Summary/abstract
Memory is a cornerstone ability upon which we build knowledge of ourselves and the world around us. Failures in memory, no matter how small, can significantly impact life success and mental health (including anxiety and depression). In adults, recognition memory is subserved by two processes, recollection and familiarity, which rely on partially distinct brain circuitry in the medial temporal lobe (MTL) and prefrontal cortex (PFC). Familiarity is the process that allows for the global assessment of memory strength. Recollection is the process that allows for the retrieval of distinct features associated with the context of the event. Recollection preferentially involves the hippocampus, a MTL structure characterized by its protracted developmental course. Neuroanatomical data illustrates that structural development of the hippocampus continues at least through the 5th year postnatally, which has been theoretically linked to functional changes observed in behavioral memory performance (e.g., improvements in autobiographical memory and episodic memory that occur during early childhood). However, this link has not yet been examined empirically. Thus, despite all we know about memory processes and associated neural circuitry in adults, the systematic study of its functional maturation early in life is notably absent. What remains relatively unexplored are age-related changes in the basic processes that underlie memory improvement in early childhood. This poses not only a gap in scientific understanding but also a barrier to development of intervention techniques that would facilitate or improve memory, particularly in those at-risk for impairment. Our goal is to elucidate mechanisms of change in memory development by systematically investigating changes in memory behavior and neural activity. Towards this end, the proposed research seeks to indentify windows during which memory processes develop that are informed by neurodevelopment. Specifically, we will examine familiarity and recollection processes during a memory retrieval task using a unique combination of electrophysiological and behavioral measures in early childhood. We hypothesize that electrophysiological and behavioral correlates of recollection (which rely on the hippocampus) will show substantial developmental change from 3 to 5 years of age, compared to changes in familiarity processes. Systematic study of memory development in humans has important implications for understanding memory in general and will ultimately further our understanding of disorders of memory (e.g., developmental amnesia), populations where memory is affected (e.g., individuals with depression), and disorders in which abnormalities of memory circuitry have been reported (e.g., depression, autism, and schizophrenia).

SPECIFIC AIMS

Memory is a cornerstone ability upon which we build knowledge of ourselves and the world around us. Failures in memory, no matter how small, can significantly impact life success and mental health. Impairments in memory are associated with learning disabilities in childhood and mental health disorders (e.g., depression and schizophrenia) in adolescence and adulthood (Gathercole, 1993; Naismith et al., 2003; McKenna et al., 1990). In adults, recognition memory is subserved by two processes, recollection and familiarity, which rely on partially distinct brain circuitry in medial temporal lobe (MTL) and prefrontal cortex (PFC; Yonelinas, 2002, cf. Squire et al., 2007). These processes allow for the retrieval of distinct features associated with the context of the event (recollection) and the global assessment of memory strength (familiarity). Recollection preferentially involves the hippocampus, located in the MTL, and is characterized by its protracted developmental course, while familiarity engages non-hippocampal structures and develops earlier (Ghetti & Angelini, 2008; Ranganath et al., 2004). Anatomical data shows that hippocampal development begins during the prenatal period yet continues at least through the 5th postnatal year (Serres, 2001). This structural maturation is theoretically linked to functional changes observed in behavior as autobiographical and episodic memory improves throughout childhood. However, this link has not yet been examined empirically in early childhood.

Despite all we know about memory processes and associated neural circuitry in adults, there are no systematic studies of the age-related changes in these basic processes that underlie successful memory performance during early childhood. This poses not only a gap in scientific understanding but also a barrier to the development of intervention techniques that would facilitate or improve memory in those at-risk for impairment (e.g., tailoring the amount of contextual detail available during encoding to enhance specific learning). Our long-term goal is to elucidate mechanisms of change in memory development by systematically investigating changes in memory performance and associated neural activity. The proposed study will examine familiarity and recollection during memory retrieval in early childhood using a unique combination of electrophysiological and behavioral measures. Based on previous findings, we hypothesize that both electrophysiological and behavioral correlates of recollection will show substantial developmental change from 3 to 5 years of age, compared to changes in familiarity. We have generated preliminary data showing that the proposed task produces reliable electrophysiological components reflecting recollection and familiarity that can be recorded during retrieval in 5-year-old children. Our specific aims are to:

Aim 1: Dissociate recollective processes from familiarity processes in early childhood. Recollection and familiarity processes will be identified via the combination of behavioral indices of memory and electrophysiological responses generated when viewing familiar and novel stimuli. We hypothesize that both recollection and familiarity will be dissociable from novelty processes, and that they will show different spatial-temporal patterns in the recorded electrophysiological response across the scalp. Consistent with research in adults and older children, we predict familiarity responses will occur at shorter latencies, recollection responses at longer latencies, and that these differences will be accompanied by divergent spatial distributions indicating involvement of separable underlying neural circuits.

Aim 2: Examine age-related changes in recollection and familiarity during early childhood. A cross-sectional design will be used to compare behavioral and electrophysiological measures of recollection in 3- to 6-year old children. This age range encompasses the window during which 1) a developmental shift has been reported in children's memory abilities on behavioral tasks (Bauer, 2007; Gathercole, 1993) and 2) neuronal connections within the hippocampus develop, altering its functional circuits (Serres, 2001). We hypothesize that both behavioral and electrophysiological measures of recollection will vary across this time period while measures of familiarity and novelty will remain relatively stable. The proposed research combines dual process models of memory and the tools of cognitive neuroscience within a developmental framework to identify mechanisms of age-related change in memory performance.

