A behavioral and neurobiological assessment of episodic-like memory in aged and young Sprague-Dawley rats

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Abstract

Episodic memory loss is a cognitive deficit commonly seen in both natural aging and pathological processes like dementia of the Alzheimer's type. Episodic-like memory, the animal counterpart of episodic memory, provides a unique avenue to study both the behavioral and neurobiological components of this cognitive decline using aged rodents. This memory type consists of "what", "when", and "where" components that must be correctly integrated for proper memory utilization. Novel behavioral interventions were based off of these components to determine if supporting or enhancing just one of these episodic-like memory components could improve the episodic-like memory mechanism as a whole. More specifically, this study aimed to determine if these behavioral interventions could improve episodic-like memory in both aged and young rats. The interventions did show promising episodic-like memory improvement in the young rat cohort and suggested that further training with the interventions may have improved memory performance in both aged and young animals. The second goal of the study was to utilize immunohistochemical analysis of choline acetyltransferase (ChAT) in the hippocampi of aged and young rats to identify neurobiological targets of episodic-like memory decline, as well as to determine how they relate to behavioral task performance deficits. No significant neurobiological relationships of ChAT to task performance were found within the potentially affected aged group. These findings suggest that behavioral interventions could be utilized to delay the loss of episodic memory occurring in both aging and dementia, especially if applied early, and that multimodal neurobiological targets may be more effective than unitary acetylcholine-targeted pharmacological therapy.

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Acknowledgements

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Introduction

Aging

Aging is an inevitable and universal process. It is a phenomenon that everyone experiences, yet each individual experiences differently. Some age more gracefully, showing few signs of age, while others undergo a more debilitating aging process, resulting in physical and/or cognitive limitations. Neurological aging-related changes manifest themselves in multiple fashions, affecting neuroanatomy, memory, and cognitive function. Effects on neurobiology include a decrease in the number of dopaminergic receptors, a decrease in white matter density, volumetric reductions in certain brain regions, as well as the development of neurofibrillary plaques and tangles (Park & Reuter-Lorenz, 2009). Memory is also commonly affected by the aging process, resulting in age-related deficits in memory as well as its associated cognitive functions. Potential deficits in memory and cognitive function include reductions in working memory capacity, long-term memories, information processing speed, and inhibitory function (Park & Reuter-Lorenz, 2009). More specifically, degeneration in recall and recollection tasks, prospective memory tasks, and some working memory tasks is often quite significant (Grady & Craik, 2000), and has been demonstrated through reduction in simple memory span (e.g. word recall), complex memory span (e.g. reading span), comprehension span, and computation span (Craik & Salthouse, 2000). Decreased inhibitory mechanisms and function in aging also appear to contribute to age-related deficits in working memory encoding and recall, attention, and comprehension. One theory for this contribution of reduced inhibitory function to memory deficits is that the decrease in inhibition may result in extraneous and excess signaling that obscures the pertinent neural information (Hasher & Zacks, 1988). In contrast, age-related decline in short-term memory and recognition tasks is often minimal (Grady & Craik, 2000).

Neurobiological and cognitive aspects of memory decline often share a unique relationship through aging, demonstrating the elaborate intertwining of both constructs. For example, episodic memory performance in free recall, cued recall, visual recognition, verbal recognition, and paired-associate learning declines as fornix white matter decreases with age (Metzler-Baddeley et al., 2011). Though all individuals experience age-related decline in both cognitive function and neuroanatomical constructs, the characterization of these deficits and the severity thereof can vary drastically across individuals.

Many of these naturally-occurring, age-related, declivities share a relationship with more debilitating neurological disorders such as dementia. Further, many natural aging changes mirror the manifestations of dementia of the Alzheimer's type, or Alzheimer's disease (AD) and share overlapping neurological disruptions including declines of both memory and neuroanatomy. Memory deficits associated with both aging and AD include decreased verbal working memory retention and manipulation on a letter-number sequencing task and reduced response inhibition on a go/no-go task (Rochat et al., 2013). Episodic memory decline in story recall, semantic memory impairment, word-object association, object matching, and name matching are also all affected by aging and AD (Adlam et al., 2006). Neuroanatomically, aging and AD both demonstrate neurofibrillary degeneration and plaque formation (Delacourte et al., 1999), loss of cerebral volume (Fox & Schott, 2004), and white matter degradation (Lee et al., 2015). Such commonalities suggest that Alzheimer's disease and age-associated cognitive impairment may stem from the vulnerability and withering of the same circuits (Hof & Morrison, 2004). This provides strong support for the use of aged subjects in researching the neurological deficits associated with the naturally occurring aging process as well as the pathologic changes associated with dementia and Alzheimer's disease.

Both aged human and animal subjects can be used to study aging and AD and share many similarities. For instance, the same hippocampal and neocortical circuits are responsible for the neurological impairment in humans and animals (Hof & Morrison, 2004). Hippocampal changes in aging rats exhibit similarities to brain changes in other mammalian species, including decreases in neuronal density, increases in glial activity and clustering, and analogous lipofuscin accumulation (Landfield et al., 1981). With the neurological similarities between aging and Alzheimer's disease and between rats and humans, an aged rat model could provide a unique avenue for gathering novel insight into the onset of these neurological deficits and help in establishing future preventative therapies.

Episodic memory

As previously described, there are many types of memory that are affected by neurodegeneration associated with aging and Alzheimer's disease (AD). However, one specific memory type, episodic memory, appears to be a key perpetrator in, and perhaps even predictor of, both aging and dementia onset. In 2000, a longitudinal study conducted by Bäckman, Small, and Fratiglioni revealed that even six years prior to an Alzheimer's disease diagnosis, AD individuals performed worse in episodic memory tests than their apparently healthy counterparts, while they showed no differences in semantic memory test performance at any time point (Backman et al., 2001). This suggests that Alzheimer's disease is characterized by a preclinical period of over half a decade during which indicative episodic memory deficits are already measurable and demonstrable. In contrast, semantic memory is often maintained even as episodic memory declines and temporally-specific details are lost (Levine, Svoboda, Hay, Winocur, & Moscovitch, 2002). Semantic memory may even improve with aging through an age-related

cognitive progression toward more thorough integration of new information (Adams et al., 1997). With the early onset of episodic memory loss in preclinical Alzheimer's disease and age-related decline being greatest for explicit, effortful, and unstructured retrieval tasks like episodic memory (Craik & Salthouse, 2000), early detection and support of episodic memory through early intervention application could produce protective effects against such deterioration, potentially shielding the brain from the natural impacts of aging, as well as the more aggressive deterioration associated with dementia and AD.

Though affected differently in both aging and AD, semantic and episodic memories are subtypes of the same memory system, declarative memory (Squire & Zola, 1996). As a result, the memory types share many features, which Tulving and Markowitsch (1998) summarize as follows. First, both memory types can receive and store information from multiple sensory and self-generated sources. Following information reception and storage, the processing and encoding of these memories are also similar – the encoding, storing, and retrieval of semantic and episodic information is context-oriented and specific, and in both memory types, a single event or fact is sufficient for memory storage. Episodic and semantic stored information is representational and is, or can be, applied to other contexts or situations, and overtly expressed. Lastly, the stored information in both systems is flexibly accessible and expressible through multiple retrieval mechanisms and paths, which can be consciously retrieved for further contemplation or application (Tulving & Markowitsch, 1998).

In contrast to the similarities, there are also stark differences between episodic and semantic memory, which allow for unique exhibition and analysis of each memory type. Episodic memory deals with the recollection of an individual's specific experiences, as opposed to semantic memory which deals predominantly with facts and events that any individual can

relate to. Episodic memory is also the only form of memory that orients to the past for memory recollection. It is thought to involve *autonoetic consciousness* through which an individual "mentally time travels" and places themselves into the past, recalls the memory, and applies that experiential knowledge to a current situation (Tulving & Markowitsch, 1998). In Tulving's 1972 chapter, *Episodic and Semantic Memory*, he succinctly and effectively captures episodic memory by stating, "Most episodic memory claims can be translated into 'I did such and such, in such and such a place, at such and such at time'." (Tulving 1972). This synopsis highlights the distinct features of episodic memory – the individualistic nature of the memory, and the recognition and binding of what, where, and when the event happened. The final, and arguably most important, distinction of episodic from semantic memory is that episodic memory is more vulnerable to multiple neurological conditions as well as the natural aging process (Tulving & Markowitsch, 1998), making it an important target for early detection and prevention of age-related memory decline and dementia onset. These distinctions of episodic versus semantic memory, such as the components of episodic memory that include recollection of what happened, in which place, and at what time, are essential to tapping into the analysis of episodic memory. Further work on episodic memory should be directed toward protecting and sustaining this vulnerable and vital memory type, as it could mitigate some of the neurological symptoms of aging and AD.

Episodic-like memory in animals

With episodic memory loss affecting both the neurologic decline of aging and dementia of the Alzheimer's type, an animal model of the aging process can serve as a valuable tool in determining the initial progression of aging and AD. Aging studies in humans require an extensive research span of many years and cannot easily control for confounding and mediating

variables, limiting human aging and dementia research. Animal models provide a more succinct and controlled avenue for studying the effects of aging on episodic memory, and can be coupled with neurobiological techniques (e.g., pharmacology, lesions, optogenetics, and immunohistochemistry) to gain a deeper understanding of the neuronal changes that occur with aging. Despite these benefits, research on episodic memory in animals is limited.

One factor contributing to this research gap is the distinction between episodic and episodic-like memory. Episodic memory, as defined by Tulving, hinges upon the utilization of autonoetic consciousness to place one's self into a past experience and recollect information from a past experience. This recollection is generally expressed verbally, and some argue that the lack of agreed-upon non-linguistic markers of conscious experience makes demonstrating episodic memory in animals essentially impossible (Clayton et al 2001). This dilemma has led some to consider animal episodic work less relevant, while it has led others to a distinction in animal research that acknowledges the inability to fully assess an animal's ability to place themselves into previous events or experiences and recall information from them.

Instead, animal researchers of episodic memory focus on the components of an experience – what happened, where it happened, and when it happened, and the binding of those components, which is referred to as *episodic-like memory*, or what-where-when memory (Zhou & Crystal, 2010). By assessing the components of episodic-like memory, particularly the what, when, where of an event, and application of this information, animal researchers circumvent the linguistic constraints of Tulving's definition.

