beta weight in DG/CA3 during false recognition; (b) Visualization of moderation analysis showing the influence of the BLA on DG/CA3 is moderated by subclinical memory impairment such that low RAVLT scores were associated with high BLA activity, but low DG/CA3 activity; (c) Visualization of the specificity of the conditional effect of BLA on DG/CA3 activity, showing that the confidence band is above 0 at less than or equal to 8.48 on the RAVLT (shaded grey).

The unstandardized simple slope for participants 1 SD below the mean on the RAVLT, the mean on the RAVLT, and 1 SD above the mean are shown in Figure 5.5b. To establish what level of subclinical memory impairment moderates the relationship between BLA and DG/CA3 activity, we applied the Johnson-Neyman technique. From this analysis, we found that when the RAVLT score was less than or equal to 8.48, higher BLA activity led to higher DG/CA3 activity during false recognition. Thus, the region of significance for the effect of BLA on DG/CA3 activity during false recognition
was RAVLT ≤ 8.48. Figure 5.5c shows a plot of the conditional effect of BLA on DG/CA3 activity during false recognition as a function of subclinical memory impairment with confidence bands. The region of significance is depicted as the values of the RAVLT corresponding to points where a conditional effect of 0 is outside of the confidence band. As can been seen, when the RAVLT ≤ 8.48, the confidence bands are entirely above zero (shaded grey). Thus, we conclude based on this analysis that subclinical memory impairment moderates the impact of the BLA on DG/CA3 activity and that this conditional relationship generally manifested in those with the lowest memory scores.

**Discussion Part II**

While it has been repeatedly shown that older adults exhibit memory impairments in aging, not all memories are treated equally. Some information may be preferentially processed due to the significance of the experience. Emotionally arousing events are typically better remembered than neutral events, however, this has not been thoroughly investigated in the context of aging. It has been previously unclear if older adults show memory deficits for all types of information regardless of significance, or if they show a preservation in remembering significant experiences. Furthermore, age-related memory impairment is highly variable such that not all older adults show memory deficits. These two aspects of memory and aging complicate general statements about episodic memory loss in aging and suggest that information content and the presence of subclinical memory impairment may shift the dynamics of memory impairments in aging.
In the current study, we utilized an emotional mnemonic discrimination task that manipulated emotion to investigate how significant versus neutral memories are processed in aging. By using a mnemonic discrimination task, we are also able to examine hippocampal subfields during correct discrimination of similar “lure” items and during false recognition of lure stimuli. This in combination with high-resolution fMRI allows us to elucidate MTL functioning and make inferences about how older adults discriminate similar experiences and utilize hippocampal pattern separation.

Furthermore, previous studies have investigated the increased variability in aging using the RAVLT to divide cognitively normal older adults into two groups: those with and without subclinical memory impairment (AI versus AU, respectively).

We found that older adults with subclinical memory impairment show a loss of emotional pattern separation, while healthy older adults maintain this ability. This suggests that older adults who are showing deficits in memory for significant events may be more impaired than their healthy counterparts who show preserved emotional pattern separation, but impaired neutral pattern separation. Furthermore, the BLA—LEC—DG/CA3 pathway was selectively altered in AI older adults, providing further evidence of dysfunction in amygdala-hippocampal connectivity through a hippocampal input involved in pattern separation.

We also found evidence for hyperactivity in the DG/CA3 in AI older adults, which is consistent with previous findings, but also suggests that this hyperactivity is generalized to significant and neutral events. However, amygdala-hippocampal hyperconnectivity in
the AI group during false recognition seems to be specific to negative stimuli, suggesting further alterations in communication between the amygdala and hippocampus when remembering significant events.

Individuals who were more subclinically impaired showed greater BLA activity related to greater DG/CA3 activity. However, individuals without impairment showed greater BLA activity related to less DG/CA3 activity (although this effect was not significant). Upon further investigation, the effect of BLA on DG/CA3 activity was significant for those who received a RAVLT score less than or equal to 8.48, suggesting this may be a more sensitive cut-off in determining the effects of subclinical memory impairment on amygdala-hippocampal functioning.

In the neuropsychological battery we also note significant differences between the AU and AI groups on the Stroop Word-Color task as well as the Trail-Making Test B, which both measure executive function and mental processing speed. Differences on these measures may signify frontal lobe alterations in those with subclinical memory impairment. Even though we are very hypothesis-specific in this study and focused on the MTL, it should be noted that age-related cognitive deficits are not limited to the MTL. We suspect there are frontal components that are also altered in the disease. Consistent with this, we find group differences in performance of tests that are sensitive to frontal dysfunction, including Trail Making Test – B, and the Stroop Color-Word Test. Due to the nature of our high-resolution scan protocols, the frontal lobes were beyond our field of view, however, future studies with whole brain fMRI (perhaps using our
same task) could significantly inform on alterations exhibited by the frontal lobe, as well as connectivity between the MTL and the prefrontal cortex (PFC). A particular limitation of the study is the absence of characterization of AD pathology. It is possible that AI individuals are more likely to be those harboring AD pathology and thus more likely to exhibit cognitive decline in the future, however, this could not be tested in the current study. This is an ongoing topic of interest in the field (Jagust, 2013) and we hope that our findings and our task may enable additional research to address these relationships.

Furthermore, we excluded analysis of the positive stimuli for the current study, as positive stimuli were not as arousing as negative stimuli. Preferentially remembering positive information in older adults (i.e. the positivity effect) has been documented in the literature (Mather & Carstensen, 2005), although other studies have suggested that older adults preferentially remember negative information (Kensinger, 2008). We hypothesized that this discrepancy may be resolved by using an individual differences approach to testing memory in older adults. While we did not include positive stimuli in this study, we have developed a modified version of the Logical Memory Subset of the Wechsler Memory Scale III to create the Emotional Logical Memory Test, which includes negative, neutral, and positive stories, preserving all other aspects such as number of details and sentence structure. Our analyses of individual differences revealed that AI individuals had better memory (i.e. less forgetting) for positive information and worse memory (i.e. more forgetting) for neutral information compared to AU individuals. This difference between AU and AI groups suggests that the positivity bias previously reported in aging may be driven at least in part by subclinical memory
impairment (see Appendix 6 for more information). This sheds light on the discrepancy across findings in older adults in the literature and suggests that adequate consideration for subclinical memory impairment is necessary for a more complete understanding of emotional memory alterations in aging.

Chapter 6: Emotional pattern separation in late-life depression

Introduction

Aging and depression are both independently associated with episodic memory impairment (Airaksinen et al., 2007; Glisky, 2007). The same MTL network, namely the hippocampus and amygdala, appears to be vulnerable to the effects of both aging and depression. Moreover, up to 50% of individuals with MCI or AD exhibit comorbid depressive symptoms (Lopez, Becker, & Sweet, 2005), which are associated with an increased risk for dementia (Diniz, Butters, Albert, Dew, & Reynolds, 2013). MTL changes in aging and depression have been linked to alterations in mnemonic discrimination. The current study sought to assess behavioral performance and neural substrates of emotional discrimination in older adults with and without depressive symptoms. We hypothesized that while it is unlikely that late-life depression will be easily characterized as the linear sum of the effects previously noted in aging and
depression independently, it is likely to involve the same MTL network. Thus, we examined behavioral performance on the emotional mnemonic discrimination task, as well as neural signals in the BLA and the DG/CA3. We included the LEC in our hypothesis-driven analyses as well, since this region is a gateway by which the BLA projects to the DG/CA3.

Materials and Methods

Participants. Forty-two participants (N = 42, 27 female; mean age 70.7 ± 7.5SD) were recruited from the local Orange County community via local campus announcements, flyers, and ads in local newspapers. Participants received monetary remuneration for their participation. Informed consent was obtained from all participants, with all procedures approved by the University of California, Irvine Institutional Review Board.

Inclusion/exclusion criteria. Participants received the same neuropsychological evaluation as noted earlier in this chapter (see Table 6.1 for results). All participants were screened against major medical or psychiatric morbidities as well as substance abuse history, with the exception of a past or current diagnoses of depression and/or anxiety (for those in the DS+ group). Participants were selected for the DS group if they scored ≥4 on the GDS ≥ 8 on the BDI-II. These scores indicate mild to severe levels of DS, in which we needed a greater range of DS variability to examine the effects of DS on a continuum. Participants’ GDS and BDI-II scores were positively correlated with one other [Pearson’s r = .907, p < .001; Fig S6.1], thus for all subsequent analyses we
utilized the BDI-II since there is a wider range of scores for the BDI-II (0-63) compared to the GDS (0-15), allowing for more variability along a continuum. Individuals with DS were not excluded if they were taking anti-depressants, however, they had to be actively experiencing depressive symptoms to be included in the DS+ group (N = 10 taking antidepressants, but not successful in fully reducing depressive symptoms). We analyzed performance in the DS- group (N=27, 18 female; mean age 72.2 + 7.6SD, mean BDI 3.0 + 3.6SD) and the DS+ group (N=15, 9 female; mean age 67.9 + 6.9SD, mean BDI-II 22.2 + 9.1SD). Participants included had no diagnosed disorders associated with memory impairments such as MCI or AD. There were no significant differences between the groups in age and cognitive status (MMSE) (p’s > .05). All participants had normal or corrected-to-normal vision.

Table 6.1. Demographics and Neuropsychological Test Results

<table>
<thead>
<tr>
<th>Groups</th>
<th>DS-</th>
<th>DS+</th>
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</thead>
<tbody>
<tr>
<td>Sample size</td>
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<td>15</td>
</tr>
<tr>
<td>M:F</td>
<td>9:18</td>
<td>6:9</td>
</tr>
<tr>
<td>*Age</td>
<td>72.2</td>
<td>67.9</td>
</tr>
<tr>
<td>*Education</td>
<td>16.7</td>
<td>13.9</td>
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</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean, SEM</th>
<th>Mean, SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit Span Forward</td>
<td>10.1, 0.3</td>
<td>9.9, 0.5</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>6.4, 0.4</td>
<td>6.1, 0.5</td>
</tr>
<tr>
<td>*Letter-Number Sequencing</td>
<td>19.0, 0.4</td>
<td>16.5, 1.0</td>
</tr>
<tr>
<td>*Geriatric Depression Scale</td>
<td>0.6, 0.2</td>
<td>7.4, 9.7</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>28.9, 0.2</td>
<td>28.1, 0.5</td>
</tr>
<tr>
<td>*RAVLT Immediate Recall</td>
<td>11.0, 0.6</td>
<td>7.7, 1.0</td>
</tr>
<tr>
<td>*RAVLT Delayed Recall</td>
<td>10.6, 0.7</td>
<td>7.6, 1.0</td>
</tr>
<tr>
<td>RAVLT Recognition Recall</td>
<td>13.8, 0.3</td>
<td>13.4, 0.6</td>
</tr>
<tr>
<td>Trail Making Test A</td>
<td>29.7, 1.2</td>
<td>36.0, 4.3</td>
</tr>
<tr>
<td>*Trail Making Test B</td>
<td>73.8, 5.7</td>
<td>107.1, 11.5</td>
</tr>
<tr>
<td>*Stroop (Word-Color)</td>
<td>32.8, 1.7</td>
<td>26.5, 1.6</td>
</tr>
<tr>
<td>*Beck Anxiety Inventory</td>
<td>3.9, 0.7</td>
<td>13.9, 2.7</td>
</tr>
<tr>
<td>*Beck Depression Inventory-II</td>
<td>3.0, 0.7</td>
<td>22.2, 2.4</td>
</tr>
<tr>
<td>Hours of Sleep (night before)</td>
<td>7.9, 0.2</td>
<td>8.3, 0.5</td>
</tr>
<tr>
<td>*Level of stress (past month, 1-7)</td>
<td>2.3, 0.2</td>
<td>4.4, 0.4</td>
</tr>
</tbody>
</table>

* = significantly different between groups. Education [t(44) = -3.8, p < .01], Letter-Number Sequencing [t(44) = -2.3, p = .033], Geriatric Depression Scale [t(44) = -12.7, p < .001], RAVLT Immediate recall [t(44) = 4.2, p < .001], RAVLT Delayed recall [t(44) = 2.0, p = .05], Trail Making Test A [t(44) = 1.3, p = .2], Trail Making Test B [t(44) = 2.4, p = .025], Beck Anxiety Inventory [t(44) = 4.6, p < .001]. Level of stress [t(44) = 4.6, p < .001].
**Neuroimaging collection and analysis:** Imaging data collection, emotional discrimination task, image analysis, and extracting ROI voxels are identical methods to those described in Chapter 2. See Appendix 7 for Supplementary Information.