The proposed research utilizes an innovative combination of behavioral and electrophysiological methods to examine the development of memory processes and associated neural activity that have been well characterized in adults. Such data are not possible to obtain using other methods, such as fMRI, given the young age of the participants. This work will contribute to a more comprehensive theory of recognition memory across the lifespan and bridge the current gap between memory literature in infants and adults. Examining memory processes during developmental periods informed by neuroanatomical data is an essential first step towards bridging structure-function relations, which is notably lacking in developmental work. This knowledge has significant public health relevance as it is critical to understanding typical and atypical memory development. It will greatly enhance our ability to make accurate predictions regarding pathological outcomes and assist in designing programs to facilitate adaptive functioning within this fundamental cognitive domain.

RESEARCH STRATEGY

a. SIGNIFICANCE

Failures in memory can range from the complete inability to form or retrieve memories (e.g., amnesia, Scoville & Milner, 1957) to mild or moderate impairments in performance (e.g., developmental amnesia, Vargha-Khadem et al., 1997). Experiences with small lapses in memory (e.g., forgetting where you put your car keys) make it readily apparent how frustrating these failures can be and how they can significantly impact daily functioning. Memory impairments are associated with learning disabilities in childhood and mental health disorders, such as depression and schizophrenia, in adolescence and adulthood (Gathercole, 1993; Naismith et al., 2003; McKenna et al., 1990), but there are few studies on the development of memory processes during childhood. The experiments in this proposal combine behavioral and electrophysiological techniques to examine familiarity and recollection during a period of significant developmental change.

Recollection and familiarity

Adults. Given its relevance to healthy functioning, there is a large existing body of literature regarding memory in adults. One prominent theory suggests two processes (recollection and familiarity) underlie adults' ability to remember (Yonelinas, 2002). Recollection allows for the retrieval of distinct features associated with the context in which the event was originally encountered. Familiarity allows for the global assessment of the strength of the memory trace of the event without contextual features. Although controversy exists surrounding dual processes models of memory (Squire et al., 2007), empirical evidence has accumulated from multiple sources including experimental, computational modeling, neuropsychological, neuroimaging, and individual differences perspectives. Behavioral research using remember-know paradigms (Tulving, 1985), source memory tasks (Mitchell & Johnson, 2009), and receiver operating characteristic (ROC) procedures (Yonelinas, 1994) show that recollection is more sensitive than familiarity to division of attention, generation, semantic encoding, response speeding, effects of aging, and amnesic effects of drugs, but is less sensitive than familiarity to shifts in response criterion, fluency manipulations, forgetting over short retention intervals, and perceptual manipulations (Yonelinas, 2002). Research in patient populations and functional neuroimaging studies in healthy adults reveal that these processes rely on partially distinct neural circuitry during encoding and retrieval. Both engage structures in the PFC and MTL; within MTL, recollection relies on the hippocampus and familiarity does not (Eichenbaum et al., 2007; Ranganath et al., 2004; Yonelinas et al., 2002).

Development. There are relatively few behavioral studies investigating the development of recollection and familiarity during childhood. Studies that do exist have utilized modifications to adult behavioral paradigms in samples of school-age children (6 years and older; Anooshian, 1999; Billingsley et al., 2002; Brainerd et al., 2004; Brainerd et al., 2009; Ghetti & Angelini, 2008; Holliday, 2003; cf. Lindsay et al., 1991) and show developmental dissociations regarding the development of these two processes. In these samples, familiarity develops before recollection, but the mechanisms contributing to this change are unknown. For example, Billingsley and colleagues (2002) used the remember/know paradigm in a sample of 8-19 year olds and reported age-related increases in recollection between 8 year olds, 12 year olds, and adults but not in familiarity. More recently, Ghetti and Angelini (2008) employed the dual-process signal detection model (Yonelinas, 1994) in a sample ranging from 6 years to adults and reported that recollection developed from childhood into adolescence, whereas familiarity showed no development during this time (once processing time was equated). Neuroimaging studies have recently begun to examine development of neural substrates underlying memory in school age children. Activations in both MTL and PFC are related to age-related improvements in recollection during memory encoding. Using a memory for scenes task, Menon and colleagues (2005) showed significant age-related decreases in activation in left MTL in 11-19 year olds that were apparent even after controlling for recognition accuracy. Others showed in 8-24 year olds that PFC activations were correlated with developmental gains in recollection (Ofen et al., 2007). Importantly, these changes were not due to differences in structural maturity (as indexed by changes in gray matter density) but were reported to reflect functional maturity. Together, these findings suggest that differential activation in MTL and PFC during encoding in younger, compared to older, age groups reflect differences in recruitment of processing resources in MTL and PFC that are necessary to sustain memory representations.