As the field of episodic-like memory animal research has developed, the significance and necessity of creating animal tasks that utilize and combine these what, where, and when components, has become more and more apparent. In such work, researchers strive to establish

tasks in which animals can demonstrate a sense of recollection of prior experiences non-verbally. Clayton, Bussey, and Dickinson (2003) argue that episodic memory in animals can be demonstrated if animals can apply the knowledge of when and where a specific event (i.e., what) occurred and utilize it to distinguish the event from other events with common features (N. S. Clayton, Bussey, & Dickinson, 2003). The act of distinguishing between the what, when, and where components of similar events is the prevailing focus in animal episodic-like memory work, and it yields promising models and results. These component features were also the targets of the behavioral interventions created for this experiment, which set out to ascertain if accentuating an individual component of episodic-like memory can improve memory utilization.

Some of the earliest and most impactful work in episodic-like memory was conducted in scrub jays (Clayton and Dickinson 1998). This episodic-like memory model capitalized on the scrub jays' natural food-caching, or storing, behavior. In the experiment, birds were allowed to cache both a perishable and non-perishable food item in distinct locations before being removed from the caching area. Trials varied such that the scrub jays were returned either 4 or 124 hours after their initial caching session, which impacted the viability of the perishable food item stored. If the scrub jays appropriately applied the information of how long it had been since caching and which item they stored in each location, they were able to successfully retrieve, or avoid, the perishable food item. Their findings indicated that the birds remembered what, where, and when they cached the food items and were able to use that information to guide their item retrieval via episodic-like memory (Clayton & Dickinson, 1998).

Episodic-like memory has since been demonstrated in other birds (e.g., Feeney et al., 2009; Singer & Zentall, 2007), rats (e.g., Crystal and Smith 2014), and primates (Gaffan, 1994), and further avenues for studying episodic-like memory through naturally-occurring animal

behavior have been proposed. Possible behaviors to utilize include parasitism in birds, where discovering each potential host nest would encompass a unique event, social status in monkeys, where the current dominant male must be remembered based off of the last dominance display or dispute, and the polygynous mating system of voles, where a male vole must integrate information of which female in which burrow is reproductively receptive at what time point (Clayton et al., 2001 and Clayton & Russell, 2009). Such behaviors may encompass various components of episodic-like memory, but could be difficult to elucidate and manipulate in a traditional research setting. Rat food hoarding and storing, however, is another natural behavior that can, and has been, utilized to study episodic-like memory in a research setting.

Rodent models of episodic memory

A prominent source of episodic-like memory research in rats utilizes a radial arm maze. The what, where, and when components in the paradigm consist of what food type, where in the 8-arm radial arm maze is food located, and when during the day the testing takes place (e.g. Crystal and Smith 2014, Babb and Crystal 2006, Zhou and Crystal 2009). Rats receive an encoding trial during which they locate two types of food pellets within the maze, and a subsequent test trial during which the rats must relocate the previously found food. However, similarly to the scrub jay caching task, only one type of food reward is available during the test trial, which varies with testing performed in the morning or afternoon. Through varying the encoding trial locations randomly each day and the time of day at which testing is conducted, rats must remember a particular episode including what food type, where in the maze each was located, and time of day in order to solve the task.

One issue with animal episodic-like memory tasks is that non-episodic processes can be used to solve the tasks. These processes include (1) completion of the task without complete encoding and recall of a previous event, (2) relying on timing retention intervals between testing and the relative familiarity of the last occurrence, and (3) learning and applying semantic rules to solve the task through the extensive training that is often required in episodic-like memory paradigms.

The first of these alternatives is referred to as the *encoding failure hypothesis* (Zhou & Crystal, 2009). In many episodic-like memory tasks, it is possible that the animals could solve the task through integration of the various what, when, and where components without undergoing the complete recollection of a previous episode as one coherent scene. To prevent this, a novel contingency can be incorporated into the task, so that the animals must completely recollect the previous episode to modify their response based on the stipulation presented. One such contingency utilized previously was a replenishment condition (Zhou & Crystal 2010), such that if one food type was present in the central hub of the radial arm maze following the encoding trial, then the more favorable chocolate food would replenish and be present in its previous arm maze location. If food was not in the hub, however, then only one food type (chow) would be in the radial arm locations that it had been in during the encoding phase. The meaning of the replenishment cue also varied by time of day, so that the replenishment cue indicated both food types would be present during testing in the morning, but that only the chow food type would be present in the afternoon. By providing a chocolate replenishment cue prior to testing, but independent of the previous encoding trial, the animals had to retrieve the information of what food they found where, and how that related to the replenishment condition based on time

of day. Successful completion of the task indicated that the rats were capable of encoding the complete episode and applying that memory appropriately based on a separate cue.

The second concern about the use of the timing retention interval and relative familiarity stems from the possibility that animals could be determining which choice to make and where to go based off of how long it had been since they were last tested or the time of day at which they are being tested. This concern was addressed by manipulating the timing retention interval, or time between the encoding and testing trials. Even with a seven-hour interval between the encoding and testing, so that testing occurred during the opposite half of the day from encoding (i.e. encoding occurred in the morning and testing occurred in the afternoon, and vice versa), the rats' performance was not impeded, and the animals still applied the encoding time of day component to correctly solve the episodic-like task (Zhou & Crystal, 2009). In a separate experiment (Zhou & Crystal, 2010), the use of a consistent time between the encoding and testing trials eliminated the possibility of the rats relying solely on the timing retention interval to solve the task, and helped to ensure the episodic nature of the experimental design by demonstrating that the rats could successfully complete the task utilizing absolute time of day.

The third concern expressed was that with the large amounts of training that animals often require, the animals may establish complex semantic rules through which they could predict what would happen next, or how to solve the task, as opposed to remembering a coherent episode (Zhou & Crystal, 2010). For example, over the course of the training required for the food-caching task, pigeons could have learned that worms should be retrieved if their caching memory was strong, while peanuts should be retrieved if the memory is less recent (Singer & Zentall, 2007). However, the use of an unexpected question in an experiment posited that through probing animals with a situation in which retrieval of an episodic memory is required for

application to a new question or situation, it could be differentiated from a system of complex rules that allow the animals to predict how to respond appropriately (Zentall et al. in 2001). For the radial arm maze task, the unexpected question utilized was assessing whether an episode encoded in one room could be retrieved and utilized to accurately earn food in another room (Zhou & Crystal, 2010). Although the rooms shared many overlapping features, including identical radial arm mazes and the same positioning and orientation of the mazes within the rooms, the unique differences of the rooms location, size, and decorative features did not deter the rats from correctly recalling the encoding episode and implementing it to the testing trial that occurred in another room (Zhou & Crystal, 2010). These tests not only ensure the involvement and integration of the what, when, and where components of episodic-like memory, but also eliminate alternative approaches for solving the Zhou and Crystal radial arm maze experimental design. As such, implementing and satisfying such verifications needs to be a fundamental component of a valid episodic-like task.

The complex nature of demonstrating episodic-like memory makes novel task development a detailed process. However, it also highlights the three distinct components of the memory type, which can be separately manipulated and studied. This allows episodic memory research to delve deeper by further assessing its underlying components. The present study utilized these episodic-like memory components to not only assess memory performance more thoroughly, but also to determine if providing support to a single component of the memory type can improve episodic-like memory.

A novel open-field episodic-like memory task

The task used in this study is closely based on the Zhou and Crystal (2009) paradigm, while allowing for more diverse measurements of episodic-like memory, particularly its spatial component. Key differences between the tasks include the open field nature of the apparatus used, the criteria used to determine successful task performance, the location of the replenishment cue, and the location(s) of the chow pellets during test trials.

This task was conducted on an open table to allow for uninhibited movement throughout the space. The less restrictive movement elicited by the task more closely mimics natural foraging behavior, thereby making this novel task more ecologically relevant. It also more closely resembles human movement within an environment. The open field provides an opportunity for more diverse analyses of the spatial component of episodic-like memory, such as assessing for any loss of spatial navigation, or wandering, that is often seen in cognitive decline. By encouraging more natural exploratory behavior seen in both rats and humans and facilitating future analyses of specific episodic-like memory components, the open field adds to the relevance, applicability, and translational capacity of this work.

Zhou and Crystal (2009 and 2010) measured the probability of an individual returning to the replenishing chocolate arm within the first four arms of the maze that a subject visited during a test trial. As such, subjects could return to half of the available locations before their performance level was affected. Subjects were also allowed to obtain all chow food rewards from their arm locations prior to the end of a test trial. This open-field task utilized more stringent criteria to define successful performance. Correct performance was defined as removing the lid off of the cup that held a food pellet during the previous encoding trial. This criterion was established to prevent subjects from applying alternate semantic strategies such as

simply removing any lids off of cups until correct locations were encountered, and to encourage direct retrieval of a food reward, as this increases the likelihood that the previous memory was being utilized to obtain the food reward.

In this novel task, rodents were started from one of four possible start boxes that were located at the midpoint of each side of the table. The start locations were consistent between an encoding trial and its test trial counterpart but were varied across sessions. The replenishment cue of chocolate pellets was placed into the front of the start box with the subject prior to undergoing their test trial. Subjects were not allowed onto the open field until they had consumed their chocolate cue. This change in chocolate cue placement from the Zhou and Crystal work is not expected to have impacted task performance, but did aid to ensure that all subjects attenuated to the replenishment cue prior to starting their test trial.

Another difference from the Zhou and Crystal task is the location(s) at which the chow or plain pellets were located during test trials. In Zhou and Crystal's paradigm, chow was available for the subjects to consume during the test trial in each of the radial arms (four total) that had not contained chow during the encoding trial. The chocolate arm was replenished as indicated by the testing conditions. In the open field task, the plain pellet reward was available during the test trial, but only in the same location in which it had been during the corresponding encoding trial. The task was designed to reduce extraneous lid removal, and because it allowed for episodic-like memory testing during non-replenishment trials (as well as replenishment trials). Since the rats located both the plain and chocolate food rewards during the encoding trial, successful utilization and integration of the episodic-like memory components would result in only choosing to remove the lid from of the plain pellet cup during a non-replenishment session, as this would be the only possible source of food reward during this session type. With this change, episodic-like

memory performance, and the utilization of its components, could be more directly assessed during both replenishment and non-replenishment sessions of this task.

Neurobiology of episodic memory

Conducting episodic-like memory research with rats opens countless doors for neuronal measurements and manipulations that can potentially be generalized to humans. The main pathways of the hippocampal memory system are analogous in rodents and primates (Eichenbaum, 2000), and the cortical and hippocampal components serve similar neurophysiological roles and fundamentally contribute to episodic-like memory across mammalian species (Dickerson & Eichenbaum, 2010). The anatomical relationships across the major neocortical and medial temporal connective pathways are also largely conserved. This includes the parahippocampal cortical areas and hippocampus, which fundamentally support episodic memory (Dickerson & Eichenbaum, 2010). The neural mechanisms and pathways of episodic memory can also be evaluated across species, since mammals share the same projections and inputs of the perirhinal and parahippocampal cortices that relay object recognition ("what") and spatial ("where") information, respectively, as well as the convergence pathways within the hippocampus (Dickerson & Eichenbaum, 2010). More specifically, hippocampal neurons encode specific combinations of events and places for the creation of episodic-like representations, even when previous experiences may share some overlapping features (Eichenbaum, 2000). Given the significant anatomic and physiologic neural conservation across mammalian species, the hippocampus, as well as the fornix and mammillary bodies that are associated with episodic memory in humans, (Morris, 2001) may be viable targets

of further episodic memory research, and prospective foci of future behavioral and pharmaceutical therapies in animal models.