**Statistical analyses.** All statistical analyses were conducted in SPSS v. 24 (IBM Corp., Armonk, NY). Planned comparisons were conducted using repeated-measures ANOVAs. Post hoc statistical tests were corrected for multiple comparisons using Scheffé’s correction, with critical F values indicated in the text corresponding to the degrees of freedom (df) of the F-test (mentioned only once for each pair of df’s), or Bonferroni correction. All tests used the General Linear Model (ANOVA and correlations). Normality assumptions were investigated using Kolmogorov-Smirnov tests and all distributions investigated did not significantly deviate from the normal distribution. Repeated measures tests were corrected for error nonsphericity using Greenhouse-Geisser correction where appropriate. Mediation and moderation analyses were conducted in SPSS using PROCESS (Hayes, 2013), which applies regression-based path analytic framework for estimating mediator and moderators models. Bootstrap confidence intervals were implemented for inference about indirect effects. Statistical values were considered significant at a final corrected alpha level of .05, which appropriately controls for Type I error.

**Results**
Depressive symptoms associated with enhanced negative discrimination

We analyzed LDI for each emotion during performance on the emotional mnemonic discrimination task. We conducted a repeated-measures ANOVA with emotion (negative, neutral, and positive) as the within-subjects factor and group (DS+, DS-) as the between-subjects factor and found a significant effect of emotion \( [F(2,78) = 3.57, p = .033] \), where neutral information was remembered better than emotional information \( [F(1,39) = 5.19, p = .028; \text{Fig 6.1a}] \). We also found a significant effect of group \( [F(1,39) = 4.62, p = .038] \), where the DS+ group performed worse than the DS- group. We found a significant positive correlation between enhanced negative LDI and BDI symptoms in the DS+ group and not in the DS- group (Fig 6.1b), which is consistent with our previous findings that depressive symptoms in young adults were associated with increased negative hyperdiscrimination (Leal, Tighe, & Yassa, 2014).

Figure 6.1. Behavioral performance. (a) Behavioral performance on the emotional mnemonic discrimination task in older adults with (DS+) and without (DS-) depressive symptoms; (b) Positive correlation between negative lure discrimination index (LDI) and the BDI score in older adults with depressive symptoms (DS+).
DG/CA3 and BLA activity during neutral discrimination predict depressive symptoms

We utilized high-resolution (1.5 mm isotropic) fMRI to investigate MTL alterations in older adults with and without DS on the emotional mnemonic discrimination task. We focused our analysis on conditions where we could test hypotheses about emotional pattern separation. We compared retrieval trials where similar lures were presented, since these trials were hypothesized to maximize interference. We analyzed both correct rejections (accurate discrimination) and false alarms (false recognition) of lures. We focused on regions involved in emotional modulation of memory, which include the hippocampal subregions (DG/CA3, CA1, and SUB), the BLA, and LEC (a main input pathway to the hippocampus and highly connected with the amygdala). We collapsed across left and right hemispheres as patterns looked similar across left and right. We used the CEA/CORT as well as the MEC as controls and found no significant differences in either region. All data across all ROI’s, conditions, and groups not shown are in Fig S6.2.

We conducted repeated-measures ANOVAs with emotion (negative, neutral, and positive) as the within-subject factor and group (DS+, DS-) as the between-subject factor for correct rejections and false alarms separately in each ROI. The only region showing any group effects during accurate discrimination was the DG/CA3 subregion of the hippocampus. In DG/CA3, we found an emotion x group interaction [F(2,80) = 3.42, p = .039; Fig 6.2a], where activity was reduced during negative discrimination and
increased during neutral discrimination in the DS+ group \( F(1, 80) = 6.32 \), critical Scheffé = 6.22, \( p < .05 \). Furthermore, the amount of DG/CA3 activity during neutral correct discrimination was positively correlated with depressive symptom severity [BDI-II; Pearson’s \( r = .35 \), \( p = .022 \); Fig 6.2b]. The greater number of depressive symptoms was associated with greater DG/CA3 activity during neutral lure discrimination.

Given the known role of the BLA in emotional modulation (McGaugh, 2004), and our prior findings of amygdala alterations in depression (Leal, Tighe, Jones, et al., 2014), we hypothesized that emotional modulation differences during DG/CA3 CRs could be linked to BLA activity, however, there were no significant group differences in BLA activity during accurate discrimination or correlations with DS severity (Fig 6.2c,d). We
then tested the possibility that BLA’s impact on DS severity may be mediated by the DG/CA3. In Step 1 of the mediation model, the regression of BLA activity during accurate neutral discrimination on level of DS, ignoring the mediator (DG/CA3 activity), was not significant \( \beta = 13.27, t(40) = 1.55, p = .128 \]. Step 2 showed that the regression of BLA activity on DG/CA3 activity was significant \( \beta = .55, t(40) = 3.8, p < .001 \]. Step 3 of the mediation process showed that DG/CA3 activity, controlling for BLA activity, was not significant \( \beta = 16.38, t(39) = 1.79, p = .082 \]. Step 4 of the analysis revealed that when controlling for DG/CA3 activity, BLA activity was not a significant predictor of the level of DS \( \beta = 4.33, t(39) = .45, p = .658 \]. Unstandardized indirect effects were computed for each of 1,000 bootstrapped samples, and the 95% confidence interval was computed. The bootstrapped unstandardized indirect effect was 8.94 and was significantly different from zero, as revealed by a 95% bootstrap confidence interval that was entirely above zero (1.77 to 23.52). Thus, greater BLA activity was associated with greater DG/CA3 activity during accurate neutral discrimination, which in turn was associated with higher levels of DS (as both paths are positive; Fig 6.3, yellow panel).

![Figure 6.3. Mediation analyses examining the relationship between BLA and depressive symptom severity.](image)

Yellow (top) portion shows the mediation model that DG/CA3 mediates the relationship between BLA and depressive symptom severity during neutral discrimination; (b) Blue (bottom) portion shows the mediation model that LEC mediates the relationship between BLA and depressive symptom severity during positive false recognition.
LEC and BLA activity during positive false recognition predict depressive symptoms

During false recognition, we found significant group effects in the DG/CA3 [F(1,40) = 8.12, p = .007; Fig S6.2a], CA1 [F(1,40) = 7.90, p = .008; Fig S6.2b], and SUB [F(1,40) = 6.02, p = .019; Fig S6.2c], where the DS+ group showed increased activity compared to the DS- group across the entire hippocampus. We did not find any significant effects in the BLA (p’s > .05; Fig 6.2g). However, in the LEC we found a significant effect of emotion [F(2,80) = 3.96, p = .024; Fig 6.3e] and a significant emotion x group interaction [F(2,80) = 5.61, p = .006]. There was increased activity only during positive false recognition in the DS+ group compared to the DS- group [F(1,80) = 17.33, p < .001]. To further examine the relationship between DS and LEC activity during positive false recognition, we performed correlation analyses and found that the level of LEC activity positively correlated with DS severity [Pearson’s r = .428, p = .006; Fig 6.2f]. To determine if this effect was specific to LEC, we ran similar correlations in the hippocampal subfields and BLA. The amount of activity during positive false recognition was positively correlated with DS in DG/CA3 [Pearson’s r = .30, p = .056, marginal; Fig S6.3a], CA1 [Pearson’s r = .33, p = .032; Fig S6.3b], and SUB [Pearson’s r = .37, p = .016; Fig S6.3c], but not in the BLA (Fig 6.2h). Since we conducted several correlational analyses, we subjected the correlation set to multiple comparison correction using the Bonferroni method, which only yielded significant results for the LEC activity correlation with DS during positive false recognition. While this remains speculative, it is possible
that the LEC showed a unique activity profile for positive stimuli at least in part due to its connection with ventral tegmental area dopamine neurons, which are associated with reward-related stimuli (Hutter & Chapman, 2013).

We hypothesized that emotional modulation differences during LEC positive false recognition would be associated with BLA activity, however, there were no significant group differences during false recognition or correlations with DS severity in the BLA. We hypothesized that the BLA's effect on DS severity might be mediated by LEC activity. In Step 1 of the mediation model, the regression of BLA activity during positive false recognition on level of DS, ignoring the mediator (LEC activity), was not significant \( \beta = 9.46, t(40) = 1.47, p = .151 \). Step 2 showed that the regression of BLA activity on LEC activity was marginally significant \( \beta = .23, t(40) = 1.97, p = .056 \). Step 3 of the mediation process showed that LEC activity, controlling for BLA activity, was significant \( \beta = 21.55, t(39) = 2.63, p = .012 \). Step 4 of the analysis revealed that when controlling for LEC activity, BLA activity was not a significant predictor of the level of DS \( \beta = 4.5, t(39) = .72, p = .478 \). Unstandardized indirect effects were computed for each of 1,000 bootstrapped samples, and the 95% confidence interval was computed. The bootstrapped unstandardized indirect effect was 4.94 and significantly different from zero, as revealed by a 95% bootstrap confidence interval that was entirely above zero (.439 to 15.66). Thus, greater BLA activity was associated with greater LEC activity during positive false recognition, which in turn was associated with greater depressive symptoms (as both paths are positive; Fig 6.3, blue panel).
The impact of DG/CA3 and BLA activity on negative discrimination is moderated by depressive symptoms

We also sought to determine if cognitive performance on the task, as measured by LDI for each emotion, was associated with amygdala or hippocampal activity levels in our DS+ and DS- groups. We calculated Pearson’s r values correlating negative, neutral, and positive LDI with DG/CA3 and BLA activity during CRs in the DS+ and DS- groups. We found a significant negative correlation between negative LDI and DG/CA3 activity during negative CRs in DS- [Pearson’s r = -.409, p = .034] and a trend in the DS+ group in the opposite direction towards a positive correlation [Pearson’s r = .444, p = .098]. We then compared r values using a Fisher’s r to z transform to determine if the groups significantly differed in their correlations and found a significant difference between correlations [DG/CA3: z\text{diff} = 2.58, p = .009; Fig 6.4a]. In the BLA, we found that DS- and DS+ groups showed opposing relationships between negative LDI and BLA activity during negative CRs [DS- Pearson’s r = -.247, p = .214, DS+ Pearson’s r = .402, p = .138]. Using a Fisher’s transform we calculated the difference between these two correlations and found a marginally significant difference [BLA: z\text{diff} = 1.92, p = .054; Fig 6.4d]. This was not the case for DG/CA3 or BLA FAs (all p > .05; Fig S6.4a,b).
Figure 6.4. DG/CA3 and BLA activity during negative discrimination is associated with depressive symptom severity. (a) Correlations between negative LDI and mean beta weight in DG/CA3 during negative CRs across DS+ and DS- groups; (b) Visualization of moderation analysis showing the influence of the DG/CA3 on negative LDI is moderated by depressive symptom severity such that high BDI scores were positively associated DG/CA3 activity and negative LDI; (c) Visualization of the specificity of the conditional effect of DG/CA3 on negative LDI, showing that the confidence band is above 0 at greater than or equal to 21.9 on the BDI (shaded grey); (d) Correlations between negative LDI and mean beta weight in BLA during negative CRs across DS+ and DS- groups; (e) Visualization of moderation analysis showing the influence of the BLA on negative LDI is moderated by depressive symptom severity such that high BDI scores were positively associated BLA activity and negative LDI; (f) Visualization of the specificity of the conditional effect of BLA on negative LDI, showing that the confidence band is above 0 at greater than or equal to 27.2 on the BDI (shaded grey).

To determine if the level of DS severity influenced the impact of DG/CA3 and BLA activity on negative LDI, we conducted a moderation analysis. For DG/CA3, activity during negative accurate discrimination and negative LDI score was entered into the first step on the regression analysis. In the second step of the regression analysis, the interaction term between DG/CA3 activity and negative LDI was entered, and it explained a significant increase in variance in negative LDI [$\Delta R^2 = .179$, $F(1,38) = 8.30$, $p = .007$]. Thus, the influence of DG/CA3 activity on negative LDI was influenced by DS severity.
severity. The unstandardized simple slope for participants 1 SD below the mean on the BDI, the mean on the BDI, and 1 SD above the mean are shown in Figure 6.4b. To establish what level of DS moderated the relationship between DG/CA3 activity during negative discrimination and negative LDI, we applied the Johnson-Neyman technique. From this analysis, we found that when the BDI score was greater than or equal to 21.9, higher DG/CA3 activity led to higher negative LDI. Thus, the region of significance for the effect of DG/CA3 on negative LDI was BDI ≥ 21.9. Figure 6.4c shows a plot of the conditional effect of DG/CA3 activity on negative LDI as a function of DS with confidence bands. The region of significance is depicted as the values of the BDI corresponding to points where a conditional effect of 0 is outside of the confidence band. As can be seen, when the BDI ≥ 21.9, the confidence bands are entirely above zero (shaded grey).