Gap in current knowledge

There is no systematic study of the development of recollection and familiarity before children enter formal schooling. One current limitation is the lack of appropriate tasks to examine recollection and familiarity in young children because the demands of current paradigms are too complicated cognitively (e.g., require reliable subjective judgments regarding memory) or overly demanding physically (e.g., motion restrictions in fMRI) for young children. This is unfortunate as there are strong theoretical (Perner & Ruffman, 1995), neuroanatomical

(Bachevalier & Vargha-Khadem, 2005), and clinical (Vargha-Khadem et al., 1997) arguments to examine the development of recollection and familiarity. This lack of knowledge poses a significant barrier not only to full understanding of memory but also development of techniques to improve memory in those at-risk for impairment. Thus, there is a critical need for studies to explore development of recollection and familiarity in early childhood and changes in the parameters that underlie memory improvement during this period.

Event-related potentials (ERPs). Familiarity and recollection can be detected in adults using ERPs. These distinct electrophysiological responses emerge as two sequentially occurring components with distinct timing and scalp distributions. Familiarity is associated with a mid-frontal negativity approximately 400 ms after stimulus onset; recollection is associated with a positive activity over parietal leads beginning approximately 500 ms after stimulus onset (Duarte et al., 2004; Friedman & Johnson, 2000; Rugg & Yonelinas, 2003). These findings are consistent with behavioral results indicating that familiarity is available earlier than recollection and with fMRI data suggesting these processes are associated with activity in partially distinct neural generators.

ERPs have been used to examine development of item and source memory in school-aged children and adolescents. Although these tasks vary slightly from those in adults, they have implications for the development recollection and familiarity. Memory for individual items may involve recollective processes but can be subserved by familiarity processes alone. In contrast, memory for contextual details, such as the source in which one encountered the item, requires recollective processes. Thus, in the developmental literature, successful source memory performance has been used as an index of recollection. For example, Cycowicz and colleagues (2003) used ERPs to examine memory for items and their context (color) in 10 to 12-year-old children and adults. They found that all age groups showed similar ERP responses for item information; however, ERPs generated during source memory showed variations in amplitude between old and new stimuli as well as different scalp topographies across age groups, which were associated with improvements in memory performance. This pattern was interpreted as reflecting the prolonged development of memory for contextual details (i.e., recollection) and the contribution of brain development (in frontal regions) to post-retrieval monitoring of source information. Czernochowski and colleagues (2005) also used ERPs to examine memory for items and their contexts (spoken words vs photos) in 3 age groups (6-8 years, 10-12 years, and adults) using an exclusion paradigm. They also documented developmental change in ERP components related to retrieval of contextual details; however, their paradigm failed to detect familiarity components in the youngest age group (perhaps due to the demands of the behavioral paradigm, which produced high false alarm rates in both child age groups). They interpreted these findings to indicate young children rely more on recollection than familiarity, however, this conclusion is largely inconsistent with behavioral findings (reviewed above). Finally, in our recent examination of memory for context (i.e., temporal order, [redacted] et al., 2009a) in 3- and 4-year-old children, we identified age-related improvements in memory for context despite similar levels of item memory (similar to Cycowicz et al., 2003). These effects were associated with components in the electrophysiological response. Consistent with adult research, distributed, positive-going activity late in the waveform was associated with recall of contextual details and recollective processes. Although this investigation broke new ground and provided a mechanism for examining neural bases of recollection in early childhood, the nature of the paradigm (i.e., assessing memory for temporal order) differed drastically from the types of paradigms used in older children and adults. Thus, relating the findings from this investigation to the established literature on recollection in adults remains speculative. In addition, the ability to examine correct versus incorrect items was limited due to inherent linking of items in the temporal order memory task. Even so, the studies described above suggest that ERPs are a viable mechanism for examining development of recollection and familiarity; however, there is great need for tasks that are appropriate for young children.

Behavioral development. Regardless of the task and stimuli used, empirical research shows that memory ability undergoes substantial qualitative change from infancy, through the preschool period, until 6-7 years of age when it becomes “adult-like” in organization (Gathercole, 1993). For example, paradigms examining memory for the source of information (Drummey & Newcombe, 2002) and relations between items (Sluzenski et al., 2006) show that abrupt improvements are made between 4 and 6 years of age and little change occurs subsequent to that time. Such empirical findings fit well with reports of children’s ability to recall events from their own lives (i.e., autobiographical memory). Between 3 and 6 years of age, the number of events that can be recalled increases linearly and the amount of information that is recalled doubles (Bauer, 2007; Howe & Courage, 1993; 1997). Relatedly, when adults are asked to recall events from their early childhood experiences, the number of memories they are able to retrieve increases steadily between 3 and 7 years (Bauer, 2007). Together, evidence from both laboratory and naturalistic settings has lead to the argument that early childhood (3 to 6 years) is a period of significant developmental change in memory.

Brain development. Contemporaneous associations exist between the behavioral findings described above and neuroanatomical changes in the neural substrate known to support memory in adults (i.e., PFC and MTL). A next critical step to understanding memory development is to evaluate the "fit" between age-related changes in memory behavior and development of the neural substrate responsible for it (Bauer et al., 2006), as this should constrain expectations regarding behavioral performance and shed light on mechanisms responsible for it (Bauer et al., 2007). Although brain development begins in the prenatal period, it follows a protracted developmental course throughout the postnatal years (Giedd et al., 1996; 1999). Within the MTL, significant development of the hippocampus has been reported in 4-25 year olds with dissimilar trajectories in posterior versus anterior subregions (Gotay et al., 2004; 2006), which is argued to parallel differences in their functional development (Geidd et al., 1996). However, functional development has not yet been examined empirically.