The hippocampus is closely associated with the prefrontal cortex in episodic memory via projections that begin throughout the neocortical areas, converge on the parahippocampal cortical areas, and then project onto each of the hippocampal subdivisions (e.g., dentate gyrus, CA1, CA3) and back onto the cortical association areas through the medial temporal lobe (Dickerson & Eichenbaum, 2010). Episodic memory encoding increases synaptic plasticity-associated transcription, dendritic spine morphology, and synaptic transmission in the medial prefrontal cortex, which indicates that the medial prefrontal cortex is an important regulator of the entorhinal-hippocampal circuit and its activation for the formation of long-term memories, including those of the episodic type (Bero et al., 2014). Furthermore, communications between the cortex and the hippocampus via the parahippocampal region can form memories without hippocampal mediation (Eichenbaum, 2000), emphasizing the important role of non-hippocampal structures in memory processing.

Just as the hippocampus and cortex collaborate for episodic memory encoding, so do the hippocampus and other brain regions via distinct circuitries for recognition, spatial, and temporal processing. These connections of the hippocampus to other brain regions are essential for the formation and analysis of the "what", "when", and "where" constituents of episodic memory, and may provide insight into which components of the episodic memory system are initially compromised in aging and dementia. Object recognition and discrimination, a fundamental component of "what" in episodic memory, relies heavily on the communications between the hippocampus, perirhinal cortex, prefrontal cortex, and medial dorsal thalamic nucleus (Aggleton & Pearce, 2001 and Allen, Morris, Mattfeld, Stark, & Fortin, 2014). While spatial memory, the

"where" component relies heavily on the hippocampus, anterior thalamic nuclei, fornix, mammillary bodies, and prefrontal cortex (Aggleton & Pearce, 2001). The timing, or "when", component of episodic memory consists of its own neural network as well. The thalamo-cortico-striatal circuit, basal ganglia, supplementary motor area, cerebellum, and parietal, premotor, prefrontal, and insular cortices all are closely associated with the processing of temporal information in the seconds-to-minutes timing system associated with episodic memory (e.g., Buhusi & Meck, 2005; Wiener et al., 2008; Morillon et al., 2009; Lustig & Meck, 2005; Balci et al., 2009; Galtress et al., 2012). Along with these brain regions, the CA1 of the hippocampus appears to mediate the minutes-to-hours duration utilized in episodic memory (Kesner & Hunsaker, 2010). The sheer number of structures involved in the timing portion of episodic memory provide testament to its significance within the episodic system, as two episodic memories may share "what" and "where" components, but must be temporally distinct to meet the episodic memory requirement of being formed through a single experience (Clayton et al., 2003).

The copious and overlapping neural connections that sustain episodic memory and its components provide insight into how these systems ideally work together, as well as how deterioration in one or multiple areas may manifest itself with age and neurological disease. The medial temporal lobes, the frontal lobes, hippocampus, anterior and dorsomedial nuclei of the thalamus, fornix, mammillary bodies, mammillothalamic tract, and retrosplenial cortex all have been implicated in Alzheimer's disease in humans (Gold & Budson, 2008). These brain regions, along with the diagonal band of Broca's area and the presubiculum, have been implicated in episodic-like memory in animals, and serve as potential targets for animal Alzheimer's models (Gold & Budson, 2008). With the magnitude of prefrontal activation during memory encoding,

atrophy of the fornix and hippocampus, and subsequent reduced prefrontal activation, may serve as valuable markers in a natural aging animal model of Alzheimer's disease (Bero et al., 2014).

With the hippocampus and prefrontal cortex both playing a role in episodic memory as a whole and its fundamental discrimination ("what"), spatial ("where"), and temporal ("when") components, they serve as indicators of global episodic memory decline. Deficits in more specific areas within the components' subsystems allow for more precise localization of neural deterioration. For instance, the perirhinal cortex, the anterior thalamic nuclei, and the basal ganglia appear to be solely involved in the recognition (what), spatial (where), and timing (when) features of episodic memory, respectively (e.g., Aggleton and Pearce, 2001; Buhusi and Meck, 2005), and therefore could provide neuroanatomic landmarks of age- or disease-related decline.

On a molecular level, neural changes and pathology can be quantified via multiple techniques. One mechanism that is common in an aging and/or dementia models is quantification of neural degeneration through choline acetyltransferase (ChAT). ChAT is the enzyme directly responsible for acetylcholine (Ach) synthesis, and serves are a reliable indicator of cholinergic function (DeKosky et al., 2002). ChAT is widely distributed throughout the brain and plays an important role in essentially all brain functions (e.g. learning, memory, motor function) through its cholinergic stimulatory effect (Oda, 1999). Age-related and Alzheimer's disease-related decline of ChAT in the hippocampus are significantly correlated in humans (Perry et al., 1992), indicating that aged models may help to understand the natural again process, as well as Alzheimer's disease.

Age-dependent decline in ChAT has been demonstrated in Sprague-Dawley rats, particularly in the subcortical structures including the hippocampus, globus pallidus, thalamus, caudate nucleus, putamen, and striatum, along with less pronounced decline in the cortical

regions of the forebrain and the temporal, parietal, and occipital lobes (Yufu et al., 1994). Interestingly, the less significant decrease in ChAT in the cortical regions may reflect support for the human scaffolding theory of aging and cognition, which hypothesizes that frontal activation may be increased with age and dementia to compensate for the deterioration of other neurological structures (Park & Reuter-Lorenz, 2009). ChAT levels also serve as a marker of spatial learning and memory abilities in hippocampus-dependent tasks in rats, such that higher levels of ChAT result in better performance in these types of tasks (Hawley et al., 2015). Since episodic memory and its components rely on ChAT for cholinergic function, and ChAT levels are known to vary with age, ChAT may be a viable measure of episodic memory decline, creating a new opportunity to develop therapies for the prevention or delay of the neuropathology of both aging and AD by identifying where the deficits begin.

Methods

Animals

Two groups of rats were used in this experiment. The aged group of rats consisted of 12 male Sprague-Dawley rats that were previously used in an impulsive choice and an open-field task at Kansas State University. The aged rats were familiar with the apparatus but naïve to the task. The young group consisted of 12 male Sprague-Dawley rats purchased from Charles River at 21 days of age. The aged group began testing at 20 months old, and the young group at 7 months. All rats were pair-housed in standard shoe box cages on a 12:12 hour light-dark cycle with the lights off at approximately 6 p.m. The behavioral testing occurred during the light half of the cycle. All animals were given a restricted diet (LabDiet 5001 Rodent Diet) once per day that maintained them at approximately 85% of their target weights based on growth curves obtained from the supplier, while having ad libitum access to water. During behavioral testing, the rats received part of their daily food ration in the form of dustless chocolate-flavored sucrose (F07256, BioServ, Frenchtown, NJ) and grain-based food pellets (F0165, BioServ, Frenchtown, NJ), which the rats obtained from cups within the testing apparatus. During testing, the rats had access to water between trials, but water was not accessible on the testing apparatus. One of the aged animals died of natural causes during the experiment and therefore did not complete the discrimination or spatial intervention tasks.

Procedure

Apparatus

The apparatus used for this study was a 122 × 122 × 30 cm (tall) open-field table, made of wood that was painted with non-toxic washable enamel paint, containing a matrix of sixteen

holes that were 6 cm in diameter and 24 cm apart drilled in the floor of the table. The apparatus had four sides (30 cm tall) to prevent animals from falling off of the table. Each side of the table included a sliding door with a 17 × 25 × 12.5 cm start box attached to each door through which the animal's access to the table was controlled. An image of the apparatus is provided below in Figure 1. Translucent plastic cups were placed into the 16 holes of the table such that the lip of the cup was level with the table. Cups were filled approximately ¾ full with non-toxic hardened clay, upon which an approximately 2in-tall PVC pipe with a mesh bottom was placed. This "food cup" fashioned out of PVC pipe and a plastic mesh bottom was utilized so that a food pellet could be placed directly below the mesh, making it inaccessible to the rats, or on the mesh, such that the food pellet was available for consumption. A diagram of this receptacle is seen in Figure 2. This "food cup" was utilized so that each of the 16 possible locations could be baited to smell like the chocolate or grain food pellets used in the experiment, but were only accessible at the session-specific correct locations where the pellet was moved from below the false bottom to within the food receptable, such that it was accessible for the rat to consume if a correct choice was made. Eight possible food locations were randomly baited with one chocolate pellet, while the other eight were baited with one grain pellet. All 16 cup locations were loosely covered with a translucent plastic lid throughout the trials. Reward and start locations were changed across each session.

To reduce the use of scent cues, the apparatus was cleaned with an odorless, non-toxic cleaner in between each session. The plastic lids were moved to different cups in between sessions and between encoding and test trials within a session such that the individual's scent on a lid could not be used as a cue for a correct location. Plastic lids were washed with a non-toxic cleaner following each daily use.

Age groups

The aged and young groups of rats ran this experiment separately. All aged rats completed all four tasks, a baseline and three intervention tasks, prior to the young cohort completing the training and tasks, resulting in the aged group running approximately 6 weeks before the young group. Both groups completed the baseline task and intervention tasks in the same order – baseline, discrimination intervention, intertrial interval (ITI) intervention, discrimination intervention, and lastly spatial intervention. All groups received one rest day before transitioning to the next intervention task. The randomized sessions were consistent within each group but varied between the aged and young groups.

Lid training

Prior to the start of the experiment, all subjects were trained to remove lids off the plastic cups on the table and enter the table apparatus via each of the four start boxes. Over the course of 5 days, rats learned to retrieve food pellets from within each cup. Lids were initially not present, but incrementally covered more of each cup until each animal could successfully retrieve food from every covered cup. Individuals were randomly placed into one of the start boxes for each training session to prevent start box bias.

Task parameters

This experiment consisted of a baseline task and three interventions - increased intertrial interval (ITI), increased discrimination, and decreased spatial navigation. Each of these interventions was designed to emphasize one of the three components of episodic memory, the temporal (“when”), discrimination or recognition (“what”), and spatial (“where”) components,

respectively. The baseline task included 25 testing days, while each intervention was 10 days.