We also performed the same analysis in the BLA. Activity during negative accurate discrimination and negative LDI score was entered into the first step on the regression analysis. In the second step of the regression analysis, the interaction term between BLA activity and negative LDI was entered, and it explained a significant increase in variance in negative LDI [ΔR² = .117, F(1,38) = 5.04, p = .031]. Thus, the influence of BLA activity on negative LDI was influenced by DS severity. The unstandardized simple slope for participants 1 SD below the mean on the BDI, the mean on the BDI, and 1 SD above the mean are shown in Figure 6.4e. When we applied the Johnson-Neyman technique, we found that when the BDI score was greater than or equal to 27.2, higher BLA activity led to higher negative LDI (Fig 6.4f).
Discussion

We found that older adults with depressive symptoms showed overall worse performance on the emotional mnemonic discrimination task, however, DS severity positively correlated with discrimination of negative stimuli. Furthermore, we found reduced DG/CA3 activity during negative discrimination and increased activity during neutral discrimination in older adults with depressive symptoms. Increased DG/CA3 activity during neutral discrimination was positively correlated with DS severity, but not during negative discrimination. These findings suggest that while healthy older adults show signals consistent with emotional pattern separation, older adults with depressive symptoms show hyperactivity during neutral discrimination. We also found that the DG/CA3 mediated the relationship between BLA activity and DS severity during neutral discrimination, while the LEC mediated the relationship between BLA activity and DS severity during positive false recognition. Consistent with anatomical data (McDonald & Mascagni, 1997) as well as animal models, the impact of the BLA on the manifestation of depressive symptoms appears to be mediated by the LEC and DG/CA3, implicating a particular amygdala-hippocampal pathway in depression. While it appeared that the DG/CA3 showed hypoactivity during negative discrimination compared to healthy older adults, this relationship was more complex. We observed that increased DG/CA3 and BLA activity was linked to better negative discrimination in those with greater depressive symptom severity. Late-life depression is complex and does not simply phenocopy
depression in young adults and is not to be considered a linear addition of aging and depression.

Many of the older adults were diagnosed with depression (N = 12), but this was not an inclusion criterion for the current study. We aimed to cut across traditional classifications of depression to understand psychopathology in a more integrative way by applying the RDoC approach. We have used this in prior work looking at depressive symptoms as a continuum, and it is clear that this approach empowered us to do analysis that could not be done if we simply had groups of no depression versus severe unmedicated depression. Also, consistent with the RDoC approach, a subset of our participants were additionally suffering from anxiety symptoms and quite possibly had co-morbid anxiety or subsyndromal anxiety (N = 5). Depression and anxiety are highly co-morbid with almost half of those with MDD also meeting criteria for anxiety disorders (Beekman et al., 2000), which suggests that they may share some common etiology. Rodent models of anxiety have shown that selective activation of hippocampal DG can suppress anxiety (Kheirbek et al., 2013). Human fMRI studies have shown a relationship between hippocampal activity and anxiety (Satpute, Mumford, Naliboff, & Poldrack, 2012). Furthermore, hyperactivity in the amygdala has also been shown in various anxiety disorders, which is also the case in depression (Holzschneider & Mulert, 2011).

Since the same MTL network is affected in both aging and depression, understanding the interaction of these states is important to fully characterize neurobiological alterations associated with each and to use this to potentially alleviate symptoms of
depression and memory impairment. Individuals with MCI or AD exhibit comorbid
depressive symptoms in up to half of patients (Lopez et al., 2005) and is associated with
an increased risk for dementia (Diniz et al., 2013). Depression later in life has serious
consequences, including patient and caregiver distress and increased mortality due to
high suicide rates in the elderly (Reynolds & Kupfer, 1999). Three major explanations
for the relationship between depression and cognitive decline have been suggested: 1)
derepressive symptoms are an early prodrome or warning sign of dementia, rather than
true depression alone, 2) depression may be a response to early cognitive loss in which
recognition of memory problems leads to depression or 3) depression may have an
etiological role in cognitive decline such that depression initiates neurological changes
that lead to neurobiological changes, which places depressed individuals at greater risk
for developing dementia (Panza et al., 2010; Sawyer, Corsentino, Sachs-Ericsson, &
Steffens, 2012). Distinctions have also been made in comparing early-onset depression
versus late-onset depression, such that early-onset depression is associated with
greater sensitivity of depressive symptoms while late-onset depression is associated
with more severe cognitive and neurological changes (Sachs-Ericsson et al., 2013).
Furthermore, the number of depressive symptoms at baseline has been shown to
predict development of AD. With each additional symptom, risk of disease increased by
about 20% (Wilson et al., 2002). Links have also been made between depression and
APOE4, such that APOE4 and depression interact and lead to increased risk of
developing MCI (Geda et al., 2006). Further investigation of the comorbidity between
depression and aging, MCI, and AD is needed to aid in more targeted treatments for
older adults with and without depressive symptoms with the goal of reducing the impact of depressive symptoms on development of cognitive decline.

Core vulnerability in the medial temporal lobes

These findings suggest that older adults with depressive symptoms may have distinct neurobiological changes occurring in the MTL that are dependent on the content of the memory being recalled. It is important to consider memory for significant experiences (such as emotional ones), as these may be remembered differently in aging and depression. For example, older adults may benefit from prioritizing emotional experiences over neutral experiences to remember. However, remembering too many details of an emotional experience may not be as adaptive (Leal & Yassa, 2014). Individuals with depression tend to remember negative experiences better and show impairments in remembering neutral or positive experiences. Determining how the MTL network is altered in aging alone versus aging with depression may help in developing therapeutic targets to alleviate symptoms and potentially shift MTL patterns back to a normal state. Late-life depression appears to implicate some of the same networks that are involved in age-related changes in emotional memory (Kensinger, 2008b; St Jacques, Dolcos, et al., 2009) as well as regions that are known to express structural and function alterations in the context of depression (Sahay & Hen, 2007; Sheline et al., 2001; Sheline, 2011).
We suggest that the amygdala-hippocampal system described herein, which expresses alterations in late-life depression, is a system that is vulnerable to a wide array of brain pathologies and conditions, including other psychiatric disorders such as schizophrenia and bipolar disorder, as well as age-related cognitive decline and Alzheimer’s disease. It appears that one of the commonalities among all of these conditions in that they impose a type of chronic stress on the hippocampal formation and its extended networks that impact memory function.

Even though we are very hypothesis-specific in this study and focused on the MTL, it should be noted that depression and age-related cognitive deficits are not limited to the MTL. We suspect there are frontal components that are also altered in the disease. Consistent with this, we find group differences in performance of tests that are sensitive to frontal dysfunction, including Trail Making Test – B, Stroop Color-Word Test, as well as Letter-Number Sequencing. Due to the nature of our high-resolution scan protocols, the frontal lobes (especially the prefrontal cortex known to be altered in depression (Koenigs & Grafman, 2009)) were beyond our field of view, however, future studies with whole brain fMRI (perhaps using our same task) could significantly inform on alterations exhibited by the frontal lobe, as well as connectivity between the MTL and the PFC.

A particular limitation of the study is the absence of characterization of AD pathology. Even though all participants are cognitively normal, it is possible that some individuals are harboring AD pathology and thus more likely to exhibit cognitive decline in the future, however, this could not be tested in the current study. Another limitation of the
study is the older adults with depressive symptoms were not all medication free. We selected individuals based on if they were currently experiencing depressive symptoms. A number of DS+ participants were medicated (N = 10), however, they expressed symptoms regardless, thus, our participants are a mixture of unmedicated and treatment-unresponsive individuals. We did this for power considerations, however, future studies with larger samples should stratify based on medication status.

While aging, in and of itself, is associated with episodic memory impairment, further insults to the MTL system such as depression can further impair memory and alter amygdala-hippocampal dynamics. Overall, our results provide novel insight into hippocampal and amygdala subregional deficits that manifest in the context of late-life depression. Our emotional mnemonic discrimination task is a new candidate for a sensitive marker of depression in older age and may serve as a tool to assess outcomes in therapeutic trials.

Chapter 7: Towards a model of amygdala-hippocampal dynamics during emotional pattern separation in healthy and cognitively-impaired states

Discussion of findings
General discussion

The goal of this dissertation was to investigate the emotional modulation of memory in the context of hypothesized hippocampus subregion specific memory processing. Thus far, cross-species evidence of the emotional modulation of memory has suggested that emotional memories are preserved, however, not all aspects of an emotional experience are better remembered. Remembering the gist of an emotional event at the expense of detail information suggests that it is more adaptive to generalize in circumstances of high arousal rather than focus on details. The mechanisms underlying this gist versus detail trade-off are unknown. We developed an emotional mnemonic discrimination task that allows us to test the mechanisms of emotional modulation of memory in the context of a pattern separation framework. Hippocampal pattern separation is one mechanism that allows a reduction in interference among overlapping representations (e.g. remembering experiences that share features as distinct from one another). Paradigms that test mnemonic discrimination offer a robust empirical framework by which hippocampal function can be assessed. Indeed, much work has already been done using this framework including the assessment of changes in neurocognitive aging (Stark et al., 2010; Toner et al., 2009; Yassa & Stark, 2011; Yassa, Lacy, et al., 2011), mild cognitive impairment (Yassa et al., 2010), perforant path degradation (Yassa et al., 2010; Yassa, Mattfeld, et al., 2011), and neurogenesis loss of function (Clelland et al., 2009) as well as gain of function (Sahay et al., 2011). Overall, we find that applying a pattern separation framework to emotional modulation of memory serves two important purposes. First, it offers a potential mechanistic account
of how emotional memories are formed. Second, it provides further insight into states that show core vulnerability in the medial temporal lobes such as depression, age-related cognitive impairment, and late-life depression. In the next several sections, I will summarize the results from the experiments presented here and discuss their impact and relevance more broadly.

Asymmetries in emotional modulation of memory

Animal and human studies have reliably demonstrated an enhancing effect of emotion on memory, where both positive and negative experiences are typically better remembered than neutral experiences (Brown & Kulik, 1977; McGaugh, 2004). These effects have been attributed to the amygdala’s influence on the hippocampus through its release of norepinephrine in the BLA (McGaugh, 2004). However, not all aspects of an emotional memory are enhanced. In fact, some components of an emotional memory, namely the details of the experience, are impaired relative to neutral stimuli (Kensinger, 2009; Mather & Sutherland, 2011). This gist versus detail trade-off can be observed readily in phenomena such as the “weapon focus” effect, where victims or eyewitnesses often recall the weapon used in a crime but fail to encode (or perhaps more quickly forget) peripheral details such as the perpetrator’s clothing (Loftus et al., 1987). Typically, the effect of emotion on memory is exaggerated after a delay, once consolidation has occurred (Hamann, 2001; McGaugh, 2004). The mechanism underlying the gist versus detail trade-off of emotional memory remains elusive. We applied the pattern separation framework to examine the asymmetry in emotional
modulation of memory by using an emotional mnemonic discrimination task in combination with high-resolution fMRI. Behaviorally, we found that when tested immediately, emotional discrimination was selectively impaired relative to neutral discrimination, an effect that was exaggerated after 24 hours. Emotional target recognition, however, was relatively preserved. From these results, it appears that emotion plays at least two distinct roles in modulating memory strength: 1) an impairment of detail-based discrimination when tested immediately, and 2) a selective retention of gist information and forgetting of detail information over a 24-hour period (presumably due to an effect on consolidation). This is consistent with prior reports of asymmetry in emotional memory in humans (Kensinger, 2009; Loftus et al., 1987), but our paradigm allows us to investigate the mechanism by which this occurs (i.e. emotional modulation of pattern separation).

Emotional pattern separation

To investigate medial temporal lobe dynamics during performance of the emotional mnemonic discrimination task, we conducted high-resolution fMRI (1.5 mm isotropic) and found that the DG/CA3 subfield of the hippocampus was sensitive to discriminating highly similar negative information over neutral information, an effect that was limited to accurate discrimination and not false alarms. This suggests that the DG/CA3 is using emotional information in order to successfully discriminate similar experiences and is capable of resolving emotional interference (i.e. emotional pattern separation). The amygdala was also modulated by emotion; however, this signal manifested regardless
of the accuracy of discrimination. We interpret these results in light of what is known about amygdala and hippocampal anatomy and function. We suggest that the amygdala sends a generalized arousal signal with emotional information to the hippocampus, which is used by the DG/CA3 to modulate the strength of the pattern separation response for emotional information. Thus, the DG/CA3 may combine the amygdala-driven emotional response with a mnemonic response to accurately resolve interference. These results suggest that the emotional mnemonic discrimination task is a sensitive task that can be used in combination with high-resolution fMRI to detect emotional pattern separation signals that implicate the amygdala and the hippocampal DG/CA3 subfield.