Neuroanatomical data regarding structural development of the hippocampus obtained from human and nonhuman primate tissue samples suggest that its prolonged development is due to neuron formation, migration, and proliferation of afferent and efferent connections; however, it is the latter that is most dramatic. Specifically, within the hippocampus, although immature cells continue to accrue within the dentate gyrus throughout the first year of postnatal life, dendritic development and synapse formation persists until at least 5 years of age (Eckenhoff & Rakic, 1991; Serres, 2001). Between the 3rd to 5th years of life, neuronal connections between granule cells of the dentate gyrus and pyramidal neurons of Ammon's horn form, which alter the functional circuits of the hippocampus. Because this circuitry is critical for adult-like memory formation, this profile suggests that adult-like memory formation in humans may not be expected before the 5th postnatal year, as morphological development is likely correlated with functional capability (Serres, 2001). The prolonged development of the hippocampus stands in stark contrast to the development of the entorhinal cortex (which has been related to familiarity processes, Ranganath et al., 2004; Yonelinas et al., 2002). At the time of birth, the basic gross anatomic features and topological relationships of the human entorhinal cortex are present, and postnatal development takes place mainly during the first postnatal year (Grateron et al., 2002).

Impact on scientific field and clinical practice

Data regarding the development of recollection and familiarity would contribute new knowledge to the field of memory development as a current controversy exists regarding the nature of memory in infancy and childhood (cf. Bauer et al., 2007, Rovee-Collier & Cuevas, 2009; see also Brainerd et al., 2009). To date, there are no paradigms available to address the issue empirically in early childhood. Exploring the development of basic processes that contribute to memory at both behavioral and neural levels would significantly enhance the field, as developmental change may not be detected by merely observing trends in overt behavior especially if the processes (or underlying circuitry) are characterized by distinct developmental trajectories (see Ghetti & Angelini, 2008 for elaboration). Thus, the study of memory will be advanced as we identify the contributions that familiarity and recollection make to memory performance during early childhood.

These data will also improve clinical practice as they will ultimately help inform understanding of 1) disorders of memory (e.g., developmental amnesia, Vargha-Kadem, et al., 1997), 2) populations where memory is affected (e.g., individuals with depression, Naismith et al., 2003), and 3) groups in which abnormalities of memory circuitry (i.e., hippocampus) have been reported (e.g., neurodevelopmental disorders such as autism, schizophrenia, epilepsy, and Down syndrome, Lavenx et al., 2007; Nugent et al., 2007). This information will allow for better predictions regarding memory outcomes in at-risk individuals as well as the development of age- and neurally-appropriate interventions by tailoring the amount of contextual detail available during memory tasks to enhance specific learning. Such information is important because damage to memory structures early in life can have consequences that are not realized until several years later, as in the case of prenatal hypoxic-ischemic events (Isaacs et al., 2003) or prenatal iron deficiency [redacted] et al., 2009b), where memory loss resulting from insult to the hippocampus in infancy becomes evident in everyday behavior only later in childhood. Such findings suggest a maturational gradient within the MTL memory system, with most abilities crucially dependent upon the hippocampus emerging in later stages of development, supporting a model of hierarchical organization of memory within the MTL (Bachevalier & Vargha-Khadem, 2005). However, empirical findings consistent with this argument are still needed. Having this knowledge early in life is important due to the plasticity that exists during this period (Nelson, 2000).

b. INNOVATION

Results from the proposed experiments will provide critical data relevant to the theoretical debate on the presence of multiple memory processes in development. More importantly, the findings will serve as a much-needed link between what is known regarding memory processes in adults and memory processes in young children. This information is essential if complete understanding of memory is to be achieved. This study will also provide empirical data linking behavioral improvements in memory to developmental changes in neural

activity (as indexed by ERPs). The unique combination of behavioral and electrophysiological methods will help fill the methodological gap that exists regarding the ability to examine the development of recollection and familiarity in early childhood, as there is a lower bound on ages that can be examined using present behavioral paradigms and neuroimaging procedures. Unlike fMRI or near infrared spectroscopy (NIRS), ERPs are well-suited for use during early childhood and can be used across the life span to track developmental changes. ERPs will add distinctive information as these measures have been associated with 1) neural activity generated at hippocampal-cortical synaptic connections, 2) individual differences in cortical volume, and 3) composite memory scales indexing real-world memory behavior in adults (Friedman & Johnson, 2000; Walhovd et al., 2006). Finally, the proposed research will provide empirical data to inform future studies that will directly examine development of the underlying neural substrates or clinical interventions that will indentify specific mechanisms that can be targeted early in life, when their impact may be greatest (Nelson, 2000).

c. APPROACH

The aims of the present proposal are to 1) dissociate recollective and familiarity processes at both behavioral and neural levels, and 2) examine age-related changes in these processes in early childhood. Towards this end we will utilize a unique combination of electrophysiological (ERP) and behavioral measures.