During each day of testing, rats completed 8 sessions, which included both an encoding and test trial. Rats entered the open field via one of the four start boxes along the sides of the open field table. The same start box was used for each session (an encoding trial and its test trial), though the start box used varied randomly across the eight sessions of each day. Different start boxes were used across sessions to provide different environmental cues for the spatial component of the task.

A diagram of a non-replenishment session is provided below in Figure 3. Sessions began with an encoding trial, which allowed rats to explore the table and determine the two locations of food reward for the session. Each reward location was baited with 3 food pellets, one cup with chocolate-flavored pellets (F07256, BioServ, Frenchtown, NJ) and one with standard grain-based pellets (F0165, BioServ, Frenchtown, NJ). All cups were covered with lids, and rats were allowed to remove as many lids as necessary to find the baited cups. Correct performance in the test trial depended upon the time of day at which testing occurred and the presence or absence of a chocolate food pellet cue in the start box, as these factors determined whether the chocolate food reward would replenish during the test trial. If the time of day and start box cue indicated the chocolate food should replenish during the test trial, correct performance involved relocating the chocolate food location. If the cues indicated that the chocolate would be absent, correct performance consisted of relocating the standard grain-based pellet location. The time of day and significance of the chocolate food reward in the start box was varied across individuals and groups. Only one pellet of either food type was available during the test trial.

Following the encoding trial and learning where food rewards were located, the rats underwent a test trial, where they were returned to the table and allowed to remove the lid off on

one cup. During the non-replenishment trials analyzed in this experiment, if the rats removed the lid off the cup containing the standard grain-based pellet food reward, they were allowed to consume the food reward for their correct choice. If an incorrect choice was made (either choosing the chocolate-baited or a non-baited location), no food was present and the rat was immediately removed from the table, ending the session. During a replenishment trial where the chocolate and the grain pellet were available, rats could make up to two choices to obtain both the chocolate and grain reward. If an individual chose a location without food reward, the rat was immediately removed from the table.

After a correct test trial, a new session with new baited locations ensued. Each incorrect choice resulted in repeating the session, both encoding and test trials, until the correct lid with standard pellets was removed or the daily allotment of 8 sessions was completed. To allow for re-acclimation to the task parameters and improved performance, rats could remove as many lids as needed to find the food pellet reward for the first test trial of each session.

Sessions were separated by an interval of approximately 2 min while pellets were rebaited and lids replaced. Rats had access to water in their transport cage during this time. All trials were video-recorded by LimeLight, a motion capture camera and software (Actimetrics, Wilmington, IL).

Intervention tasks

Though the aged group was run through the task first, both groups ran the task components in the same order – baseline task, discrimination intervention, ITI intervention, and lastly spatial intervention. Intervention order was not counterbalanced due to the small sample size in this experiment, which did not provide sufficient power for order effects analyses. Given

the variability across the intervention tasks, intermixing the intervention types within a phase would also not have been feasible. Given these limitations, one single random order was selected and utilized for all individuals. All rats completed 25 days of the baseline task and 10 days of each intervention with one rest day before the next intervention. Half of the days of each task consisted of non-replenishment trials, while the other half consisted of replenishment trials. Each intervention task manipulated one component of the baseline task to accentuate either the timing, discrimination, or spatial component of the task and episodic memory, but was otherwise identical to the baseline task. As such, each intervention still consisted of an encoding and test trial and required integration of the what, when, and where components but merely made one aspect of the task more distinct. These changes are described below and represented in Figure 4.

For the timing intervention, the intersession interval (or the interval in between the last test trial and a new encoding trial) was lengthened from 2 min between sessions to 10 min to make each session a more salient and unique event and to reduce interference from memories of previous trials. For the increased discrimination intervention, the BioServ food pellets were substituted for cheese-flavored yogurt drops (eCOTRITION, Cincinnati, OH) and berry-flavored fruit loop treats (Brown's, Sinking Spring, PA) to make the food reward types more distinct and salient. The yogurt treat served as the chocolate pellet, while the fruit loop treat substituted for the grain pellet. For the spatial intervention, only half of the cups on the table were used to create more distinct target locations and reduce spatial memory load.

Video recordings were hand-scored for correct test trial performance and then manually transferred to a long-format spreadsheet for statistical analysis. Scoring was performed daily and then validated by a single individual who re-scored all of the test trial data after the completion of the experiment.

Histology

Upon completion of behavioral testing, rats were deeply anesthetized via intraperitoneal injections of sodium pentobarbital and transcardially perfused first with 0.1 M phosphate buffered saline (PBS) and subsequent 4% paraformaldehyde (PFA).

Following perfusion, brains were removed and stored in PFA for 24 h. They were then transferred to a cryoprotectant 20% sucrose solution and remained there until adequate tissue cryoprotection and dehydration had occurred, as was indicated by the tissue sinking within the solution. The samples were then flash-frozen and stored at -80° C until they were sliced into 40 μm coronal sections that were stored in cryoprotectant-filled wells until immunohistochemical staining.

Immunohistochemistry was conducted using a polyclonal IgG anti-choline acetyltransferase (ChAT) antibody (Chemicon, AB144P). First, sections were washed and rinsed with 1x PBS (phosphate-buffered saline) three times to remove the cryoprotectant solution. Sections were then placed in 3% hydrogen peroxide for 15 min to deactivate any peroxidase enzymes remaining within the tissue. After thorough rinsing with 1x PBS, sections were placed in 5% normal horse serum for blocking for 1 h, followed by placement into a 2.5% normal horse serum solution containing 1:250 dilution of the primary (ChAT) antibody for 12 h at -4° C. Following the incubation with the primary antibody, sections were rinsed twice in 0.1 M PBS and then placed in a 1:200 dilution of the biotinylated secondary antibody (Vector Laboratories, BA-9500-1.5) in 2.5% normal horse serum for 1.5 h at room temperature. Sections were rinsed three times in 0.1 M PBS and then placed into the ABC (avidin/biotin complex) solution (Vector Laboratories, SK-4600) followed by another PBS washing and placement in ABC solution for 12 h at -4° C. Three 0.1 M PBS wash were performed prior to staining with the purple HRP

(horseradish peroxidase) chromagen stain (Vector Laboratories, SK-4600) for 25 min. Two final rinses with 0.1 M PBS were performed to quench the chromagen stain reaction. Following immunohistochemistry and staining, sections were mounted onto chromium-gelatin slides, and cover-slipped for microscopic imaging and analysis.

ChAT optical density measurements were taken of the hippocampus via ImageJ software (US NIH, version 1.50) with measurements taken from the hippocampal subdivisions of the dentate gyrus, CA1, and CA3 for comparison across groups and individuals. All images used for optical density measurements were double-blinded prior to analysis to limit experimenter bias. Measurements were also obtained by three different individuals. Interrater reliability between these scorers was approximately 90% agreement. Representative images of hippocampal slices from an aged and young rat are provided below in Figure 4.

Data analysis

For this report, all non-replenishment sessions, the sessions during which chocolate was not available during test trials are reported. Though both replenishment and non-replenishment conditions are informative, non-replenishment sessions were utilized for these analyses as they had only one correct location per test trial. This made the non-replenishment condition a simpler test for the rats to complete and a more cohesive dataset for initial analyses of performance in this novel task. The data includes the first session test trials, during which rats could remove as many lids as needed to obtain food reward, since only the first location choice was used to determine correct performance in all test trials. Individuals underwent 10 days of testing on each intervention task – five days were non-replenishment sessions and the other five were replenishment sessions. As such, the five days of non-replenishment sessions from each

intervention task were analyzed. To match the number of sessions from the intervention tasks, data from the last five days of non-replenishment sessions were utilized from the baseline task.

The non-replenishment sessions of the dataset were analyzed via generalized mixed effects modeling to assess the effects of the interventions, age, and individual differences on correct trial performance. The dependent, binomial variable in these models was test trial performance (0 = incorrect lid choice, 1 = correct grain pellet location choice). Independent variables included were the four-level (baseline, discrimination, ITI, and spatial intervention) categorical variable, task, the two-level (aged and young) categorical variable, age, and the continuous variable of day (days 1-5 of the non-replenishment condition). The continuous variable of day was scaled relative to the last day of each task to compare performance on the last day of the task. The random effect of individual subject was also used in the models. Logistic regression (function = "glmer", family = "binomial") was selected given the binomial nature of the dependent variable. The categorical variables were dummy coded with the aged rodent group serving as the reference group for the Age variable and the baseline task serving as the reference group for the Task variable. The best models were selected via lowest AIC. All analyses were conducted in RStudio Desktop version 1.3.959.

The same set of non-replenishment data was also taken and separated out by individual subject. Logistic regression (function = "glm", family = "binomial) using the same dependent variable of correct test trial choice was used to determine the effects of task on individual performance. The variable, Task, was again dummy coded for direct comparison of performance in the interventions to the baseline task. No random effects were used in these models.

Linear regression and Pearson's correlations were used to identify relationships between ChAT quantity across brain regions and performance across baseline and intervention tasks. All

correlational analyses were conducted with the “cor.test” function in RStudio. One aged rat had ChAT optical density measurements that were greater than two standard deviations away from the mean value of each brain region analyzed. As such, this subject was removed from the ChAT analyses only.

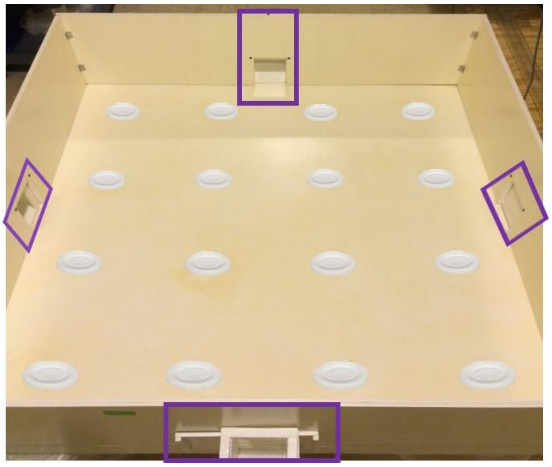


Figure 1. Picture of the open-field table apparatus including the 16 holes in the table that held the cups into which food reward was placed. All cups were covered with plastic lids during each trial, as depicted. The four start boxes from which the rats began each trial are highlighted by the purple boxes.

