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Activation for newly learned words in left medial-temporal lobe during toddlers' sleep is associated with memory for words

Highlights

- Toddlers learned labels for toys and puppets and remembered them 1 week later
- Sleeping toddlers showed hippocampal and MTL activation while hearing these words
- Activation in these regions correlated with memory for puppet words and vocabulary

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In brief

Johnson et al. examine how toddlers remember newly learned words by assessing neural activation associated with these words while toddlers are asleep. Hippocampal and anterior medial-temporal lobe activation for newly learned words is associated with memory for these words, underscoring a link between memory and word learning in early life.



Article

Activation for newly learned words in left medial-temporal lobe during toddlers' sleep is associated with memory for words

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SUMMARY

Little is known about the neural substrates underlying early memory functioning. To gain more insight, we examined how toddlers remember newly learned words. Hippocampal and anterior medial-temporal lobe (MTL) processes have been hypothesized to support forming and retaining the association between novel words and their referents, but direct evidence of this connection in early childhood is lacking. We assessed 2-year-olds ($n = 38$) for their memory of newly learned pseudowords associated with novel objects and puppets. We tested memory for these associations during the same session as learning and after a 1-week delay. We then played these pseudowords, previously known words, and completely novel pseudowords during natural nocturnal sleep, while collecting functional magnetic resonance imaging data. Activation in the left hippocampus and the left anterior MTL for newly learned compared to novel words was associated with same-session memory for these newly learned words only when they were learned as puppet names. Activation for known words was associated with memory for puppet names at the 1-week delay. Activation for newly learned words was also associated with overall productive vocabulary. These results underscore an early developing link between memory mechanisms and word learning in the medial temporal lobe.

INTRODUCTION

The emergence and fate of early memories have long fascinated researchers and laypeople alike.^{1,2} The ability to remember specific episodes emerges in infancy and undergoes substantial improvement in the first 2 or 3 years of life,^{3,4} but the neural mechanisms underlying this improvement have been examined rarely.^{5,6} Moreover, robust forgetting during early development⁷ constrains the conditions under which neural mechanisms can be examined while robust retrieval is still observed.

One promising domain in which to examine these neural substrates is word learning. Infants and young children amass a vast vocabulary⁸ and growing evidence suggests that hippocampal processes that are foundational for episodic learning and retention may also support word learning.⁹ Although early studies of amnesiac patients following hippocampal lesions suggested the preserved ability to accumulate vocabulary and semantic knowledge¹⁰ through cortical mechanisms,¹¹ newer evidence suggests an impaired ability to learn new words in hippocampal amnesia.^{12,13} Moreover, recent studies show that learning new word-referent associations in healthy adults promotes changes

to hippocampal structure,¹⁴ and that newly learned words, first encoded in the hippocampus, are then integrated into cortical representations through memory consolidation processes.¹⁵ Therefore, relational processes in the hippocampus may also support forming representations that capture the initially arbitrary relation between a word and its referent, first learned at a single point in space and time. This possibility is consistent with models of infant word learning emphasizing the contribution of associative memory processes.^{16–18}

In infancy, research has begun to provide insight on how neural processes unfold during learning and retrieval of novel associations between words and their referents,^{19,20} but the use of event-related potential techniques has precluded the examination of the hippocampus and medial-temporal cortices. These questions can be addressed with complementary methods, such as functional magnetic resonance imaging (fMRI), which is more appropriate to investigate the role of subcortical structures, including the hippocampus. The emphasis on hippocampal processes, however, invites questions about whether this brain structure is sufficiently functional in infants and toddlers. It has been argued that cortical mechanisms may dominate word learning