Method. The study will be conducted in the [redacted]. [redacted] houses a "child friendly" 64-channel Biosemi EEG data acquisition system and appropriate rooms for behavioral testing. A total of 100 children (25 3, 4, 5 and 6 year olds) will be recruited from the [redacted] database (maintained by faculty at UMD) for participation. Children will visit the laboratory setting on 2 occasions. During the first, children will be asked to remember two sets of novel items (Toy A vs. Toy B) in two different contexts (Location 1 vs. Location 2). To enhance and promote encoding of the material, each item will be paired with a novel action (e.g., spinning the item like a top on the floor). Following a 7-day delay (see [redacted] et al., 2009a), children will return to the laboratory for two memory "assessments." In these memory assessments, previously-viewed ("old") items will be presented in neutral context (beige background) along with a set of distracter ("new") items. Measures of children's electrical brain responses will be recorded as children passively view digital photographs of the stimuli. The items will be presented in random order (stimulus duration = 1000ms; with a variable inter-trial interval ranging from 1500-2000ms; DeBoer et al., 2005). To ensure enough trials for analysis, each participant will view the entire set of pictures twice (in 2 separate blocks). Based on pilot data, we found this strategy was more effective than doubling the number of to-be-remembered items, which significantly decreased memory performance. In accordance with previous studies, EEG will be recorded from 64 active Ag–AgCl scalp electrodes plus two vertical and two horizontal electrooculogram (EOG) channels to a common reference sampled at 250 Hz (see DeBoer et al., 2007 for review). These recordings will be used to index processes associated with recollection, familiarity, and novelty.

Immediately following the ERP session, children will be asked to behaviorally sort the items according to the appropriate context (e.g., Toy A in Location 1). Consistent with paradigms in the adult literature, items will be presented one at a time in random order. Based on the child's sorting, indices of recollection (old item in correct context), familiarity (old item in incorrect context), and novelty (new item correctly rejected) will be derived. As described above, memory for contextual details, such as the location of the item, requires recollective processes; item memory may be subserved by either. False alarms and misses will also be recorded to examine accuracy of memory performance. Finally, memory for the novel actions associated with the objects during encoding will be assessed for use in follow-up analyses of ERP results (see *Statistical approach*). The fixed presentation order of the ERP and behavioral task is necessary because the constraints of ERP methodology are such that behavioral recall can not be done simultaneously and behavioral testing could influence the ERP response (e.g., by giving children experience with the novel stimuli); whereas prior research has demonstrated no effect of ERP exposure on subsequent recall performance (Carver et al., 2000). Additional behavioral measures (i.e., verbal fluency and verbal IQ) will also be included, to ensure the children in the 4 age groups are from similar populations and to explore the contribution of verbal ability to declarative memory (Quamme et al., 2004). Parent questionnaires will be used to evaluate child characteristics and EEG-related variables of interest, including handedness, medication history, and conditions related to brain health (e.g., seizures, loss of consciousness). Participants with such conditions will be excluded from analysis.

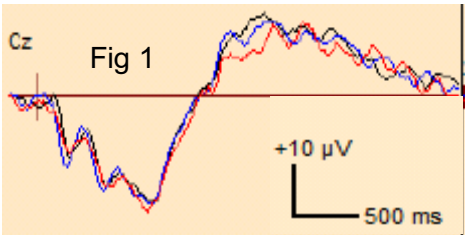
Statistical approach. Behavioral responses will be used to determine which items were recollected (i.e., old items in which the context was correct), those that were familiar (old items in which the context was incorrect), and those that were novel (new items). False alarms (new items judged as old) and misses (old items judged as new) will also be recorded. Mean levels of each behavioral response will be analyzed using repeated measures (RM) ANOVAs and appropriate follow-up tests (SPSS Inc., Chicago, IL). Successful memory

performance will be inferred when the mean number of item correct responses (for both old and new items) exceeds false alarms and misses. The mean number of recollected items must exceed the mean number of familiar items and chance levels (i.e., 50%). Given that a sufficient number of ERP responses are required for both recollection and familiarity conditions, task demands will be tailored (see below) to ensure that behavioral performance in both conditions will yield an appropriate number of ERP trials for analysis.