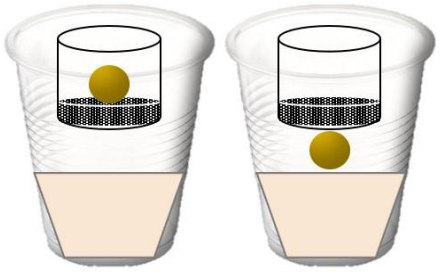


Figure 2. Diagram of the cups placed into each of the 16 holes on the open-field table. Clay was present in the bottom portion of the cups, such that the food reward could be placed within the PVC food receptacle on its mesh bottom, or below it. This prevented the odor of the pellet from serving as a cue, while making the food reward only accessible to the rats at the correct locations.

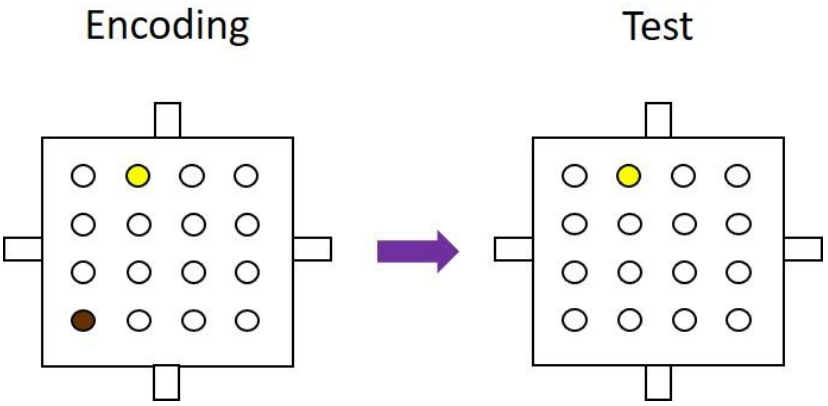


Figure 3. Diagram of a representative non-replenishment session. During the encoding trial, rats were allowed to explore the table until they obtained both the chocolate food reward, indicated by the brown circle, as well as the grain food reward, represented by the yellow circle. For the test trial, the chocolate reward location did not replenish, and rats were only able to obtain a food reward by returning to the location of the grain reward (yellow circle).

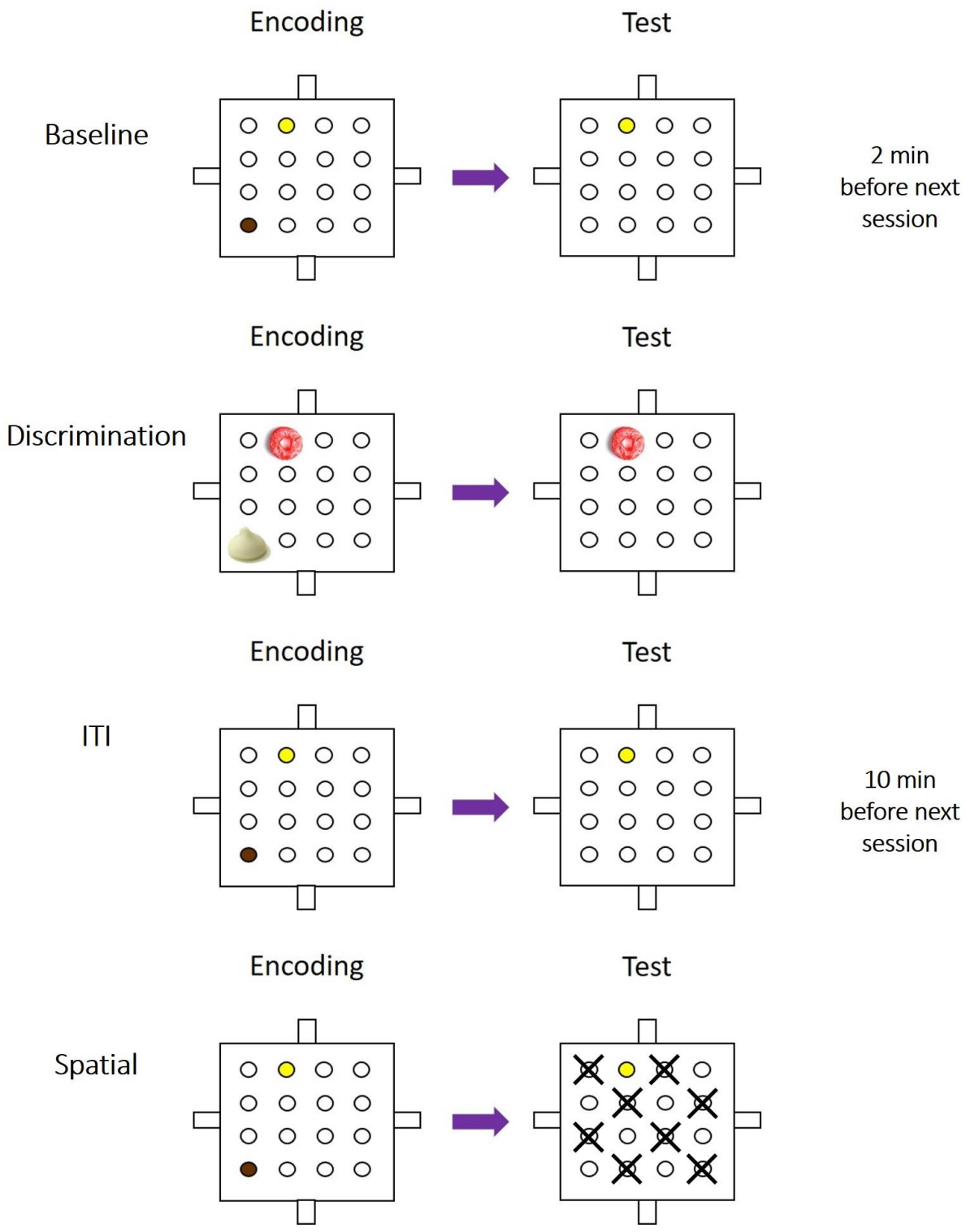

Encoding
Test
Baseline
2 min
before next
session
Encoding
Test
Discrimination
Encoding
Test
ITI
10 min
before next
session
Encoding
Test
Spatial

Figure 4. Diagrams representing the four phases of the experiment and the manipulations applied for each of the intervention tasks. Only one component of the task was different from the baseline for each intervention. The discrimination intervention utilized rodent yogurt drops and rodent fruit loops in place of chocolate and grain pellets, respectively. The intertrial interval intervention lengthened the time between separate sessions from 2 minutes to 10 minutes. Lastly, the spatial intervention limited the cup access to 8 of the 16 cups on the table to make the accessible cups more spatially distinct.

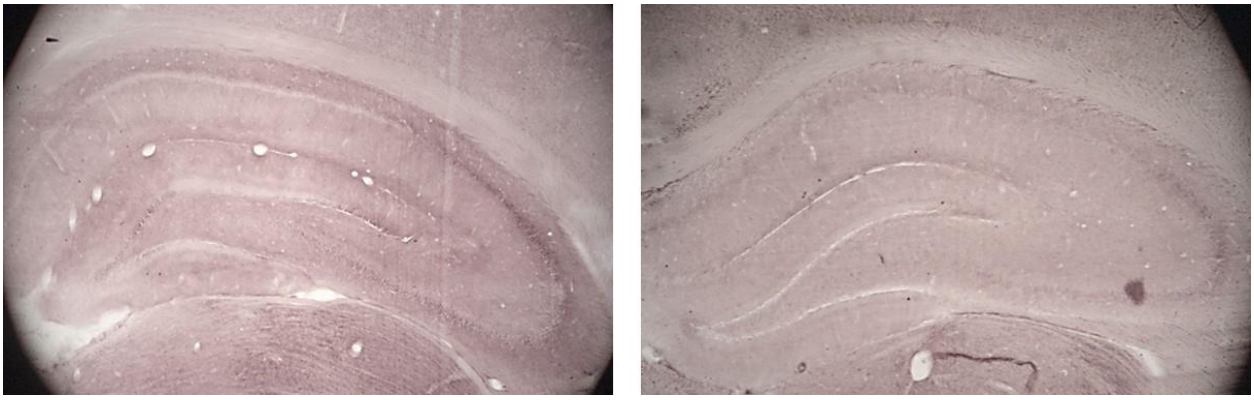


Figure 5. Representative images of ChAT-stained hippocampal slices from a young (left) and aged (right) rat, respectively.

Results

Group-level Task Performance and Intervention Effects

The best fitting model of the episodic-like memory task performance data included three two-way interactions of Task × Day, Task × Age, and Age × Day and the random effect of individual rat. Significant effects of intercept (b = -0.87, SE = 0.18, p <0.001), the ITI Task (b = 0.61, SE = 0.20, p = 0.002), Spatial Task × Age (b = 0.51, SE = 0.20, p = 0.009), and ITI Task × Day (b = -1.15, SE = 0.35, p < 0.001) were present. Though the rats were significantly more likely to make an incorrect choice on the last day of the baseline task during a non-replenishment trial, the likelihood of making a correct choice was 30%. This is greater than chance performance on the task, which is 6%, indicating that the rats were able to perform the baseline task well above chance. Of the interventions, only the ITI intervention task resulted in significant improvement above the baseline performance reference group on the last day of the task (Figure 5). The Spatial Task × Age interaction highlighted improved performance from baseline by the young group in the spatial task, while the aged group demonstrated a mild decline from baseline performance (Figure 6). The interaction also precluded a main effect of the spatial intervention despite overall performance within the intervention being similar to performance in the significant ITI main effect. No other task performances across age group were significant. The ITI Task × Day interaction showed that performance significantly improved across days in the ITI task, while performance was stable at the end of the baseline task (Figure 7).

Pairwise comparisons of the interactions from the best-fit model revealed that there were no differences across the aged and young groups in baseline performance (b = -0.08, SE = 0.20, p = 0.99). Nor did any of the interventions improve the aged group's performance above baseline. Discrimination task performance for the aged group was nearly significantly worse than baseline

(b = -0.39, SE = 0.15, p = 0.058) as well. The young group, however, showed significant improvement from baseline in the spatial task (b = 0.53, SE = 0.14, p = 0.007), driving the significant interaction. The young group also demonstrated significant improvement from baseline in the ITI task (b = 0.38, SE = 0.14, p = 0.03). In contrast to the aged group, the decline in the young group's discrimination task beyond baseline was not significant (b = -0.18, SE = 0.14, p = 0.63). Taken together, these findings provide support for the use of behavioral interventions, targeted at the individual components of episodic-like memory, in episodic-like memory improvement.