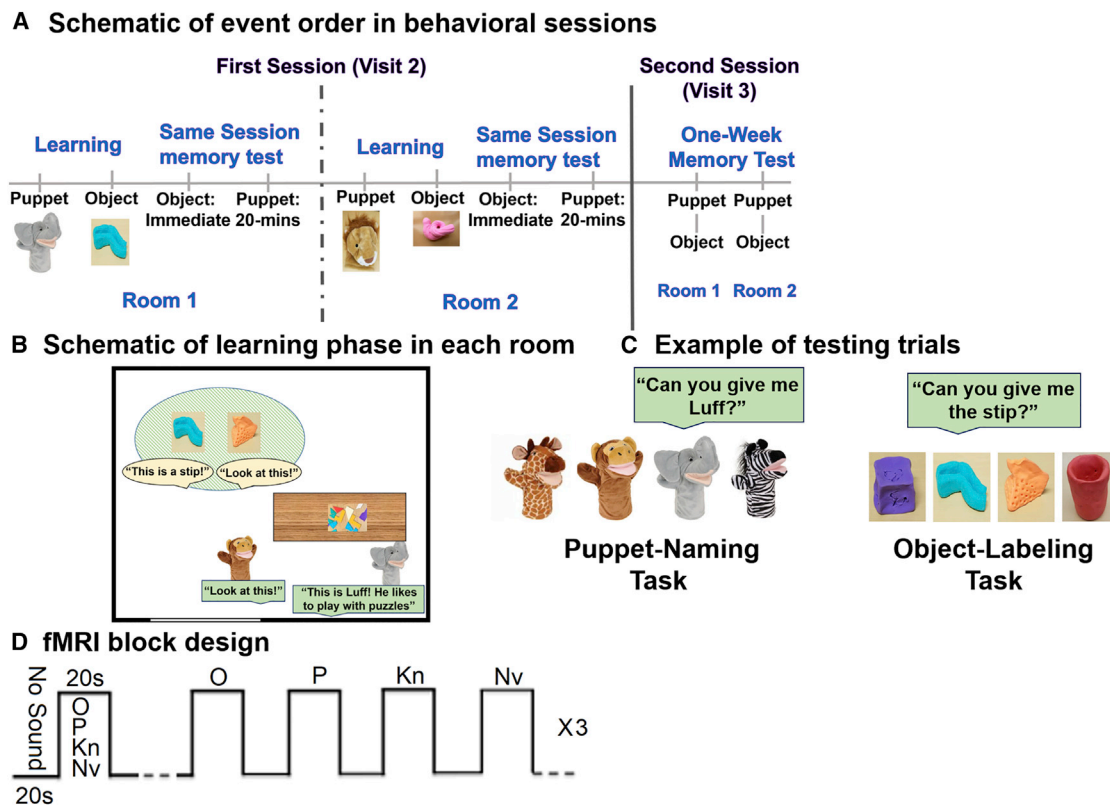


Figure 1. Schematic of methods

(A) Schematic of behavioral sessions. The overall order of events during behavioral tasks shows toddlers first visit room 1 and are introduced to the puppet name. After playing with puzzle games, toddlers learn the object label, and are immediately tested for the object label. Finally, after testing for the object label, toddlers are tested for the puppet name at a 20-min delay from the learning of the puppet name. This process is repeated with different items and words in room 2. After a 1-week delay toddlers are tested for the puppet name, followed by object label, in the same room as learning. This process is repeated in the second room.

(B) Schematic of learning phase in each room. In each of 2 rooms, learning proceeds as shown. One puppet is named, and 1 is shown but not named during puzzle games. Then, 1 object receives a label while sitting on a mat, and 1 is shown but not labeled.

(C) Example of testing trials. Testing proceeded as shown, with selection from a field of 4, including the target (received a label), the non-target (demonstrated but no label), and 2 distractors. The testing of the object label precedes the testing of puppet names.

(D) fMRI block design. The portion on the left demonstrates that toddlers are presented 20-s periods of words separated by 20-s periods of no audio. Blocks of words include O (the 2 object targets), P (the 2 puppet targets), Kn (2 known words), and Nv (2 novel pseudowords pulled from the same pool of words used for puppet names and object labels). Each word block (O, P, Kn, Nv) is repeated 3 times for a total of twelve 20-s periods of active blocks. All of the participants received the same order of stimuli; however, the words that corresponded to the puppet, object, and novel conditions were counterbalanced across participants.

within the first 2 years of life, when hippocampal circuitry may not be sufficiently functional.²¹ However, the normative timing of this development is still largely uncharted. One recent fMRI study has shown that 28-month-olds exhibited greater hippocampal activation during sleep for newly learned songs compared to novel songs²² that were played in the scanner. This activation was associated with the toddlers' memory for events associated with the songs.^{22,23} Thus, the hippocampus may support early memory and, by extension, learning of new words.