Electrophysiological data will be sorted offline for analysis according to behavioral recall (using the categories described above) using Brain Electrical Source Analysis (BESA) software (MEGIS Software GmbH, Gräfelfing, Germany). Data files will be hand-edited to remove movement and blink artifacts for data analysis. A minimum of 15 trials will be included for all conditions. Missing data from individual channels will be interpolated for a maximum of 10% of bad channels (i.e., 6 per participant; see DeBoer et al., 2005 for rationale). Appropriate filters will be utilized (.1 Hz, 6 dB/octave to 80 Hz, 24 dB/octave). Data will be re-referenced offline to an average reference. Two components of the ERP relevant to our data have been described previously. First is a well-defined, negative amplitude, middle latency component (occurring 400–600 ms after stimulus onset) related to attentional processes (Courchesne et al., 1981; Nelson & Collins, 1991) and modulated by memory (de Haan & Nelson, 1997; Carver et al., 2000). Second is a positive-going component occurring later in the waveform (approximately 900 ms after stimulus onset) and distributed over the 900-1500ms window in the recording epoch (referred to as positive slow wave activity, PSW; Nelson, 1994). PSW is invoked by previously seen, partially encoded stimuli and is suggested to reflect hippcampally-mediated recollection memory ([redacted] et al., 2009a; 2009b). Specific windows will be selected based on previous reports (e.g., Marshall et al., 2002; [redacted] et al., 2009a; [redacted], 2009) and visual inspection of the data. Differences in mean amplitude, mean latency, and/or area under the curve between the 3 conditions (recollection, familiarity, novelty) at both midline and lateral leads will be evaluated using RM-ANOVAs and appropriate follow-up tests. Differences in scalp topography (both within and between age groups) will be assessed statistically via RM-ANOVAs conducted on difference waves normalized for between group amplitude differences. Greenhouse-Geisser corrections will be used in analyses when the assumption of sphericity is violated. Based on *Preliminary Studies* [redacted] we assume 80% of the sample (n=20 per group) will provide useable ERP data and allow detection of effects 1-2μV in magnitude with .80 power (assuming standard deviations between 1.5-3μV).

Aim 1: Dissociate recollective processes from familiarity processes in early childhood. The processes underlying recollection and familiarity will be identified by combining behavioral indices of memory with electrophysiological responses generated by viewing familiar and novel stimuli. We hypothesize that recollection and familiarity will be reflected in the data via different spatial-temporal properties of the recorded ERP components. As with adults and older children, we predict shorter latencies for familiarity responses, which will show amplitude differences to “new” compared to “old” items (regardless of whether items were “familiar” or “recollected”). In contrast, recollection processes will be revealed by components with longer latencies and show amplitude differences between “recollected” compared to both “familiar” and “new ” items (see Friedman & Johnson, 2000; Rugg & Curran, 2007). Spatial distributions across the scalp will also be analyzed via RM-ANOVA and Current Source Density maps (Picton et al., 1995) to examine involvement of underlying neural circuitry. We also predict that familiarity components will be maximal over right frontal leads and recollection components will be maximal over left parietal leads. Finally, regression analyses will be used to examine how differences in the number of recalled contextual details influences the amplitude of the recollection and familiarity responses. Based on adult research, we expect amplitude of the recollection response to be greatest when both the location and the novel action associated with the object are recalled; however, memory for actions will not modulate amplitude of the familiarity response (Woodruff et al., 2006).

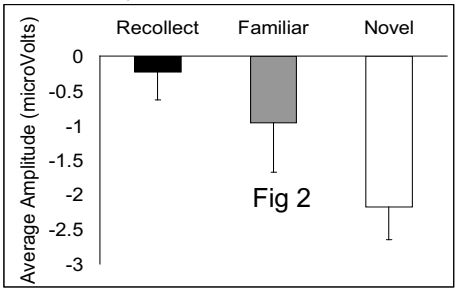
Aim 2: Examine age-related changes in recollection and familiarity during early childhood. Cross-sectional data will be used to examine development of memory processes. We hypothesize that both behavioral measures and ERP components related to recollection will vary across age as this is a period of significant behavioral and neuroanatomical development (3 to 6 years). In contrast, we do not predict developmental changes in behavioral measures and ERP components related to familiarity or novelty.

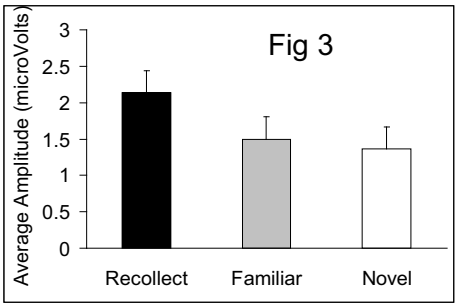


Preliminary data. To demonstrate feasibility of the approach outlined above, we have collected preliminary behavioral and ERP data on a sample of 17 children (10 male, 7 female) between 5 and 6 years (Mean = 5.62 years, Range = 5.04- 6.06 years) using the methods described above. Children visited the lab on 2 occasions approximately a week apart (Mean = 6.59 days, Range = 5-8

days). Behavioral results indicated that children correctly identified 87.55% of the previously-seen target items as "old" and 80.00% of the new distracter items as "new." Out of the correctly identified target items, 55.38% were sorted to the correct location (i.e., were recollected), a level that was significantly above chance, t(16) =3.011, $p<.05$. Rates for misses and false alarms were low, 12.45% and 19.80% respectively.

Based on visual inspection of the data and previous studies (Marshall et al., 2002), 3 time windows were selected for analysis of ERP data: 300-600ms, 600-900ms and 900-1500ms (Figure 1). Average amplitude was extracted for each condition in each time window. RM-ANOVAs were used to examine data from 5 midline (AFz, Fz, FCz, Cz, CPz) leads using a 3 (Condition) x 5 (Leads) design. Laterality differences were examined via RMANOVAs on 6 lateral (F3, F4, C3, C4, P3, P4) leads using a 3 (Condition) x 2 (Hemisphere) x 3 (Coronal plane) design. Only main effects and interactions with condition were examined. Analysis of midline leads revealed a main effect of condition in the 600-900ms window, $F(2,32)=3.63$ $p<.05$ (Figure 2). Analysis of lateral leads revealed a 3-way interaction in the 900-1500ms window, $F(4,64)=2.91$ $p=.05$ (Figure 3, right leads). As predicted, average amplitude in the early window was greatest to novel items compared to old items (recollected or familiar, Figure 2), whereas amplitude in the later window was greatest to recollected items compared to either familiar or novel items in right hemisphere leads (Figure 3). These data show that familiarity and recollection effects can be detected in young children using the proposed paradigm.