Individual Differences in Task Performance and Intervention Effects

Models for the data of each individual were run using the Task variable to evaluate the effects of the interventions on each rat. Of the twenty-four animals in this experiment, sixteen rats had no significant difference in intervention task performance from baseline, five demonstrated significant improvement in one or more interventions, and three showed significant decreases in intervention performance below baseline. More specifically, one of the aged individuals performed worse on both the discrimination (b = -1.10, p = 0.02) and spatial (b = -0.97, p = 0.04) interventions than baseline. Two other individuals, one aged (b = -1.1, p = 0.03) and one young (b = -1.3, p = 0.02), performed more poorly on the discrimination intervention than baseline. Two different rats, one aged and one young, had significant improvement in performance on two tasks, both the ITI (aged individual: b = 1.03, p = 0.05, young individual: b = 0.92, p = 0.05) and spatial (aged individual: b = 1.03, p = 0.05, young individual: b = 0.92, p = 0.05) interventions. Another young individual had an almost significant improvement in the spatial intervention from the baseline task (b = 0.93, p = 0.06). These

individual differences provide insight into both individual- and group-level performance, emphasizing the need for personalized approaches to episodic-like memory decline while also possibly obscuring significant group-level effects, especially within the aged group.

ChAT Levels across Age and Correlations with Task Performance

The ChAT levels within the CA1, CA3, and DG (dentate gyrus) were all compared across age groups via linear regression. No significant differences in ChAT levels were found between the aged and young group in any of the three brain regions investigated (CA1: b = -3.81, p = 0.65, CA3: b = -8.25, p = 0.42, DG: b = -6.93, p = 0.47).

The relationships of optical density measures in each of the three brain regions (CA1, CA3, and dentate gyrus) with the proportion of correct responses for each of the four tasks (baseline, discrimination, ITI, and spatial) were assessed with Pearson's correlations. No significant correlations were found, suggesting that choline acetyltransferase (ChAT) levels were not related to task performance.

These correlations were also assessed within the aged and young data separately to ensure that age was not affecting the relationships. Though separate aged and young assessments did strengthen some of the relationships within both age groups, no significant interactions were present within the aged group (Figure 8). In the young group (Figure 9), however, one significant interaction was seen between ChAT in the CA3 and ITI intervention performance ($r = 0.64$, $p = 0.03$), such that increased levels of ChAT in the CA3 were associated with increased ITI performance. This finding suggests that in young individuals, higher ChAT levels within the CA3 region of the hippocampus predict better episodic-like memory performance when events are made more salient and previous memory interference is reduced by an increased intertrial interval. However, no significant relationships between performance and ChAT were seen in the

aged group that is more susceptible to episodic-like memory deficits. Despite the one significant correlation finding, these data predominantly indicate a limited role for ChAT and acetylcholine in episodic memory on its own.

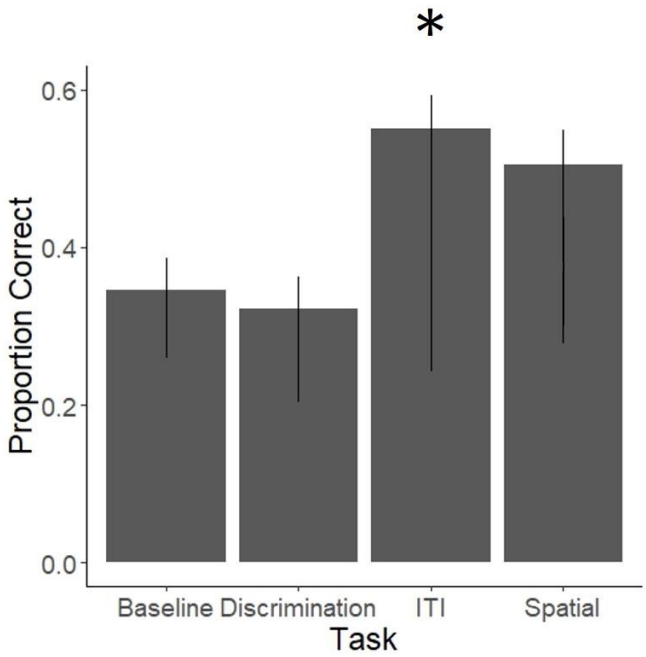


Figure 6. Overall performance depicted by the proportion of correct choices made across the baseline task and three interventions. Performance in only the ITI intervention was significantly improved above baseline.

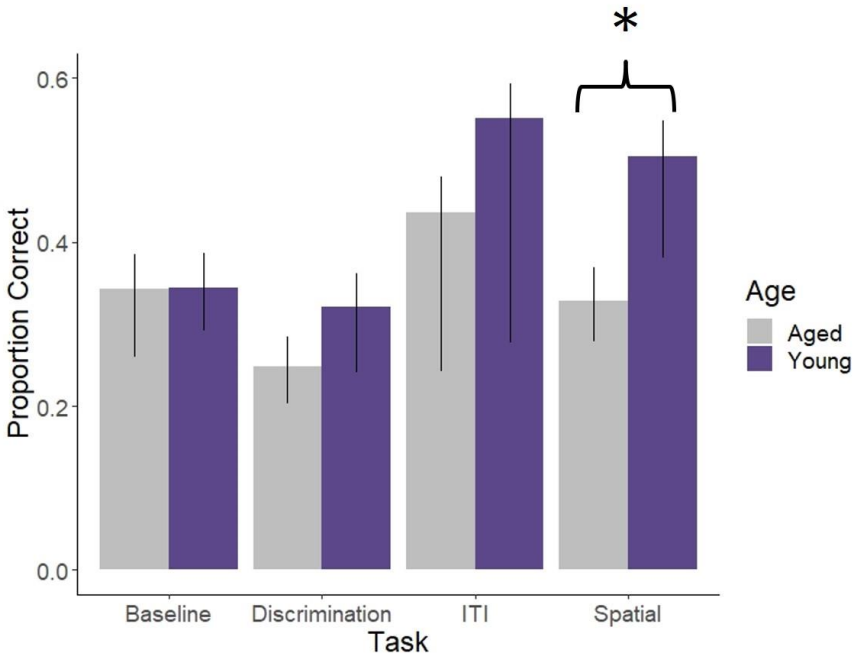


Figure 7. Proportion correct performance in the baseline and intervention tasks separated by age group. The aged rats made fewer correct choices during the spatial intervention than during the baseline, while the young group improved their performance, resulting in a significant Spatial Task x Age interaction. Pairwise comparisons also revealed that the young group's performance did significantly improve in both the ITI and spatial tasks. The aged group, on the other hand, showed a decline in performance during the discrimination task that was trending toward significance ($p = 0.058$).

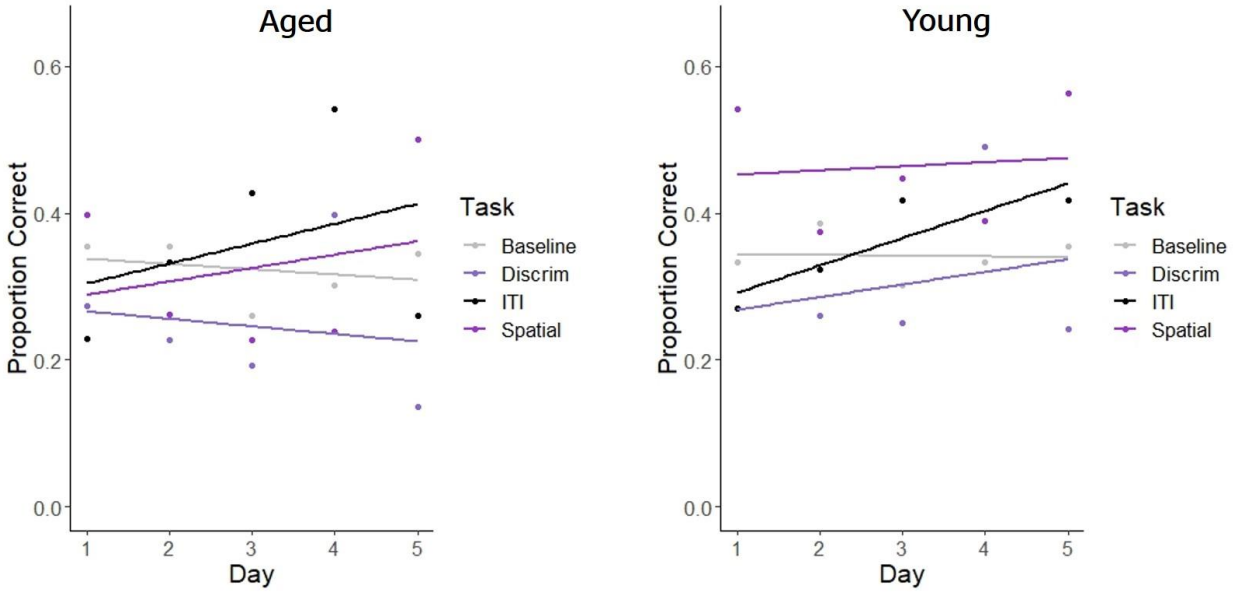
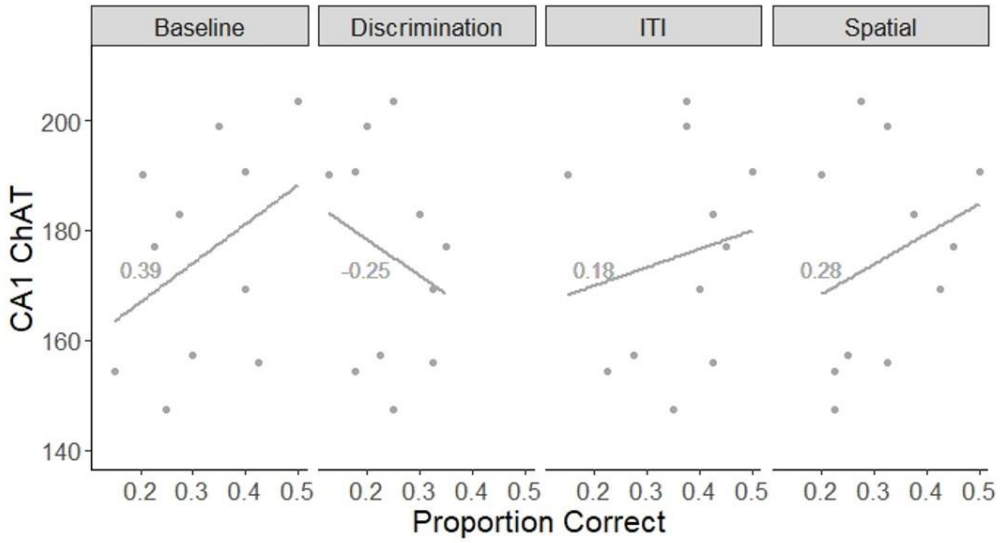
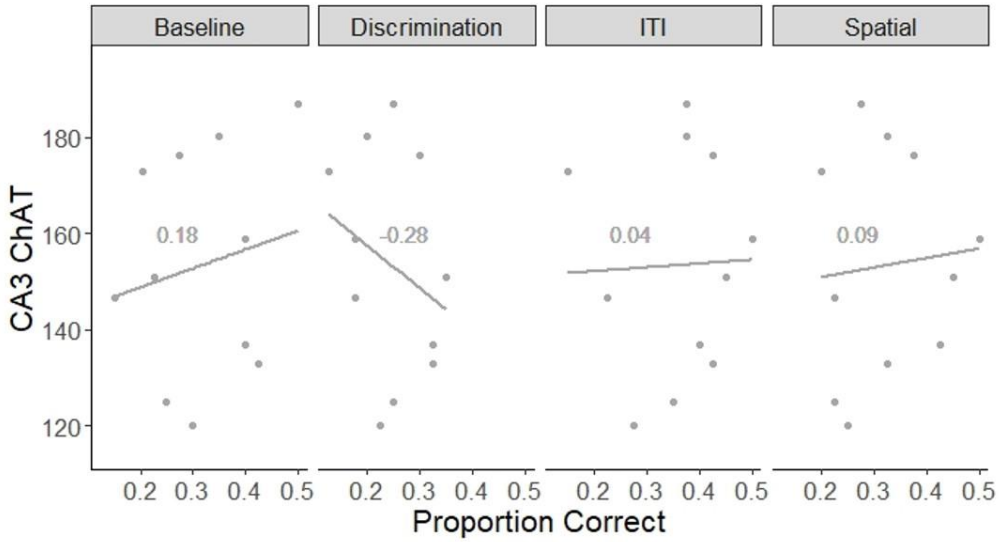