**Emotional memory alterations in memory and mood disorders**

Beyond gaining insight into basic amygdala-hippocampal functioning, we aimed to determine if the emotional mnemonic discrimination task was sensitive to states in which emotional memory processing was altered. Young adults with depression show a negativity bias in memory (Airaksinen et al., 2007). There have been mixed findings in older adults, where some studies have shown a positivity bias in aging (Mather & Carstensen, 2005), while other studies have not and even show the opposite effect (Foster et al., 2012). Furthermore, amygdala and hippocampal alterations (both structural and functional) in aging and depression have been found previously (Leal & Yassa, 2015a; Sheline, 2011). We hypothesized that the emotional mnemonic discrimination task would be a sensitive behavioral measure to characterize dysfunction
in emotional memory processing and an appropriate task to evaluate altered amygdala-hippocampal network activity during high-resolution fMRI across mood and memory disorder conditions.

**Hippocampal-amygdala dynamics during emotional pattern separation in depression**

Individuals with depression are known to have alterations in emotional memory processing and exhibit changes in the amygdala-hippocampal network. While past studies have observed a negativity bias in depression, the interpretation has traditionally been that this is due to an overgeneralization of negative information (Fulford et al., 2011). However, results from our paradigm offer an alternative account. In individuals with depressive symptoms, neutral discrimination was impaired, consistent with recently reported results using an object discrimination task (Shelton & Kirwan, 2013). At the same time, negative discrimination was enhanced, and the degree of such enhancement was correlated with symptom severity. It is possible that this emphasis on negative details is associated with the mood dysregulation that is characteristic of depression. Overemphasizing negative details can come at the cost of processing neutral or positive information, and thus may affect processing stimuli across a wide range of experiences and may be linked to some of the psychiatric symptoms experienced by depressive patients. Indeed, we found that there was a positive association between depressive symptoms and the increase in negative discrimination on an individual basis.
To examine amygdala-hippocampal alterations in individuals experiencing depressive symptoms that may underlie the behavioral effects noted above, we conducted high-resolution fMRI during performance on the emotional discrimination task. During negative discrimination, activity in the DG/CA3 subfield of the hippocampus was decreased, while activity in the amygdala was increased in individuals with depressive symptoms, suggesting that the amygdala may be driving the enhancement of negative discrimination. The level of DG/CA3 activity was negatively correlated with depressive symptom severity, suggesting that reduced DG/CA3 activity was a pathological condition. This suggests that enhanced processing of negative information may be due to a network imbalance between the amygdala and the DG/CA3 where the influence of the amygdala is increased and the influence of the DG/CA3 is decreased. The most parsimonious explanation of the DG/CA3 diminished signal and the heightened amygdala activity is that the amygdala’s activity may have enhanced processing of negative information that facilitated discrimination in the absence of the normal DG/CA3 response. Thus, we surmise that a discrimination behavioral response can be the result of either effective pattern separation in the DG/CA3 or enhanced processing by the amygdala. Whether this effect is driven by impairment in the amygdala, DG, CA3, or all of the regions is still unknown, although data from animal studies suggest that structural changes in the CA3 may be critical (Conrad et al., 1996; Conrad, LeDoux, Magariños, & McEwen, 1999; Vyas et al., 2002; Watanabe et al., 1992). Reductions in DG neurogenesis may also contribute to this effect (Dranovsky & Hen, 2006). Altogether, these data suggest that reduced DG/CA3 activity may be a useful biomarker to test the
efficacy of drugs that may target the hippocampal system in MDD. Recent data has suggested that one mechanism by which selective serotonin reuptake inhibitors (SSRIs) may reduce memory symptoms in those with depression is by improving DG neurogenesis (Samuels, Leonardo, & Hen, 2015; Treadway et al., 2015). Thus, the use of the task, as well as the fMRI biomarker may be useful for future proof of concept trials in this cognitive space.

**Hippocampal-amygdala dynamics during emotional pattern separation in aging and subclinical memory impairment**

Episodic memory deficits are commonly reported in older adults, but the extent to which these deficits manifest across different types of information remains elusive. Data across studies have been mixed, with some showing preservation of emotional memory in aging, mostly for positive information. There is increased variability in the degree of memory impairment in aging (Gallagher et al., 2006), suggesting that an *individual differences* approach may expose important relationships. We found that older adults were impaired on neutral target recognition but intact on emotional target recognition. We also found that the pattern we found in young adults (reduced emotional compared to neutral discrimination of similar items) was reversed in older adults. The shift in emotional modulation could be due to at least two possible explanations: 1) a compensatory effect such that emotional arousal can boost discrimination performance on similar items and help increase memory for more important emotional events or 2) an aberration of emotional-mnemonic processing in older adults such that the increased
emotional discrimination is actually maladaptive, since it would be better to remember
the gist for emotional events rather than the details. Young adults’ discrimination (ability
to suppress false recognition) was enhanced on neutral items compared to emotional
items presumably due to a trade-off between gist and detail. Thus, it may be more
adaptive to forget minute details of emotional experiences in favor of retaining the
bigger picture (Adolphs et al., 2001; Kensinger, 2009; Loftus et al., 1987). Older adults,
on the other hand, appear to suppress false alarms better for emotional items,
suggesting that they may be engaging in a more costly mnemonic operation without
clear adaptive value.

During high-resolution fMRI, we tested cognitively normal older adults that we stratified
into aged-unimpaired (AU) and aged-impaired (AI) groups to investigate individual
differences in memory performance in aging. We found signals consistent with
emotional pattern separation in the DG/CA3 in AU but not in AI individuals, suggesting a
loss of emotional pattern separation in subclinical memory impairment. During false
recognition, we found increased DG/CA3 activity in AI compared to AU, regardless of
emotional content, supporting previous findings of dysfunctional hippocampal
hyperactivity (Bakker et al., 2012; El-Hayek et al., 2013). The BLA showed increased
activity for negative versus neutral stimuli during false recognition. Furthermore, we
found that the relationship between BLA and DG/CA3 activity during false recognition
depended on the level of subclinical memory impairment. To investigate amygdala-
hippocampal network connectivity, we measured functional connectivity to examine
indirect and direct amygdala-hippocampal connectivity and well as intra-hippocampal
connectivity. AI individuals showed a selective deficit in the indirect pathway (BLA—LEC—DG/CA3) during negative discrimination, consistent with a loss in pattern separation ability. We found evidence of hyperconnectivity in the AI group across the MTL during negative false recognition, suggesting overgeneralization in the hippocampal and amygdala network.

Our findings of altered emotional pattern separation in aging are a significant addition to what is known about pattern separation impairment in aging and further characterize MTL dysfunction in aging. We find that 1) emotional pattern separation is preserved in cognitively healthy older adults, 2) emotional pattern separation is impaired in older adults with subclinical memory impairment, 3) decreased connectivity in the BLA—LEC—DG/CA3 indirect pathway is specific to older adults with age-related memory impairment. This suggests that healthy young and older adults have intact emotional pattern separation. Previous work from our laboratory has suggested that older adults with subclinical memory impairment are more likely to show degradation in perforant path integrity (Yassa, Muftuler, & Stark, 2010), DG/CA3 representational rigidity (i.e. failure of pattern separation) (Yassa et al., 2011) as well as DG/CA3 hyperactivity (Yassa et al., 2010). In the current work, we complement these findings by demonstrating that emotional pattern separation in the DG/CA3 is impaired in older adults with subclinical memory impairment and that the indirect pathway from BLA to LEC and from LEC to DG/CA3 appears to be compromised in the same individuals. The latter could be stemming at least in part from the previously reported deficits in perforant path integrity, but additionally implicates connectivity with the BLA.
Hyperactivity during false recognition is consistent with previous literature (Yassa et al., 2011), however, our findings add three major contributions: 1) the effect seems to be generalized such that hyperactivity exists for negative and neutral information, 2) hyperactivity is only evident in those with individuals with subclinical memory impairment, and 3) hyperconnectivity between MTL regions occurs only during negative false recognition, consistent with faulty overgeneralization. This further supports the notion that DG/CA3 hyperactivity in aging is a dysfunctional condition.

**Hippocampal-amygdala dynamics during emotional pattern separation in late-life depression**

We also investigated the impact of depressive symptoms on memory performance in older adults, as both depression and aging influence MTL function. While both aging and depression are associated with episodic memory deficits, not all types of information may be subject to loss and forgetting. We found that older adults with depressive symptoms had reduced DG/CA3 activity during negative discrimination, but increased DG/CA3 activity during neutral discrimination compared to healthy older adults. Increased DG/CA3 activity during neutral discrimination was correlated with depressive symptom severity. Furthermore, increased BLA activity during neutral discrimination led to increased DG/CA3 activity, which was related to greater depressive symptoms. This suggests that BLA—DG/CA3 activity is linked such that hyperactivity in these regions during neutral discrimination is associated with greater symptom severity.
This again supports the notion that in older age, DG/CA3 hyperactivity may be a pathological condition, perhaps secondary to the loss in inhibition frequently noted in animal studies (Cadiacio, Milner, Gallagher, & Pierce, 2003; Spiegel, Koh, Vogt, Rapp, & Gallagher, 2013; Stanley & Shetty, 2004; Vela, Gutierrez, Vitorica, & Ruano, 2003).

During positive false recognition, the LEC showed increased activity in older adults with depressive symptoms, and its interaction with the BLA predicted depressive symptom severity. This suggests that the BLA—LEC interaction in late-life depression appears to be dysfunctional. Selectivity of network dysfunction only during positive false recognition in late-life depression may be due to the LEC’s connection with ventral tegmental area dopamine neurons, which are associated with reward-related stimuli (Hutter & Chapman, 2013). Furthermore, as positive stimuli seem to be treated differently in older adults (Mather & Carstensen, 2005), a dysfunction specific to positive stimuli may underlie the phenotype of depression in late life. Finally, while overall older adults with depressive symptoms showed less DG/CA3 activity, the effect of DG/CA3 and BLA on negative lure discrimination depended on the level of depressive symptoms where greater depressive symptoms were associated with higher DG/CA3 and BLA activity. This suggests that increased activity in this network may be associated with higher levels of psychopathology, consistent with the idea that it is an aberrant (and perhaps reversible) condition.

Amygdala-hippocampal dynamics, especially at high-resolution capable of resolving subfields, have never been previously investigated in late-life depression. This was a
novel avenue of investigation that significantly informed us about emotional memory processing in late-life depression. We found that 1) older adults with depressive symptoms show a different amygdala-hippocampal activity profile than older adults without depressive symptoms, and 2) the interactions of the BLA, LEC, and DG/CA3 depended on the emotional context and depression symptom severity. While aging is associated with MTL deficits, aging in combination with depression yields quite a different manifestation of neural signals. These differences are exposed in further detail when examining the type of information being remembered. Namely, when discriminating neutral information, mediation analyses demonstrate that the BLA acts in concert with the DG/CA3 in older adults with high depressive symptoms. This suggests that older adults require the BLA and DG/CA3 to act in concert in order to show accurate discrimination. During positive false recognition, the BLA acts in concert with the LEC in older adults with high depressive symptoms. The latter interaction is associated with the generalization that seems to occur in those with depressive symptoms. Furthermore, BLA and DG/CA3 predict negative discrimination, but depends on the level of symptom severity such that greater depressive symptoms are associated with greater BLA and DG/CA3 activity. These findings suggest that different sub-networks are selectively engaged during emotional discrimination and generalization and expose vulnerabilities in both networks in those with late-life depression.

Implications of neurogenesis on emotional pattern separation
The DG/CA3 alterations we find in depression, age-related cognitive impairment, and late-life depression may in part be due to underlying alterations in neurogenesis, as all these conditions have been previously associated with impaired neurogenesis in rodent models. Recent studies have shown that DG newborn granule cells may play a role in pattern separation (Aimone et al., 2011; Deng et al., 2010). Ablation of DG neurogenesis impairs behavioral discrimination (Clelland et al., 2009), whereas enhancing neurogenesis improves it (Sahay et al., 2011). Inhibiting immature granule cells results in pattern separation deficits whereas inhibition of old granule cells does not (Nakashiba et al., 2012). DG neurogenesis is also important for emotional memory formation (Hernández-Rabaza et al., 2009; Kitamura et al., 2009; Saxe et al., 2006). BLA lesions suppress DG neurogenesis as well as activation of immature cells in a fear-conditioning task (Kirby et al., 2012). Importantly, DG neurogenesis is also implicated in depression and the action of anti-depressants (Dranovsky & Hen, 2006; Sahay & Hen, 2007; Samuels & Hen, 2011). Reduction in the rate of neurogenesis in the adult DG is one hypothetical explanation of hippocampal volume loss in depression (Dranovsky & Hen, 2006). It has also been reported that adult-born granule cells in the DG buffer the ability of this region to counteract stress. A recent study showed that inhibiting adult neurogenesis in the DG resulted in HPA axis dysregulation whereby recovery from glucocorticoids was slower and depressive symptoms were readily apparent (e.g. increased food avoidance in a novel environment after acute stress, increased behavioral despair in the forced swim test, and decreased sucrose preference, a measure of anhedonia) (Snyder et al., 2011). Anti-depressants suppress the toxic effects on the hippocampus and increase hippocampal neurogenesis and synaptic
plasticity, however, there are neurogenesis-independent mechanisms of antidepressant action (MacQueen & Frodl, 2011). While neurogenesis is an attractive mechanism by which to alter memory and mood, a reduction in neurogenesis does not seem to be the major cause of the volume reduction. Rates of neurogenesis in adults are relatively low, making it unlikely that such small alterations can significantly contribute to the 10-15% reduction in entire hippocampal volume (Czéh & Lucassen, 2007).