Potential problems, alternative strategies, and future directions

First, we predict that memory performance in younger age groups will be poor compared to older age groups when examined on the same task. Given that at least 15 items must be available in all conditions in order to examine associated ERP responses, task demands may be tailored to ensure that behavioral performance will yield enough ERP trials for analysis. Strategies utilized to increase performance will include decreasing the length of delay, increasing encoding time, or altering the number of to-be-remembered items. Given these efforts to maximize performance across all age groups, it may be that differences in behavioral measures of memory will not be detected. If this occurs, a separate sample of children will be tested on identical behavioral tasks to examine age-related changes in performance. However, we do not predict that this scenario will impact age-related differences in the electrophysiological data, as ERPs have been shown to be a more sensitive measure than behavior (e.g., in aging research ERPs detect between-group differences even when none are apparent at the behavioral level; Guillaume et al., 2009). Second, although the hypotheses specified regarding the development of recollection (and associated ERPs components) are in line with neuroanatomical data suggesting a linear developmental trajectory, it has been suggested that functional development may follow a trajectory distinct from that of underlying structures (e.g., Johnson, 2001). Thus, we may find that ERP components related to recollection will appear with different spatial distributions or time courses in each of the age groups. In anticipation of this alternative outcome, omnibus ANOVAs examining all leads will be used to examine the full range of data collected. In addition, visual inspection of ERP data at all ages will guide windows used for analyses both within and across groups. These strategies will allow for detection of both linear and nonlinear change. Finally, if age-related changes are observed, there will be a limit to the conclusions that can be made regarding development to the cross-sectional design. Cross-sectional designs are more efficient than longitudinal designs, thus developmental conclusions will be tempered as necessary. Nevertheless, these data will be critical in future studies where we will design longitudinal paradigms to directly examine age-related changes and individual differences. Additionally, if changes are observed in recollection based on the contemporaneous association between development of underlying substrates and behavior, such data would motivate future investigations linking structural and functional development more directly.

Timeline. Given the preliminary data presented above, we are well-prepared to undertake the proposed research. Timeline is as follows: Months 1-3: participant recruitment and behavioral pilot studies, Months 4-15: data collection (8-10 participants/month), Months 16-19: data analysis, Months 20-24: manuscript preparation/submission. The first benchmark for success will be completion of the pilot studies that are currently underway to determine parameters (delay and encoding time) for successful behavioral performance in all age groups. Once complete we are confident that ERP data collection will be feasible in all age groups. The PI has previous experience collecting and analyzing ERP data in young children and has documented differences in these responses relevant to behavioral memory performance [redacted] et al., 2009a; 2009b).

Protection of Human Subjects

[redacted]

a. Human Subjects Involvement and Characteristics. Children and their families (N=100) from the local community will be asked to participate in this study, which requires 2 visits to the laboratory. The *Targeted/Planned Enrollment Table* below describes the racial and gender categories of the participants. We will attempt to recruit as many children as possible but have budgeted for 25 children per age group based on preliminary data and power analysis calculations indicating a requirement of 20 children per age group, which is inline with the retention rate from the pilot study (See *Approach* and *Preliminary Studies*). The children will range in age from 3 to 6 years. This age range encompasses the window during which 1) a developmental shift has been reported in children's memory abilities on behavioral tasks (Bauer, 2007; Gathercole, 1993) and 2) neuronal connections within the hippocampus develop, altering its functional circuits (Serres, 2001). A Research or Graduate Assistant will contact the families and explain the purpose and procedures of the study. We have a successful history recruiting participants from this community and maintain good rapport with them via the [redacted] Program [redacted] which is maintained by the PI and three other faculty members at [redacted]. Therefore we do not anticipate problems in recruiting the cohort. Once the family agrees to participate, we will obtain informed consent from the caregiver and assent from the child.

All participants will be screened (via parent report) in order to exclude diagnosable central nervous system illnesses or histories of head trauma (e.g., epilepsy, mental retardation), and major medical conditions (including neurodevelopmental disorders). Participants who are taking medication that may alter their attentional functioning (e.g., Clonidine Straterra, antipsychotics, Effexor) will be excluded from data analysis. We will provide information regarding referral sources if during the research process we suspect that any child has a psychiatric, learning, or medical problem that needs attention.

b. Sources of Materials Data sources include: behavioral data from memory task, electrophysiological data from EEG recordings, and parent-reported questionnaire data. Only the PI and senior research personnel will have access to private participant information (e.g., names and addresses necessary for recruitment). All study-related information will be kept in file cabinets located in locked offices within the laboratory and/or secure files on password protected computers.

c. Potential Risks There are no medical risks for either the child or caregiver as a result of participation in this study. There is potential for anxiety symptoms related to the EEG testing procedure. In order to reduce this potential anxiety, participants will be given explicit instructions and a tour of all equipment to reduce the possibility of fear and anxiety associated with the EEG procedure. Our team has experience collecting EEG data from young children. Furthermore, the parent will be able to stay with the child through the entire procedure. Based on previous experience and preliminary studies, we have found that clear explanations combined with the presence of the caregiver greatly reduce anxiety in similar samples and promotes successful data collection.