Figure 8. Proportion correct performance across the days of each task for the aged (left) and young (right) groups. Average performance is indicated by the data points, while a linear regression model (Proportion Correct ~ Day) was fit to the data to demonstrate performance slope across days. This figure highlights the positive slope for the ITI task (black line) represented in the model by the significant ITI task × Day interaction. The graphs also demonstrate the positive intervention slopes seen for only the spatial and ITI task in the aged rats, while all three interventions had a positive slope in the young group.


Baseline
Discrimination
ITI
Spatial
CA1 ChAT
200
180
160
140
0.39
-0.25
0.18
0.28
0.2 0.3 0.4 0.5
Proportion Correct


Baseline
Discrimination
ITI
Spatial
CA3 ChAT
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120
0.18
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Proportion Correct

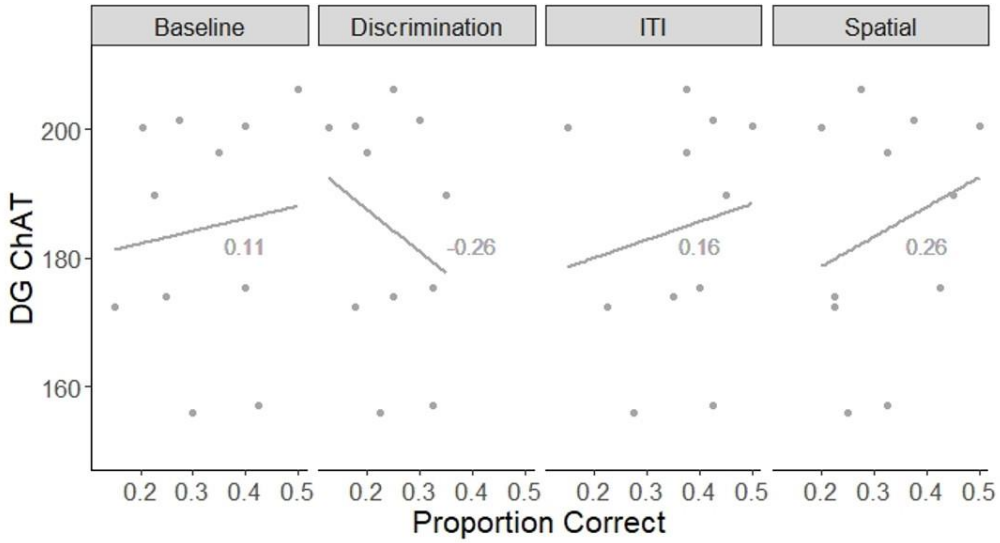
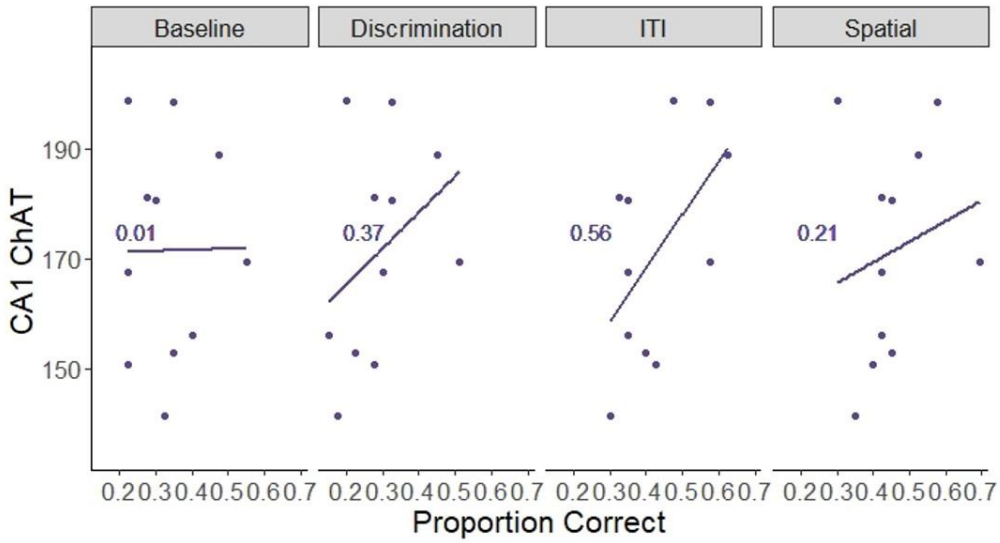
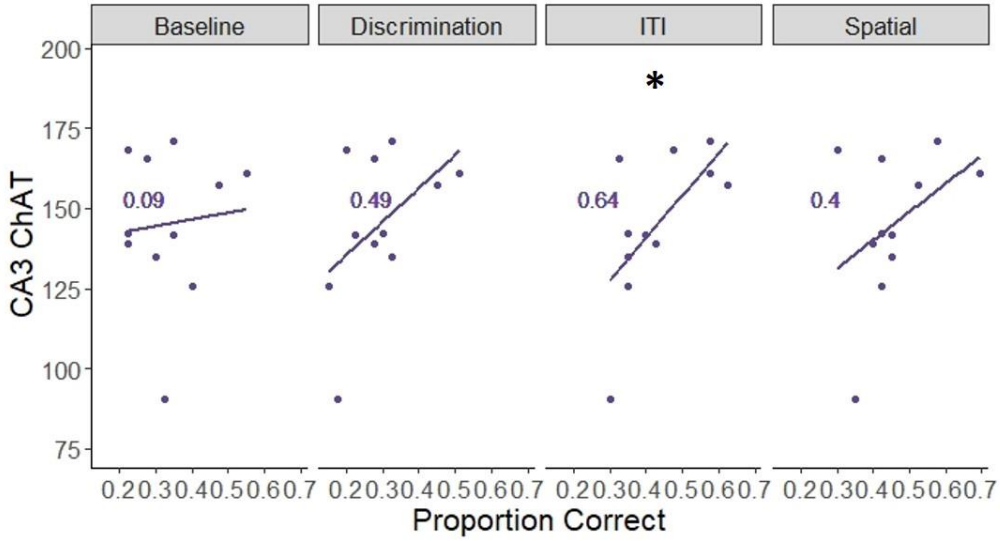

Baseline
Discrimination
ITI
Spatial
DG ChAT
200
180
160
0.11
-0.26
0.16
0.26
0.2 0.3 0.4 0.5
Proportion Correct

Figure 9. ChAT optical density correlations with proportion correct performance across the baseline and intervention tasks for the aged rats. Correlations are depicted for each hippocampal region assessed – CA1, CA3, and DG (dentate gyrus), respectively. Within the aged group, no significant correlations were found.


Baseline
Discrimination
ITI
Spatial
CA1 ChAT
190
170
150
0.01
0.37
0.56
0.21
0.20.30.40.50.60.7
Proportion Correct


Baseline
Discrimination
ITI
Spatial
CA3 ChAT
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175
150
125
100
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*
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Proportion Correct

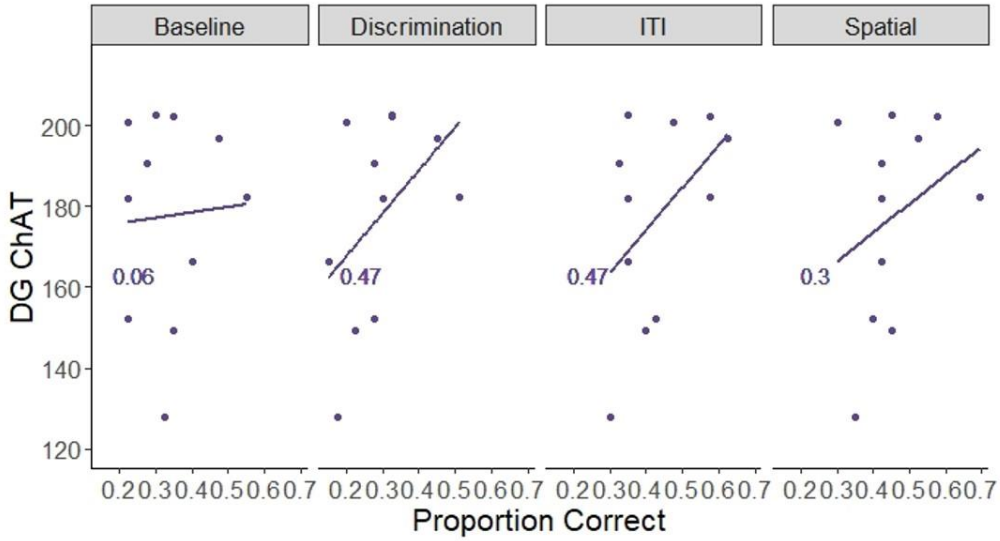
Baseline
Discrimination
ITI
Spatial
DG ChAT
200
180
160
140
120
0.06
0.47
0.47
0.3
0.20.30.40.50.60.7
Proportion Correct

Figure 10. ChAT optical density correlations with proportion correct performance across the baseline and intervention tasks for the young group. The significant CA3 × ITI interaction is evidenced by an asterisk and was the only significant correlation noted.