It has been proposed that the age-related decline in neurogenesis may underlie memory deficits and could contribute to pathological conditions such as AD (Donovan et al., 2006; Haughey, Liu, Nath, Borchard, & Mattson, 2002; Haughey, Nath, et al., 2002). While neurogenesis may contribute to DG function, neurogenesis alone is not sufficient to preserve function during normal aging (Galvan & Jin, 2007), and is not correlated with the extent of age-related memory impairments (Bizon & Gallagher, 2003). More recently, nuclear-bomb-test-derived [14]C in genomic DNA was to used assess neurogenesis rates throughout the lifespan and found that rates are comparable in middle-aged humans and mice, with only a modest decline during aging (Spalding et al., 2013). Assessing the role of declining neurogenesis in age-related memory impairment requires the development of sensitive techniques that are capable of assessing the state of newborn granule cells in vivo. Despite some modest success using spectroscopic techniques (Manganas et al., 2007), new tools need to be developed to dynamically assess and track neurogenesis in humans in vivo, such that the contributions of neurogenesis to memory and cognition in humans can be evaluated.
and how this could impact therapeutic interventions aimed to reverse mood alterations and age-related cognitive decline.

**Core vulnerability in amygdala-hippocampal network in disorders of mood and memory**

We suggest that the amygdala-hippocampal system described herein, which expresses alterations in depression, age-related memory impairment, and late-life depression, is a system that is vulnerable to a wide array of brain pathologies and conditions, including other psychiatric disorders such as schizophrenia and bipolar disorder, as well as age-related decline such as MCI and AD. It appears that one of the commonalities among all of these conditions is that they impose a type of chronic stress on the hippocampal formation and its extended networks that impacts memory function. Across the different brain pathologies and conditions, there are areas of overlap and areas of dissimilarity (Table 7.1). For example, both depression and age-related memory impairment are associated with shifts in amygdala-hippocampal dynamics, but the way they shift and interact with one another is unique. In depression, there is hypoactivity in the DG/CA3 and hyperactivity in the amygdala. However, in aging, amygdala hyperactivity appears to be limited to false recognition. The emotional mnemonic discrimination task with concomitant high-resolution imaging allows us to tease apart subtle MTL differences in network functioning and connectivity in mood and memory disorders.
Table 7.1. Differences and similarities between groups during emotional mnemonic discrimination

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<th>Comparison</th>
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<td>Healthy young vs Healthy old</td>
<td>1. Young show impaired emotional discrimination, old show preserved discrimination.</td>
<td>1. Both show preserved emotional recognition. 2. Both show DG/CA3 emotional pattern separation.</td>
</tr>
<tr>
<td>Depressed young vs Depressed old</td>
<td>1. Young show impaired neutral recognition and discrimination, but enhanced negative discrimination, while old show impaired discrimination overall. 2. Young show amygdala negative hyperactivity while old show DG/CA3 neutral hyperactivity.</td>
<td>1. Both show positive correlations with BDI and negative discrimination. 2. Both show DG/CA3 negative hypoactivity.</td>
</tr>
<tr>
<td>Healthy old vs age-impaired old</td>
<td>1. Healthy show DG/CA3 emotional pattern separation, while age-impaired show impaired DG/CA3 emotional pattern separation. 2. Healthy show preserved amygdala-hippocampal functional connectivity, while age-impaired show impaired BLA—LEC—DG/CA3 connectivity (negative discrimination) and hyper connectivity during negative false recognition.</td>
<td>1. No differences on discrimination performance. 2. Both show preserved BLA—CA1 (direct) and DG/CA3—CA1 (intra-hippocampal) connectivity.</td>
</tr>
<tr>
<td>Healthy old vs Depressed old</td>
<td>1. Healthy old show preserved emotional discrimination, while depressed old show impaired discrimination overall. 2. Healthy old show emotional pattern separation, while depressed old show DG/CA3 negative hypoactivity and neutral hyperactivity. 3. Healthy old show lower DG/CA3 and BLA activity, while depressed old show higher DG/CA3 and BLA activity during negative discrimination.</td>
<td>None detected</td>
</tr>
</tbody>
</table>

These unique activity profiles during performance on the emotional mnemonic discrimination task may be useful biomarkers when investigating memory and mood disorders. Furthermore, behavioral performance on the emotional mnemonic discrimination task may be a sensitive outcome measure to use to determine dysfunction in emotional memory processing.

Model of amygdala-hippocampal dynamics during emotional pattern separation

Based on our findings during emotional pattern separation, we propose conceptual models for healthy and depressed young adults, healthy and depressed older adults, and older adults with subclinical memory impairment. In healthy young individuals, the amygdala and the DG/CA3 may cooperate or at least act in concert during processing
of negative information (Fig 7.1a). However, in depression, amygdala activity is heightened and DG/CA3 activity is diminished, leading to an enhancement in processing negative information (Fig 7.1b). The model suggests that hippocampal output is heavily influenced by the amygdala. An appropriate potentiation of the amygdala’s response coupled with a similar response in the DG/CA3 may result in the adaptive ability to resolve interference in an emotional situation. Absent the DG/CA3 response, the same potentiation results in generalization and an inability to resolve the interference. In depression, an inappropriate potentiation of the amygdala, coupled with a diminished response in the DG/CA3 may result in a maladaptive hyper-discrimination of negative information that is consistent with depression symptomatology. While amygdala potentiation and hippocampal impairment have been previously observed in studies of depression, our study is the first to demonstrate that the hippocampal impairment is associated with specific DG/CA3 dysfunction.

In healthy older adults, the amygdala and the DG/CA3 may function in concert during processing of negative information such that the amygdala and hippocampus are functionally connected (via direct and indirect pathways) (Fig 7.1c). However, in subclinical memory impairment, BLA—LEC—DG/CA3 connectivity (indirect pathway) is weakened and associated with impaired emotional pattern separation (Fig 1.7d). The model suggests that while the amygdala’s direct connection with the hippocampus and intra-hippocampal connectivity is functioning normally, selective impairment of the amygdala to hippocampus through the EC (via the perforant path) occurs in subclinical memory impairment. When this pathway is functionally connected as in healthy older
adults, DG/CA3 signals consistent with emotional pattern separation exist. In subclinical memory impairment, selective weakening of this pathway may result in impaired emotional pattern separation. This is in line with previous studies showing perforant path degradation in age (Yassa et al., 2010; Yassa, Mattfeld, et al., 2011) and suggest that the LEC may be selectively impaired.

In depressed older adults, higher DG/CA3 and BLA activity yields better negative discrimination (Fig 7.1e). The model suggests that when depressed older adults process negative information, BLA and DG/CA3 activity is increased to promote negative discrimination.
Future directions

Resting state functional connectivity

The default mode network (DMN) is a network of regions that are active at rest (Gusnard & Raichle, 2001; Raichle et al., 2001) and typically include the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), precuneus, anterior cingulate cortex (ACC), parietal cortex, and the MTL, including the hippocampus (Buckner, Andrews-Hanna, & Schacter, 2008; Greicius & Menon, 2004). Alterations in the DMN have been implicated in aging (Leal & Yassa, 2013), depression (Drevets et al., 2008), and other neuropsychiatric and neuropathological disorders. Thus, examining this network would be informative in determining whether or not these populations show altered DMN activity that is related to activity during the emotional mnemonic discrimination task. Furthermore, performing higher resolution functional connectivity (1.8mm isotropic) would allow us examine functional connectivity at rest within hippocampal and amygdala subregions. In depression and aging, we might expect to see altered DMN activity compared to controls, which may be associated with impaired cognitive and neural functioning during the emotional mnemonic discrimination task.
Diffusion tensor imaging

Diffusion tensor imaging (DTI) could also provide useful information regarding white matter tracts that may be altered in depression, age-related cognitive impairment, and late-life depression. Fornix integrity is associated with mnemonic discrimination performance over the lifespan such that increased age is associated with impaired pattern separation, but not recognition (Bennett, Huffman, & Stark, 2015). These data suggest that limbic tract integrity can contribute to pattern separation ability. In depression, altered white matter microstructure in circuits connecting the PFC, the parietal lobe, and the limbic system have been found such that greater alteration in the white matter was associated with a more ruminative state (Zuo et al., 2012). These alterations could also contribute to the effects we see during performance of the emotional mnemonic discrimination task. Furthermore, applying ultrahigh resolution microstructural DTI (Yassa et al., 2010) to measure perforant path integrity would be very informative, as we find alterations in the BLA—LEC—DG/CA3 network, which is critically involved in emotional pattern separation.

Treatment with anti-epileptics or anti-depressants

We find some overlapping patterns of results in aging, depression, and late-life depression: 1) hyperactivity during false recognition and 2) altered DG/CA3 activity during negative discrimination. Across species, the data suggest that hippocampal hyperactivity is an index of network dysfunction and disinhibition and not evidence of adaptive compensation. A recent clinical trial used a low-dose of levetiracetam (LEV) in
individuals with amnestic MCI successfully reduced DG/CA3 hyperactivity and reversed deficits on the object mnemonic discrimination task (Bakker et al., 2012). Hippocampal hyperexcitability in cognitive decline is a generally reversible condition in both animals and humans (Bakker et al., 2012, 2015; Koh et al., 2014; Sanchez et al., 2012), which has immense translational potential for age-related memory loss and AD. We saw evidence of DG/CA3 hyperactivity in age-related cognitive decline (negative and neutral) and in late-life depression (neutral). There was also hyperactivity in young adults with depression, but this was only noted in the amygdala and not the DG/CA3. It is not clear whether anti-epileptic treatment would be helpful in this condition. During negative discrimination, we observed reduced DG/CA3 activity in young adults with depression, age-related cognitive decline, and in late-life depression. It is possible that anti-depressants may increase neurogenesis and alter DG/CA3 function when a stressor is present (Anacker et al., 2011; Malberg, Eisch, Nestler, & Duman, 2000). While examining DG/CA3 activity could inform us about neurogenesis, it is not a direct measurement of neurogenesis. Once spectroscopic techniques become more developed (Manganas et al., 2007) to assess neurogenesis in humans in vivo, we could perhaps determine the role of neurogenesis in emotional pattern separation and how it may be altered with anti-depressant use.

Comorbidity of depression and MCI/AD

Understanding how aging and depression interact to alter MTL function is important, especially since up to half of all patients with MCI or AD exhibit comorbid depressive
symptoms (Lopez et al., 2005). Late-life depression is also associated with an increased risk for dementia (Diniz et al., 2013). It has been suggested that there are three major explanations for the relationship between depression and cognitive decline: 1) depressive symptoms are an early prodrome or warning sign of dementia, rather than true depression alone, 2) depression may be a response to early cognitive loss in which recognition of memory problems leads to depression or 3) depression may have an etiological role in cognitive decline such that depression initiates neurological changes that lead to neurobiological changes, which places depressed individuals at greater risk for developing dementia (Panza et al., 2010; Sawyer et al., 2012). Furthermore, the number of depressive symptoms at baseline has been shown to predict development of AD. With each additional symptom, risk of disease increased by about 20% (Wilson et al., 2002). Furthermore, a synergistic interaction between depression and APOE4 has been associated with increased risk of developing MCI (Geda et al., 2006). Further investigation of the comorbidity between depression and aging, MCI, and AD is needed to aid in more targeted treatments for older adults with and without depressive symptoms with the goal of reducing the impact of depressive symptoms on development of cognitive decline.

Another avenue to explore is how amyloid and tau pathology may influence MTL function in comorbid depression and aging/MCI/AD. It is difficult to determine whether memory impairment in aging is a feature of “normal” aging or preclinical AD, which researchers have struggled with for some time (Fjell, McEvoy, Holland, Dale, & Walhovd, 2014; Jagust, 2013). How depression influences and is influenced by beta-
amyloid plaques (Aβ) and neurofibrillary tangle tau pathology (NFTs) in unknown. Tau pathology is evident early in the course of AD in the LEC (Khan et al., 2014; Yassa, 2014), which was one of the regions noted in our connectivity analysis of older adults with subclinical memory impairment as well as older adults with late-life depression. With the recent advent of tau PET imaging, examining tau pathology in older adults with late-life depression could be significantly informative.