Adequacy of Protection against Risks.

a. Recruitment and Informed Consent. Families who reside in the local community will be invited to participate. We have a successful history recruiting participants from this community and maintain good rapport with them via the [redacted], which is maintained by the PI and three other faculty members at [redacted]. A Research or Graduate Assistant will explain the purpose and procedures of the study to the caregiver and the child. If they agree to participate, informed consent from the primary caregiver will be obtained via forms and procedures approved by the Institutional Review Board at the [redacted]. Assent from the child will be obtained verbally. Consent forms will be kept in locked file cabinets at the [redacted]. Copies of the signed consent forms will also be offered to the primary caregiver. Children will be given a small toy and certificate for compensation. We will accommodate families' schedules by offering research sessions in the afternoons, evenings, and on weekends.

b. Protections against Risk**.** To ensure confidentiality of records, all data collected on research-related forms will contain confidential ID numbers with no personally identifying information for data entry. The files that contain personally identifying information (necessary for contacting participants) will be kept locked in the laboratory and on password protected computers. No names of participants will be used in any reports. The IRB will be informed of all changes in the protocol or of any serious or unexpected adverse experiences occurring during the study, which are likely to affect the safety of the subjects or the conduct of the study. All adverse experiences are documented by the Research or Graduate Assistant at each visit.

Potential Benefits of the Proposed Research to Human Subjects and Others

There is no direct benefit for the families participating in the present study. However, there may be benefits to other children, especially those at-risk for memory impairment or neurodevelopmental disorders. Findings from the proposed study may help identify processes that reduce risk and promote healthy development.

Importance of the Knowledge to be Gained

Memory is a foundational ability upon which we build knowledge of ourselves and the world around us. Failures in memory can range from the complete inability to form or retrieve memories (e.g., amnesic syndromes) to mild or moderate impairments in performance that impact learning and daily functioning (e.g., developmental amnesia). Memory impairments are related to academic failure in childhood and mental health in adolescence and adulthood (e.g., depression and schizophrenia). Results from the present study will enhance current understanding of memory processes and have important implications for intervention in those at-risk for memory impairment. For example, information regarding the development of recollection and familiarity will be crucial in developing support platforms by providing information about the optimal level of contextual detail that should be available during encoding and recall of information in order to enhance specific learning.

Data and Safety Monitoring Plan

We will screen for adverse events related to participation in the proposed project among participants. If there is suspicion of an adverse event, the research team will notify the Primary Investigator who will notify the Institutional Review Board and the funding agency, as appropriate.

Inclusion of Women and Minorities

Inclusion of Women

We anticipate that the majority of primary caregivers of child participants will be women. Approximately half of the child sample will be female.

Inclusion of Minorities

Participants will be recruited from three counties surrounding the University [redacted]. These counties are chosen for their proximity to the campus and diversity in race/ethnicities and SES. Our goal is to recruit a sample of participants from a broad range of racial and economic backgrounds in order to increase generalizability of the findings. We do not predict differences associated with racial or ethic background. The Table below provides a summary of demographic data for the three counties based on information from school websites.

	White	Hispanic	African American	Asian	ESOL	FARMS
[redacted]	39%	22%	23%	16%	12%	27%
[redacted]	5%	17%	74%	3%	10%	48%
[redacted]	55%	6%	21%	16%	4%	12%
Average	33%	15%	39%	12%	9%	29%

ESOL: English for Speakers of Other Languages, FARMS: Free and Reduced Meals

Targeted/Planned Enrollment Table

Study Title: [redacted]

Total Planned Enrollment: 100

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	7	7	14
Not Hispanic or Latino	43	43	86
Ethnic Category: Total of All Subjects *	50	50	100
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	8	8	16
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	20	20	40
White	22	22	44
Racial Categories: Total of All Subjects *	50	50	100

Inclusion of Children

Children between 3 to 6 years of age will be included in this investigation. The rationale for selecting this specific age range is 1) the focus on memory processes in early childhood and 2) the period during which structural development of the hippocampus undergoes significant refinement of synaptic connections. The PI and investigative team have extensive expertise conducting research with children. Specifically, Dr. [redacted] (PI) has 10 years of research experience with developmental populations, including experience collecting electrophysiological data from infants and young children. We have successfully obtained preliminary data on a sub-sample of children (see *Preliminary Studies* Section). Finally, it is possible to include a sufficient number of children to contribute to a meaningful analysis relative to the purpose of the study (see *Preliminary Studies*).

Participants will be informed of the purpose and procedures of the study using language suitable to their age. The participants will be told explicitly that they can stop their participation in the study at any time without any negative consequences. We try to minimize participation time with the participants' needs taken into account. We will show videos to participants while they are undergoing preparation for the EEG recording when testing is not being performed. Any child who does not wish to participate will be released from the study.