Discussion

This project sought to determine if age-related episodic-like memory decline could be ameliorated through behavioral interventions. These interventions were created to highlight one component of episodic-like memory, that might serve as a scaffold to improve episodic-like memory overall. Though similar tasks have been implemented in humans (e.g. Rudebeck et al. 2012, Belleville et al. 2006), to the best of the author's knowledge, no experiments have been conducted to compare interventions focusing on each of the three "what", "when" and "where" components separately. Further, such interventions, or training, have not been extensively studied in rodents, nor have the intervention effects been compared between young and aged populations in animals or humans.

The results of this study did not support the initial hypothesis that targeted interventions for each component of episodic-like memory would improve memory performance in aged animals, nor did the data show significant differences in baseline episodic-like memory performance between the aged and young groups. One consideration for this lack of age-related differences in episodic-like memory performance is the difficulty level of the task. Due to the stringent criteria applied to the task, overall performance was low, which may have limited the task's ability to parse out age-related change. However, results indicate significant differences in intervention performance, suggesting that though no group differences were elucidated at

baseline, possibly due to the threshold imposed by the task difficulty, plasticity differences are likely present between the two age groups.

None of the targeted interventions resulted in better task performance in the aged group, and the discrimination task worsened performance, though this finding only neared statistical significance. The young group, however, did improve in both the ITI and spatial intervention tasks, indicating that supporting components of episodic-like memory could be a possible mechanism for refining episodic-like memory. Results also suggested that making the discrimination component of episodic-like memory more salient may worsen memory performance. This finding was seen in both the aged and young rat groups to varying degrees (Figure 6), though was not significant when compared to baseline performance on the final day of each task.

One possible reason for this worsening in episodic-like memory during the discrimination task, especially in the aged group, is that making the food items more prominent and distinct may have promoted proactive interference, such that previous memories of reward locations hindered the formation and utilization of subsequent trial memories. This could be due to the decrease in inhibitory mechanisms known to occur with aging, which further contributes to the obscuring of neural information through extraneous signaling (Hasher & Zacks, 1988). Regardless of the underlying mechanism, the discrimination task may be a less viable approach to improving episodic-like memory.

The other two interventions, the spatial and ITI interventions, showed more promise in this study. In the young group of rats, both tasks resulted in significant improvement above baseline performance. As such, the spatial and ITI interventions could serve as valuable tools to enhance episodic-like memory, at least in younger animals, and should be the focus of future

work. Though ideally positive effects of the spatial and ITI interventions would have been seen in both the young and aged groups, an age-related decline in neuronal plasticity may be a contributing factor to this discrepancy, as declines in neuronal plasticity have been reported in both humans and rodents (e.g. Blank et al. 2007). The lack of response to behavioral interventions also attests to the importance of early intervention in preventing episodic-like memory decline.

Figure 7 provides further hope for the successful use of the described interventions in improving episodic-like memory performance. Though not all the interventions resulted in improvement, the slope of each intervention task across days of the task is positive, suggesting that learning and improvement were occurring at a gradual level. Perhaps with more days per task, more enhancement in performance would be seen. Future experiments should consider longer courses of each task to assess this possibility. The discrimination task performance also yielded a mildly positive slope, suggesting that though the task may initially decrease performance, it should not be completely disregarded as an intervention mechanism. Perhaps the latency of learning in the task is merely delayed, and episodic-like memory performance improvement could still be obtained at a slower rate (Gallistel et al 2004).

As evidenced by the individual subject analyses, there was much variability in the data from this experiment, which likely made it more difficult to discern significant group differences. Despite this impact on group differences, the individual analyses highlight the complex nature of behavioral interventions for episodic-like memory due to how differently individuals can respond to the same treatment. For instance, one individual from the young group and two rats from the aged group showed significantly worse performance than baseline with the discrimination and/or spatial interventions, highlighting how one mechanism is unlikely to

provide episodic-like memory improvement across all individuals. Though these interventions may not be diffusely successful in improving episodic-like memory, they can be utilized and tailored to an individual. The improvement seen in 5 of 24 rodents in this study represents an episodic-like memory improvement in 20% of a population, and the improvement of 2 of the 12 aged subjects represents an improvement in 17% of a potentially affected group. From a translational perspective, the potential to improve the episodic memory performance of 17-20% of a population is of noteworthy importance, especially given the impact that memory improvement can have on an individual's quality of life through furthered independent living, reduced confusion, reduced wandering, etc. These improvements to an individual's life, no matter how robust across groups, statistics, or experiments, are of importance.

No significant relationships were found to exist between the amount of ChAT present within the three regions of the hippocampus studied (dentate gyrus, CA1, CA3) and task performance in the aged group. This finding is inconsistent with current literature on ChAT and aging, and may have been affected by multiple factors. First, the age gap between the aged and young rats in this experiment may not have been large enough to demonstrate differences, especially with such a small sample size. Second, the immunohistochemical measure utilized in this experiment does not allow for any dynamic assessment. It instead provides a snapshot of overall ChAT levels, which may have not been representative. The measure used also does not provide any indication of functionality – perhaps functional ChAT was reduced in the aged group, but the technique used could not have made such a distinguishment. Better methods to allow for more dynamic and repeated measurements such as ELISA, PCR, DREADDs, or pharmaceutical administration should be considered for future work.

One significant correlation was found in the young group between CA3 ChAT levels and ITI intervention performance, suggesting that increased CA3 ChAT levels predict improved performance under ITI manipulations. Since this relationship was not seen in the aged rats, perhaps ChAT levels within the CA3 need to be supported as early as possible to facilitate successful utilization of the ITI intervention.

The lack of robust age-related relationships is inconsistent with previous data (e.g., Yufu et al. 1994) and may be due to the different measurement modalities used, as well as the age of subjects studied. The aged group of rodents used in this study was 20 months of age, which is younger than used in similar studies. Though this late middle age of rats helped to reduce the risk of age-related subject loss and ensured the individual's physical ability to perform the tasks, this lower age could have contributed to the lack of age-related ChAT differences. Optical density provides a generalized overview of ChAT quantity, but it does not allow for quantification of the enzyme itself nor to determine if the enzyme present is still functional. This inability of optical density to capture enzyme functionality could have resulted in the contradictory findings of this study, and, as such, may not be the best measure to utilize. Rather, future work should use measures like radiometric measures of activity (e.g., Yufu et al. 1994) and Western blots (e.g., Hawley et al. 2015), which assess ChAT activity, quantity, or function more thoroughly.

Another component to consider in the absence of ChAT effects in the current study is the complex neurobiology involved in memory consolidation and recall. Though ChAT and its enzymatic product acetylcholine have previously been demonstrated to play a role in age-related decline and episodic-like memory, they are not the sole component of either mechanism. With multiple other enzymes and neurotransmitters contributing to these processes, assessing other measures as well as ChAT could provide a more representative picture of cognitive changes.

Perhaps, this lack of finding is congruent with current knowledge, clinical findings, and the poor success of the four current FDA-approved Alzheimer's disease (AD) medications. Of the four medications, three are acetylcholinesterase inhibitors, which work to increase the amount of the neurotransmitter, acetylcholine, present at the neuronal synapses (Long and Holtzman 2019). Despite 75% of current AD medications targeting acetylcholine, only modest effects in cognitive improvement have been documented. Though ChAT certainly plays a role in the episodic memory and cognitive decline associated with aging and AD (e.g., Perry et al., 1992), it may not be the most potent component. Rather, a more multimodal therapeutic approach targeting multiple neurotransmitters may be more effective at managing AD symptoms.

Much remains to be learned about the cognitive and neurobiological decline associated with aging and AD, there are copious methods for studying these processes. Episodic memory is one of the first memory types to be affected. So much so that episodic memory decline can foreshadow AD onset by over half a decade. By studying episodic memory decline, light can be shed on the onset of more severe disease and, hopefully, how to mitigate it. Rats share many of the memory-associated neurobiological pathways and provide a unique opportunity to study cognitive changes in a more controlled setting and over a shorter period of time. Rodents have also been shown to utilize episodic-like memory, the animal counterpart of episodic memory, which was studied via a novel task in this work.

Though this novel paradigm provides new opportunities for episodic-like memory analysis, it has points of weakness. As a result of the lack of constraint in an open field, the criterion set for correct test trial completion, and the complexity of the task, successful performance was low (and lower than seen in the Zhou and Crystal work), and likely made improvements in performance more difficult to elicit and observe in this study. Further work and

paradigm modifications conducted since this experiment have improved task performance and reduced the length of training needed for successful task completion, demonstrating further promise for the open-field paradigm. Due to the novelty of this task, it has yet to be tested against the main criticisms of episodic-like memory tasks – this should be addressed in future work. However, given the extensive parallels to a paradigm that has already successfully demonstrated episodic-like memory performance and the complex nature of the task, it is reasonable to surmise that the task does meet the aforementioned criteria needed to ensure it elicits episodic-like memory. Further work should also aim to study the effects of intervention order, as the sample size of the current study prohibited such analyses. Despite this limitation, initial concern for recency or carry-over effects is mitigated by the data, as the only intervention that showed a decrease in performance, the discrimination intervention, was the first intervention administered and therefore would not have been susceptible to these mechanisms.

Given the strong overlap in the cognitive decline seen with aging and AD, using aged subjects can provide insight into both the naturally occurring aging process as well as the pathologic changes associated with dementia and Alzheimer's disease. Findings of this study suggest that behavioral interventions aimed at supporting one of the "what", "where", or "when" components of episodic memory may improve memory task performance. Though this effect was only seen in the young rat group, the results speak to the importance of utilizing such interventions prior to the onset of cognitive impairment. This study also emphasized the importance of investigation of different pharmaceutical product types for the cognitive decline associated with AD, as the ChAT levels present within the rodent brains did not correlate with memory task performance. Taken together, these findings may aid in the development of new

techniques to perturb some of the inevitable cognitive deficits seen in both the natural aging process and dementia, such as AD.

Conclusion

The behavioral interventions targeting specific components of episodic-like memory utilized in this experiment suggest that episodic-like memory performance can be improved, and that further improvement may be seen over time. These effects were only observed in young, not aged, individuals, however, which attests to the importance of early intervention application, as does the human literature which suggests that episodic memory deficits can precede pathology-related cognitive decline by more than half of a decade. Choline acetyltransferase (ChAT), an enzyme that produces the neurotransmitter, acetylcholine, did not show any relationship with successful task performance within the aged group. Different analysis techniques may have yielded other results, though the absence of effects in the potentially compromised aged rats also mirrors the poor clinical improvement seen with acetylcholine-targeted medications in the treatment setting of cognitive decline. This work provides a scaffold for future studies to assess the efficacy of similar behavioral interventions and new neurobiological targets as mechanisms to combat the cognitive decline associated with both natural aging and dementia processes.

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