Concluding remarks

The set of experiments described in this dissertation argue strongly that simply examining general episodic memory deficits is not sufficient to understanding the complex nature of the medial temporal lobe and the mechanisms by which it processes memories. Remembering significant events is a key component of MTL processing, as these memories are prioritized at the expense of remembering neutral, non-significant information. The emotional mnemonic discrimination task we developed allows us to examine the impact of emotion on memory interference both cognitively and neurobiologically. Furthermore, the task is sensitive to emotional memory and amygdala-hippocampal dysfunction in states of cognitive impairment such as depression, age-related cognitive impairment, and late-life depression. Each state of cognitive impairment yields a different cognitive and neurobiological profile that may be useful in targeting different aspects for treatment. With this in mind, future studies may be able to target therapeutics to modify the amygdala-hippocampal biomarkers we identified or cognitive performance on the emotional mnemonic discrimination task with implications for memory and mood disorders.
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Appendices

Appendix 1: Supplementary Information for Chapter 2 and 4

**Fig. S2.1. Valence, arousal, and similarity measures.** (a) Valence ratings across emotional category (N=50); (b) Arousal ratings across emotional category (N=16); (c) False alarm rates for stimuli in each emotional category stratified by similarity bin (N=17). See main text for analysis details.

**Fig. S2.2. Effect of stimulus similarity.** (a) Lure Discrimination Index in the immediate testing condition for low and high similarity stimuli (N=24); (b) Lure Discrimination Index in the 24-hour delayed testing condition for low and high similarity stimuli (N=14); (c) Lure Discrimination Index in the immediate testing condition for individuals with depressive symptoms (DS) for low and high similarity stimuli (N=15).
Fig. S2.3. Match-to-sample (MTS) task performance. (a) Target hit rate on the two MTS experiments when stimulus presentation time is 2500ms (solid bars) and 1000 ms (hatched bars). (b) Lure rejection rate on the two MTS experiments when stimulus presentation time is 2500ms (solid bars) and 1000 ms (hatched bars). There were no differences across emotion in either experiment (P > .05 for all comparisons). Sample sizes were N=18 in the 2500 ms condition and N=19 in the 1000 ms condition.

Fig. S2.4. Gender differences across studies. (a) Performance of healthy participants on immediate recognition test; (b) Performance of healthy participants on delayed recognition test; (c) Performance of participants with depressive symptoms (DS) on immediate recognition test. In all cases, target recognition (top) and lure discrimination (bottom) showed no main effect of gender and no interaction between gender and emotion.
Fig. S2.5. Reaction time data (in milliseconds) across all studies. (a) Healthy participant reaction time in the immediate recognition test. No significant main effects of emotion or similarity in lure false alarms (top) whereas in lure correct rejections there were significant main effects of both emotion and similarity (bottom); (b) Healthy participant reaction times in the delayed recognition test. No significant main effects of emotion or similarity either in lure false alarms (top) or lure correct rejections (bottom); (c) Participants with depressive symptoms’ reaction times in the delayed recognition test. No significant main effects of emotion or similarity either in lure false alarms (top) or lure correct rejections (bottom).

**SI Text**

**Analyses of gender differences across studies.** For the immediate test (13F, 8M), target recognition showed a significant main effect of emotion \[F(2, 38) = 5.05, \ P = .017\] but no significant effect of gender or interaction between gender and emotion. For lure discrimination, there was a significant effect of emotion \[F(2, 38) = 5.38, \ P = .009\]. There was no significant main effect of gender \[F(1, 19) = 1.96, \ P = .178\] and no interaction between gender and emotion. For this analysis, women on birth control were excluded from analysis.
For the 24-hour delayed test (5F, 8M), we conducted the same analyses of gender differences. For target recognition, there was a significant effect of emotion \([F(2,22) = 4.08, P = .04]\) but no significant effect of gender or interaction between gender and emotion. For lure discrimination, there was a main effect of emotion \([F(2,22) = 11.09, P = .001]\) but no significant effect of gender or interaction between gender and emotion.

For individuals with depressive symptoms (11F, 4M), target recognition showed a main effect of emotion \([F(2,26) = 4.72, P = .03]\) but no significant effect of gender or interaction between gender and emotion. Lure discrimination showed no significant effect of emotion, gender, or interaction between gender and emotion.

**Analyses of reaction time data across studies.** We analyzed reaction time data using separate repeated measures ANOVA for lure false alarms and correct rejections. On false alarms, there was no main effect of emotion, similarity, or interaction between emotion and similarity. On correct rejections there was a significant main effect of emotion \([F(2,46) = 3.27, P = .05]\), a significant main effect of similarity \([F(1,23)=6.77, P = .016]\), but no interaction between emotion and similarity. The effect of similarity is not unexpected given that high similarity items may be tougher to discern and may require additional processing time.

For the 24-delayed testing group, during lure false alarms there was no main effect of emotion, similarity, or interaction between emotion and similarity. During lure correct rejections, there was no significant main effect of emotion, similarity, or interaction
between emotion and similarity. The effect noted on immediate testing was no longer reliable at a delay, suggesting that even low similarity items may require more time to process after some forgetting has occurred.

For individuals with depressive symptoms, there was no main effect of emotion, similarity, or interaction between emotion and similarity for false alarms. For lure correct rejections, there was no main effect of emotion, similarity, or interaction between emotion and similarity. It is possible that individuals with depressive symptoms require additional time to process low similarity items and thus the effect of similarity is not present.

Appendix 2: Supplementary Information for Chapter 3

Table 3.1. Reaction times on mnemonic discrimination task (healthy young adults)

<table>
<thead>
<tr>
<th>Measures</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Lure FA</td>
<td>1026.14</td>
<td>43.68</td>
</tr>
<tr>
<td>Neutral Lure FA</td>
<td>1055.60</td>
<td>42.75</td>
</tr>
<tr>
<td>Positive Lure FA</td>
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<td>50.94</td>
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<td>Negative Lure CR</td>
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</tr>
<tr>
<td>Neutral Lure CR</td>
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<td>50.85</td>
</tr>
<tr>
<td>Positive Lure CR</td>
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<td>37.90</td>
</tr>
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<td>Negative Target Hit</td>
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<td>41.31</td>
</tr>
<tr>
<td>Neutral Target Hit</td>
<td>914.56</td>
<td>27.76</td>
</tr>
<tr>
<td>Positive Target Hit</td>
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<td>29.67</td>
</tr>
<tr>
<td>Negative Target Miss</td>
<td>1132.33</td>
<td>86.27</td>
</tr>
<tr>
<td>Neutral Target Miss</td>
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<td>77.87</td>
</tr>
<tr>
<td>Positive Target Miss</td>
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<td>70.82</td>
</tr>
<tr>
<td>Negative Foil CR</td>
<td>953.13</td>
<td>36.96</td>
</tr>
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<td>885.98</td>
<td>33.17</td>
</tr>
<tr>
<td>Positive Foil CR</td>
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<td>29.77</td>
</tr>
<tr>
<td>Negative Foil FA</td>
<td>979.43</td>
<td>57.31</td>
</tr>
<tr>
<td>Neutral Foil FA</td>
<td>1149.56</td>
<td>81.27</td>
</tr>
<tr>
<td>Positive Foil FA</td>
<td>1409.30</td>
<td>69.73</td>
</tr>
</tbody>
</table>
Fig S3.1. ROI activity profiles across conditions and hemispheres in healthy adults. ROI activity profiles in all ROI’s tested (CA1, DG/CA3, subiculum, amygdala) during correct rejection and false alarms of high and low similarity items divided by hemisphere and classified according to emotion. CR: Correct Rejection; FA: False Alarm. Error bars are ± S.E.M.
Appendix 3: 24-hour delay in healthy and depressed young adults

We measured target recognition and LDI in these participants and compared their performance to the DS group tested immediately and compared them to healthy young adults (both tested immediately and at a delay) using an group x emotion x time of testing ANOVA. For target recognition, we found a significant effect of emotion \([F(2,126) = 12.57, p<.001]\), where negative targets were better remembered than neutral or positive targets. There was also a significant effect of time \([F(1,63) = 7.77, p = .007]\), where target recognition was worse after 24 hours (Fig A1).

For lure discrimination, we found significant effects of emotion \([F(2,126) = 6.72, p = .002]\) and time \((F(1,63) = 49.4, p <.001]\), and significant interactions between emotion x group \([F(2,126) = 7.55, p = .001]\), emotion x time \([F(2,126) = 3.32, p =.04]\), group x time \([F(1,63) = 5.22, p = .026]\), and a marginal interaction between group x emotion x time \([F(2,126)= 2.97, p = .057, \text{Fig A2}].\)
Figure A2. Lure discrimination in healthy and depressed individuals tested immediately and 24 hours later.

We also examined whether symptom severity correlated with lure discrimination index across emotions. Both positive and negative LDI correlated with symptom severity, where the more depressive symptoms, the better at discriminating similar negative [Pearson r = .381, p=.055] and positive lures [Pearson r = .484, p = .012, Fig A3].

Figure A3. Correlation between BDI-II and lure discrimination measures.
Fig S4.1. ROI activity profiles across conditions and hemispheres in depressed adults. ROI activity profiles in all ROI’s tested (CA1, DG/CA3, subiculum, amygdala) during correct rejection and false alarms of high and low similarity items divided by hemisphere and classified according to emotion. CR: Correct Rejection; FA: False Alarm. Error bars are ± S.E.M.
Table S4.1. Reaction Times on Emotional Mnemonic Discrimination Task

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<th>SEM</th>
<th>DS</th>
<th>SEM</th>
</tr>
</thead>
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</tr>
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</tr>
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<td>33.17</td>
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<td>1409.30</td>
<td>69.73</td>
<td>1324.01</td>
<td>109.87</td>
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### Table S5.1. Raw Response Proportions on Emotional Mnemonic Discrimination Task

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<tr>
<th>Measures</th>
<th>Young immediate</th>
<th>Young delay</th>
<th>Old Immediate</th>
<th>Old delay</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
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</thead>
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<td>0.92</td>
<td>0.02</td>
<td>0.93</td>
<td>0.02</td>
<td>0.92</td>
<td>0.02</td>
<td>0.75</td>
<td>0.06</td>
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<td>0.87</td>
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<td>0.04</td>
<td>0.71</td>
<td>0.06</td>
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<td>0.03</td>
<td>0.87</td>
<td>0.03</td>
<td>0.73</td>
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<td>0.02</td>
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<td>0.02</td>
<td>0.22</td>
<td>0.04</td>
<td></td>
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<td>0.19</td>
<td>0.04</td>
<td>0.26</td>
<td>0.04</td>
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<td>0.03</td>
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<td>0.01</td>
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<tr>
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<td>0.002</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.12</td>
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</tr>
<tr>
<td>Positive Target FA</td>
<td>0.01</td>
<td>0.003</td>
<td>0.04</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
<td>0.13</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Target CR</td>
<td>0.997</td>
<td>0.002</td>
<td>0.97</td>
<td>0.01</td>
<td>0.96</td>
<td>0.01</td>
<td>0.82</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral Target CR</td>
<td>0.997</td>
<td>0.002</td>
<td>0.96</td>
<td>0.01</td>
<td>0.97</td>
<td>0.01</td>
<td>0.84</td>
<td>0.06</td>
<td></td>
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</tr>
<tr>
<td>Positive Target CR</td>
<td>0.99</td>
<td>0.003</td>
<td>0.96</td>
<td>0.01</td>
<td>0.96</td>
<td>0.01</td>
<td>0.84</td>
<td>0.05</td>
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<tr>
<td>Negative LS Lure FA</td>
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<td>0.02</td>
<td>0.39</td>
<td>0.03</td>
<td>0.35</td>
<td>0.03</td>
<td>0.42</td>
<td>0.06</td>
<td></td>
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<tr>
<td>Neutral LS Lure FA</td>
<td>0.13</td>
<td>0.02</td>
<td>0.22</td>
<td>0.03</td>
<td>0.31</td>
<td>0.03</td>
<td>0.41</td>
<td>0.04</td>
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<td>Positive LS Lure FA</td>
<td>0.15</td>
<td>0.02</td>
<td>0.38</td>
<td>0.04</td>
<td>0.31</td>
<td>0.04</td>
<td>0.42</td>
<td>0.05</td>
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<tr>
<td>Negative LS Lure CR</td>
<td>0.83</td>
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<td>0.61</td>
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<tr>
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<td>0.62</td>
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<td>0.69</td>
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<tr>
<td>Negative HS Lure FA</td>
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<td>0.62</td>
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<tr>
<td>Neutral HS Lure FA</td>
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<td>0.44</td>
<td>0.03</td>
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<tr>
<td>Positive HS Lure FA</td>
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<td>0.02</td>
<td>0.53</td>
<td>0.04</td>
<td>0.49</td>
<td>0.05</td>
<td>0.49</td>
<td>0.05</td>
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<tr>
<td>Negative HS Lure CR</td>
<td>0.62</td>
<td>0.04</td>
<td>0.38</td>
<td>0.04</td>
<td>0.42</td>
<td>0.04</td>
<td>0.35</td>
<td>0.04</td>
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<tr>
<td>Neutral HS Lure CR</td>
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<td>0.56</td>
<td>0.03</td>
<td>0.55</td>
<td>0.04</td>
<td>0.51</td>
<td>0.05</td>
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</tr>
<tr>
<td>Positive HS Lure CR</td>
<td>0.70</td>
<td>0.02</td>
<td>0.47</td>
<td>0.04</td>
<td>0.51</td>
<td>0.05</td>
<td>0.50</td>
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</table>
Figure S5.1. Reaction time (RT) from immediate (a) and 24-hour delay testing (b).
Figure S5.2. Match-to-sample task performance in young and older adults. (a) Older adults perform better than young adults on target hit rate; (b) No differences in lure rejection rate across age.

Table S5.2. Lure Discrimination Index on Emotional Mnemonic Discrimination Task

<table>
<thead>
<tr>
<th>Measures</th>
<th>Overall (Mean, SEM)</th>
<th>AU (Mean, SEM)</th>
<th>AI (Mean, SEM)</th>
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</thead>
<tbody>
<tr>
<td>Negative LDI</td>
<td>0.39, 0.04</td>
<td>0.38, 0.06</td>
<td>0.42, 0.03</td>
</tr>
<tr>
<td>Neutral LDI</td>
<td>0.47, 0.04</td>
<td>0.47, 0.05</td>
<td>0.47, 0.06</td>
</tr>
<tr>
<td>Positive LDI</td>
<td>0.40, 0.03</td>
<td>0.41, 0.04</td>
<td>0.40, 0.05</td>
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</tbody>
</table>

Table S5.3. Lure Discrimination Index on Emotional Mnemonic Discrimination Task

<table>
<thead>
<tr>
<th>Measures (RT)</th>
<th>Overall (Mean, SEM)</th>
<th>AU (Mean, SEM)</th>
<th>AI (Mean, SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative CR</td>
<td>1380, 35</td>
<td>1343, 46</td>
<td>1427, 51</td>
</tr>
<tr>
<td>Neutral CR</td>
<td>1329, 38</td>
<td>1311, 54</td>
<td>1350, 53</td>
</tr>
<tr>
<td>Positive CR</td>
<td>1389, 41</td>
<td>1371, 63</td>
<td>1411, 50</td>
</tr>
<tr>
<td>Negative FA</td>
<td>1406, 32</td>
<td>1384, 45</td>
<td>1433, 45</td>
</tr>
<tr>
<td>Neutral FA</td>
<td>1379, 35</td>
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</tr>
<tr>
<td>Positive FA</td>
<td>1334, 34</td>
<td>1320, 48</td>
<td>1351, 50</td>
</tr>
</tbody>
</table>
Figure S5.3. ROI activity profiles across conditions and hemispheres in older adults. ROI activity profiles in all ROI’s tested (DG/CA3, CA1, SUB, BLA, CEA/CORT, LEC, and MEC) during correct rejection and false alarms of lure items and classified according to emotion. CR: Correct Rejection; FA: False Alarm. Error bars are ± S.E.M.
Figure S5.4. ROI activity profiles across conditions and hemispheres in AU and AI groups. ROI activity profiles in all ROI's tested (DG/CA3, CA1, SUB, BLA, CEA, LEC, and MEC) during correct rejection and false alarms of lure items and classified according to emotion. CR: Correct Rejection; FA: False Alarm. Error bars are ± S.E.M.
Appendix 6: Development of the Emotional Logical Memory Test to examine individual differences in age-related memory impairment

Introduction

In addition to developing the emotional mnemonic discrimination task to understand how gist versus detail information is remembered, we have developed an emotional version of The Logical Memory Subset of the Wechsler Memory Scale (WMS) to further investigate memory mechanisms in aging. In the original version, participants are read two stories and are tested on their memory of the stories immediately and after a delay. The stories do not assess or control for emotional content, even though there are emotional components in the stories. We developed a modified version of Logical Memory Subset of the WMS to assess and control emotional content by creating negative, neutral, and positive stories while all other components are matched (e.g. number to details, sentence structure, etc.). Numerous studies have suggested that older adults preferentially remember positive information ("positivity effect"), however others have reported mixed results. One potential source of conflict is that aging is not a unitary phenomenon and individual differences exist. Modification of this commonly used neuropsychological test will aid in fully understanding how verbal information is recalled in aging and how memory for gist and detail information shifts over time. We have also added a one-week delay to further investigate alterations in memory over time.
Materials and Methods

Participants. Thirty-two participants (N = 32, 21 female; mean age 74.8 + 4.4SD, range = 63-83) were recruited from the local Orange County community via local campus announcements, flyers, and ads in local newspapers. Participants were split based on their delayed recall performance on the RAVLT into AU (N=16, 11 female; mean age 75.7 + 3.9 SD, range = 68-83) and AI (N=16, 10 female; mean age 73.8 + 4.8 SD, range = 63-81, RAVLT score ≤ 11). Informed consent was obtained from all participants, with all procedures approved by the University of California, Irvine Institutional Review Board. All participants were screened against major medical or psychiatric morbidities as well as substance abuse history. All participants had normal or corrected-to-normal vision.

Inclusion/exclusion criteria. Neuropsychological battery is identical to the one described in the previous section and results are shown in Table B1. Importantly, the AI group did not present with memory complaints, nor did they present with memory deficits sufficient for a diagnosis of clinical impairment. The particular selection of the RAVLT to split participants into AU and AI groups was motivated by the fact that it is a hippocampus-sensitive and a highly standardized neuropsychological test (Estévez-González et al., 2003). Furthermore, recent modeling work using the AD Neuroimaging Initiative suggests that changes in RAVLT performance are found very early in the clinical/pathological progression of AD, even prior to detectable changes in amyloid pathology (Jedynak et al., 2012). There were no significant differences between the
older adult groups in age, education, and all other neuropsychological measures with
the exception of the RAVLT. All participants had normal or corrected to normal vision.

Table B1. Demographics and Neuropsychological Test Results

<table>
<thead>
<tr>
<th>Groups</th>
<th>AU</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>M:F</td>
<td>5:11</td>
<td>6:10</td>
</tr>
<tr>
<td>Age</td>
<td>75.7</td>
<td>73.8</td>
</tr>
<tr>
<td>Variables</td>
<td>Mean, SEM</td>
<td>Mean, SEM</td>
</tr>
<tr>
<td>Digit Span Forward</td>
<td>9.8, 0.5</td>
<td>9.8, 0.6</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>6.7, 0.5</td>
<td>6.0, 0.5</td>
</tr>
<tr>
<td>Letter-Number Sequencing</td>
<td>17.4, 0.7</td>
<td>17.1, 0.8</td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
<td>0.8, 0.4</td>
<td>1.6, 0.4</td>
</tr>
<tr>
<td>Mini Mental State Exam</td>
<td>28.6, 0.3</td>
<td>27.9, 0.4</td>
</tr>
<tr>
<td>*RAVLT Immediate Recall</td>
<td>13.1, 0.3</td>
<td>8.3, 0.5</td>
</tr>
<tr>
<td>*RAVLT Delayed Recall</td>
<td>13.1, 0.3</td>
<td>7.9, 0.6</td>
</tr>
<tr>
<td>*RAVLT Recognition Recall</td>
<td>14.3, 0.2</td>
<td>12.5, 0.5</td>
</tr>
<tr>
<td>Trail Making Test A</td>
<td>31.6, 2.0</td>
<td>29.0, 2.3</td>
</tr>
<tr>
<td>*Trail Making Test B</td>
<td>78.7, 5.9</td>
<td>82.8, 9.2</td>
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<tr>
<td>*Stroop (Word-Color)</td>
<td>30.4, 2.4</td>
<td>30.5, 1.8</td>
</tr>
<tr>
<td>Beck Anxiety Inventory</td>
<td>3.8, 1.4</td>
<td>6.0, 1.3</td>
</tr>
<tr>
<td>Beck Depression Inventory-II</td>
<td>3.8, 0.8</td>
<td>3.1, 0.7</td>
</tr>
<tr>
<td>Hours of Sleep (night before)</td>
<td>7.5, 0.4</td>
<td>3.1, 0.7</td>
</tr>
</tbody>
</table>

**Emotional Logical Memory Task:** There were three stories used: the negative story
was very similar to the original story from the WMS, while the neutral and positive story
were novel and matched for gist and detail information and sentence structure. Each
story had a word count between 60-70 words and was three sentences long. Each story
was rated for valence and arousal on a scale by a separate group of participants (N =
28, mean age = 22.32, 14 female). Emotional arousal was rated using a 1-9 scale,
where 1 was not arousing at all (calm) to 9 being very arousing (exciting). Emotional
valence was rated using a 1-9 scale, where 1 was the most negative, 9 was the most
positive, and 5 was neutral. For emotional arousal ratings, a repeated-measures ANOVA with emotional valence as a fixed factor revealed a significant effect of emotion \([F(2,54) = 36.67, p < .001; \text{Fig B1a}]\). Negative and positive stories were found to be more arousing than neutral stories revealed via quadratic trend \([F(1,27) = 72.41, p < .001]\). For emotional valence ratings, an ANOVA with emotion as a fixed factor also revealed a significant effect of emotion \([F(2,54) = 143.66, p < .001; \text{SI Fig B1b}]\). There was a linear trend with negative valence receiving the lowest rating, with an increase in rating for neutral valence, and an increase in rating for positive valence \([F(1,27) = 156.99, p < .001]\).

![Figure B1. Arousal (a) and valence (b) ratings for negative, neutral, and positive story. Both were rated on a 1-9 scale.](image)

**Administration details:** After each story was verbally read to participants, they were instructed to repeat the story from memory as accurately as possible. Each story was read twice, and participants were asked to repeat as much as they could remember from the story after each reading (immediate recall, Imm). Responses were recorded and assessed for specific information (detail) and thematic information (gist) consistent with the WMS-III. There were a total of 25 possible points for detail information and 7 points for gist information for each story. The order of the stories was randomized.
across participants. Half of the neuropsychological battery was completed in the first session following the immediate condition of the ELMT. After 20 minutes of neuropsychological testing, participants were asked to recall as much as they could remember from each of the three stories (20-minute delay, 20m). Responses were recorded as described above. Participants returned to the lab one week later, and were again asked to recall the three stories (1-week delay, 1wk). The second half of the neuropsychological battery was then administered during the second visit (Fig B2a).

**Logical Memory Stories:**

**Story A (Negative):** Anna Thompson of South Boston, employed as a cook in a school cafeteria, reported at the police station that she had been held up on State Street the night before and robbed of fifty-six dollars. She had four small children, the rent was due, and they had not eaten for two days. The police also had a murder case that night and so were unable to help her.

**Story B (Neutral):** Joe Garcia of San Francisco, a retired college English professor, was watching the news as he dressed to go to the post office to mail his electric and water bills which were three days late. He put on his black coat, sealed the envelopes, and left his home. He had thirty minutes until the post office opened so he decided to walk instead of drive.

**Story C (Positive):** Margret Pitcher of Chicago, a paralegal in a law firm, drove to the hospital early in the morning to see her niece being born. She arrived at the hospital with much excitement just as her sister delivered the baby. The doctor said it was a
healthy baby girl. Her sister and her husband decided to name the baby Sophia after her grandmother.

**Statistical Analyses:** All statistical analyses were conducted in SPSS v. 23.0 (IBM Corp., released 2015, Armonk, NY). Repeated measures ANOVAs were corrected for error non-sphericity using Greenhouse-Geisser correction where appropriate. Post hoc statistical tests were conducted using trend analyses of planned comparisons and were corrected for multiple comparisons. Statistical values were considered significant at a final corrected alpha level of .05, which appropriately controlled for Type I error.

**Results**

To investigate how emotion alters memory performance over time, we also added a one-week delayed test (Fig B2a). We hypothesized that by examining older adults with and without subclinical memory impairment, we might be able to gain insight into the basis of the positivity effect and provide a more detailed assessment of emotional memory and recall over time in older adults.
Figure B2. Performance on ELMT for gist and detail measures (a) Overall experimental schematic; (b) Overall memory performance (proportion correct) for gist and detail information in aging over time; (c) Forgetting rate (immediate – 1wk delay) calculated within each subject for gist and detail information; (d) Proportion correct for gist information when split by emotional content (negative, neutral, and positive) in aging over time; (e) Proportion correct for detail information when split by emotional content (negative, neutral, and positive) in aging over time; (f) Forgetting rate for gist and detail information split by emotional content.

We analyzed memory for gist and detail information (collapsing across emotion) by performing a 2-way ANOVA with memory (gist and detail) and time (immediate- Imm, 20 minute- 20m, one-week- 1wk) as within-subjects factors. We found a significant effect of memory [gist > detail; F(1,31) = 257.04, p < .001; Fig B2b] and a significant effect of time, [F(2,62) = 90.62, p < .001], with worse memory of information over time [F(1,31) = 111.01, p < .001]. We also found a significant interaction between memory and time [F(2,62) = 10.69, p = .001] with worse memory for detail information over time [F(1,31) = 16.99, p < .001].
We calculated a forgetting rate for each participant (the difference between performance on the immediate and 1-week delay tests) and found that there was more forgetting of detail information than gist information over one week [t(31) = 4.12, p < .001; Fig B2c]. Better memory (i.e. less forgetting) for the gist versus the details of a story as well as memory impairment over time are consistent with prior literature, suggesting that our task has external validity and can recapitulate known findings.

In a 3-way ANOVA including emotion as a factor, we observed an additional significant effect of emotion [negative > positive & neutral; F(2,62) = 34.15, p < .001], and an interaction between emotion and memory [F(2,62) = 32.22, p < .001; Fig B2d,e]. We further parsed this interaction and found that memory for negative gist information was superior to negative detail information, and this gist-detail relationship existed to a lesser extent for neutral and positive information [F(1,31) = 55.73, p < .001]. This suggests that while gist information is better remembered than detail information, the effect is greatest when the information is negative compared to either positive or neutral information. In comparison of forgetting rates, we observed a significant interaction between emotion and memory [F(2,62) = 3.44, p < .001; Fig B2f], with more forgetting of negative detail information compared to gist, which was not the case for positive and neutral gist information [F(1,31) = 7.96, p = .008]. This suggests that while negative information is better remembered overall compared to neutral and positive information, negative details are more likely to be forgotten after a one-week delay. These analyses
strongly suggest that both the emotional content of information and the amount of time passed influences the fidelity of memory in older adults.

Figure B3. Individual differences in memory performance. (a) Gist and (b) Detail remembering in aged-unimpaired (AU) and aged-impaired (AI) over time; Performance in AU adults split by emotional content for (c) gist and (d) detail information; Performance in AI adults split by emotional content for (e) gist and (f) detail information. See corresponding statistics.

To assess how emotional memory is altered in the course of age-related cognitive decline, we grouped our participants based on performance on the RAVLT delayed
recall test and created AU and AI groups using a median split (Gallagher et al., 1993; Stark et al., 2010). In a 3-way ANOVA with time and memory as within-subjects factors and group (AU and AI) as a between-subjects factor, we found a significant effect of group \(F(1,30) = 4.94, p = .03; \) Fig B3a,b, with worse memory in the AI group compared to AU. These findings are consistent with past work and with a generalized memory deficit in the AI group. We then conducted separate 3-way ANOVAs for gist and detail in order to examine the effect of emotion. For gist, we found a significant three-way interaction between emotion, time, and group \(F(4,120) = 4.52, p = .004; \) Fig B3c,d, where the AI group had worse memory of neutral gist information over time, whereas the AU group had worse memory of positive gist information over time \(F(1,30) = 12.34, p = .001\). There was a marginal effect of group \(F(1,30) = 3.46, p = .07\), where the AI group showed worse memory for gist information compared to AU. For detail information, we observed a significant effect of group \(F(1,30) = 5.96, p = .02; \) Fig B3e,f, where the AI group showed worse memory for detail information compared to AU. We also found a marginal interaction between emotion, time, and group \(F(4,120) = 2.5, p = .065\), where the AI group had worse memory for neutral detail information over time compared to the AU group. This is a similar pattern to what we saw for remembering gist information, although it was not as statistically reliable.
Figure B4. Forgetting rates for gist and detail information in aged-unimpaired (AU) and aged-impaired (AI) groups (a) Forgetting rate for gist information across AU and AI split by emotional content, (b) Forgetting rate for detail information across AU and AI split by emotional content. See corresponding statistics.

In comparing forgetting rates for gist information, we found a significant interaction between emotion and group \( [F(2,60) = 7.15, p = .002; \text{Fig B4a}] \). The AI group exhibited the most forgetting for neutral information, whereas the AU group exhibited the most forgetting for positive information \( [F(1,30) = 12.33, p < .05] \). For detail information, we found a significant interaction between emotion and group \( [F(2,60) = 3.28, p = .045; \text{Fig B4b}] \). Similar to the results for gist information, the AI group exhibited less forgetting of positive and more forgetting of neutral detail information than the AU group \( [F(1,30) = 5.74, p < .05] \). In a direct comparison of gist and detail, we found a significant 3-way interaction between emotion, memory, and group \( [F(2,60) = 3.44, p = .04] \). Post hoc contrasts showed that the effects noted above (AI exhibiting less forgetting of positive and more forgetting of neutral information than AU) were larger for gist than detail information \( [F(1,30) = 6.33, p = .02] \).
Overall, these findings suggest that relative to healthy older adults, those with subclinical memory impairment (i.e. AI) are more prone to forgetting neutral rather than emotional information. However, healthy older adults (i.e. AU) are more prone to forgetting positive gist information. These effects are similar across gist and detail information but appear to be more prominent in gist memory.

Discussion

While numerous neuropsychological assays have been used to assess memory impairments, emotional content has been largely ignored as a factor that can alter how memories are stored and recalled in neuropsychological tests. For example, the stories in the LMS of the WMS-III contain emotional and neutral content, but memory is assessed as a whole without consideration for emotion. This could yield an incomplete account of how memory is altered in aging and disease, since memory systems underlying emotional memory are complex and are altered in the context of age and neuropsychiatric disorders. In the current study, we created three stories that separately test negative, neutral, and positive content. We tested memory in healthy older adults across three different time points to better understand how emotional memory changes over time. We also investigated whether this task may be sensitive to subtle cognitive deficits by examining how individual differences in memory performance were associated with emotional memory processing.
We found that gist information was remembered better than detail information in older adults overall. This is consistent with previous findings suggesting that aging is associated with decreased recollection and preserved familiarity, and also fits with computational approaches suggesting that older adults are more biased towards memory generalization at the expense of discrimination (Koen & Yonelinas, 2014; Yassa & Stark, 2011). When analyzing performance based on the emotional content of the stories, we found that negative information was selectively preserved compared to neutral or positive information. Preferentially remembering positive information in older adults (i.e. the positivity effect) has been documented in the literature (Mather & Carstensen, 2005), although other studies have suggested that older adults preferentially remember negative information (Kensinger, 2008). We hypothesized that this discrepancy may be resolved by using an individual differences approach to testing memory in older adults. Our analyses of individual differences yielded several interesting results. We found that AI individuals had better memory (i.e. less forgetting) for positive information and worse memory (i.e. more forgetting) for neutral information compared to AU individuals. This difference between AU and AI groups suggests that the positivity bias previously reported in aging may be driven at least in part by subclinical memory impairment. This sheds light on the discrepancy across findings in older adults in the literature and suggests that adequate consideration for subclinical memory impairment is necessary for a more complete understanding of emotional memory alterations in aging.
There are several benefits of the study from a neuropsychological task development perspective. Since we modified a standard neuropsychological test of memory to additionally examine questions related to the processing of emotion, we retained the structure and scoring techniques that have been validated extensively in the past. The ELMT has several key advantages. First, it directly assesses and controls for emotional content, which is not possible in the original subset. Another advantage is that unlike the standard version, the modified ELMT includes a one-week delayed test to assess the effects of consolidation and forgetting. Given the extensive literature on how memories change over time (McGaugh, 2000), a delayed test is significantly informative. Using the ELMT as a novel neuropsychological test may allow us a deeper understanding of emotional memory consolidation and forgetting in young and older adults.

A particular limitation of the study is the absence of characterization of AD pathology. It is possible that AI individuals are more likely to be those harboring AD pathology and thus more likely to exhibit cognitive decline in the future, however, this could not be tested in the current study. This is an ongoing topic of interest in the field (Jagust, 2013) and we hope that our findings and our task may enable additional research to address these relationships.

In conclusion, the ELMT allows for the detection of subtle differences in emotional memory in older adults. Neuropsychological tests that evaluate the impact of emotion on memory will aid in a more accurate and thorough understanding of the aging brain and changes that may be associated with cognitive decline. Using these measures
concomitantly with structural and functional neuroimaging of the medial temporal lobes to further understand age-related alterations in the amygdala-hippocampal network will be critical to more fully understand how emotional memory changes with age, and the association between emotion, memory, and cognitive decline.

Appendix 7: Supplementary Information for Chapter 6

Figure S6.1. Correlation between BDI-II and GDS in older adults.
Figure S6.2. ROI activity profiles across conditions and hemispheres in DS+ and DS- groups. (a) Mean beta weight in DG/CA3 during CRs and FAs across emotion in DS+ and DS- groups; (b) Mean beta
weight in CA1 during CRs and FAs across emotion in DS+ and DS- groups; (c) Mean beta weight in SUB during CRs and FAs across emotion in DS+ and DS- groups; (d) Mean beta weight in CEA/CORT during CRs and FAs across emotion in DS+ and DS- groups; (e) Mean beta weight in LEC during CRs and FAs across emotion in DS+ and DS- groups; (b) Mean beta weight in MEC during CRs and FAs across emotion in DS+ and DS- groups. Error bars are ± S.E.M.

**Figure S6.3. Correlations between hippocampal subregions during positive false recognition.** (a) Positive correlation between BDI and DG/CA3 activity during positive FAs; (b) Positive correlation between BDI and CA1 activity during positive FAs; (c) Positive correlation between BDI and SUB activity during positive FAs.

**Figure S6.4. Correlations between negative LDI and DG/CA3 and BLA activity during false recognition.** (a) Correlation between negative LDI and DG/CA3 activity in DS+ and DS- groups; (b) Correlation between negative LDI and BLA activity in DS+ and DS- groups.
Curriculum Vitae

EDUCATION

Doctor of Philosophy in Psychological & Brain Sciences (2016)
Johns Hopkins University, Baltimore, MD.

Master of Arts in Psychological & Brain Sciences (2013)
Johns Hopkins University, Baltimore, MD.

Bachelor of Science in Biopsychology (2011)
General and Departmental Honors
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RESEARCH & PROFESSIONAL EXPERIENCE

Graduate Research Fellow, Translational Neurobiology Laboratory (2011-2016)
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Research Assistant, Cognitive Neuroscience Lab (2010-2011)
University of California, Santa Barbara, CA.

Caregiver, Senior Helpers (2010-2011)
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Research Assistant, Behavioral Pharmacology Lab (2009-2010)
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Research Assistant, Behavioral Pharmacology Lab (2009-2010)
University of California, Santa Barbara, CA.

TEACHING

Teaching Assistant, Foundations of Mind, Dr. Lisa Feigenson (Spring 2014)

Teaching Assistant, Psychology of Aging, Dr. Michela Gallagher (Spring 2013)

Teaching Assistant, Psychopharmacology, Dr. Linda Gorman (Winter 2012)

Teaching Assistant, Functional Human Neuroanatomy, Dr. Susan Courtney (Spring 2012)

Guest Lecture, False Memory, Neurobiology of Learning and Memory (Fall 2013)
Guest Lecture, Emotion and Fear, Neurobiology of Learning and Memory (Fall 2013)

**HONORS & AWARDS**

Alzheimer’s Association International Conference Travel Fellowship (2015)

Fellowship, Summer Institute in Cognitive Neuroscience, UCSB (2014)

Pre-doctoral Fellow, Research Training in Age-Related Cognitive Disorders (2013-2016)

Honorable Mention, NSF Graduate Research Fellowship Program (2013)

Walter L. Clark Student Collaborative Research Award, JHU (2012-2013)

Robert S. and Dorothy L. Waldrop Graduate Fellowship, JHU (2011-2014)

Distinction in the Psychology Major, UCSB (2011)

Exceptional Academic Performance in the Psychology Major, UCSB (2011)

High Honors (top 6% upon graduation), UCSB (2011)

Dean’s Honors, UCSB (all quarters) (2009-2011)

**ACADEMIC SERVICE**

TA Committee for Psychological & Brain Sciences Department (2011-2014)

Colloquium Committee for Psychological & Brain Sciences Department (2013-2014)

Active Minds, President & Founder (2012-2014)

JHU Counseling Center Advisory Board (2012-2014)

American Medical Student Association, Vice-President & Co-Founder, UCSB (2009-2011)

**PEER-REVIEWED PUBLICATIONS**


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**PRESENTATIONS**

**Leal, S.L.** (2016). Emotional Modulation of Episodic Memory and Translational Applications to Aging and Depression-Related Cognitive Impairment. *Dissertation Seminar at UCI*.


**Leal, S.L.** (2014). Emotional modulation of memory in depression and cognitive aging. UCI NeuroBlitz.