It is also possible, however, that toddlers learn new words with limited hippocampal engagement, as do patients with hippocampal lesions but a spared perirhinal cortex.²⁴ The perirhinal cortex, which surrounds the anterior hippocampus, supports rapid object processing, including forming representations that combine object features into a whole (e.g., a cup and a handle are represented as a whole object^{25,26}). This unitization mechanism may be particularly helpful to remember words and their referents.²⁴ Non-human research suggests that memory processes

supported by the perirhinal cortex may be functional in infancy.²⁷ Although evidence from non-human animals cannot directly inform us about memory for words, it bolsters the case for a role of the perirhinal cortex in early learning processes. Moreover, the involvement of the perirhinal cortex does not preclude the additional involvement of the hippocampus, given ample evidence that both contribute to memory performance in adults.²⁸

Task-related fMRI methods, which are the primary approach for assessing hippocampal and perirhinal function, present significant challenges for infants and young children.⁵ Memory-related activation in toddlers during nocturnal sleep²² and neural activations associated with phonological processing during infant sleep^{29,30} provide reassurance that fMRI methods can be used to examine neural substrates underlying learning new words in toddlers.

In the present study, a sample of thirty-eight 25- to 32-month-olds took part in 2 different tasks assessing retention of newly learned words during the same session as learning and after a

1-week delay (Figures 1A–1C). In the object-labeling task, toddlers were taught two new pseudowords in association to two novel objects.^{31,32} In each of the 2 trials, the experimenter repeated the target word multiple times (3–5) over the course of 30 s while directing the toddlers' attention to the target object. Toddlers' memory was assessed immediately after learning (same-session test) and at a 1-week delay by selecting the object from a field of four (i.e., the labeled object, a novel object that was manipulated but did not receive a label, and two known objects). The experimenter repeated the pseudoword in association with the object after the same-session test, but no reminders were given before the 1-week delay test.

In the puppet-naming task, toddlers were taught two additional novel pseudowords as the names associated with two animal puppets. On each trial, a puppet name was learned through a comparable number of repetitions of the label to the object-labeling task (3–5), but this repetition occurred over the course of a 5-min play session. The toddlers' memory was tested after another 20 min (same-session), and after a 1-week delay, by selecting the named puppet from a field of 4 (the named puppet, a familiar puppet that did not receive a name, and 2 novel puppets). As in the object-labeling task, the experimenter repeated the pseudoword in association with the puppet immediately after the same-session memory test, but no reminders were given before the 1-week delay test. In contrast to the object-labeling task in which novel shapes were associated with novel labels, in the puppet-naming task, novel pseudowords corresponded to first names of common animal puppets. Learning and remembering new name-face combinations is associated with hippocampal activation.^{33,34} We therefore reasoned that this task, with learning opportunities distributed while playing, provides an additional and naturalistic way to examine the retention of new words. We then carried out a neuroimaging session as soon as possible after the completion of the 1-week delay session. Our fMRI task delivered blocks of these newly learned words, blocks of completely novel pseudowords, which allowed for an estimation of a memory contrast (newly learned > novel), and blocks of known words, included as a reference point for words integrated in the lexicon (Figure 1D).

Our primary hypothesis was that newly learned words would trigger left hippocampal activation based on left lateralization of verbal processing and memory.^{35,36} This activation was expected to be associated with memory for these newly learned words. An alternative hypothesis was that left anterior medial-temporal lobe (MTL) regions, including the perirhinal cortex, would be associated with memories for words, consistent with the possibility that word learning at this age is supported by unitizing mechanisms in the perirhinal cortex.²⁹ Finally, given that both hippocampus and anterior MTL support associative memory,³⁷ including associations between names and faces,³⁴ we considered that newly learned words would activate both regions during sleep.³⁸ Although associations between hippocampal and anterior MTL activations and behavioral performance were expected on both tasks, we acknowledge that the naturalistic nature of the puppet-naming task, with learning distributed across a play session and memory assessed after a 20-min delay, may be particularly well suited to capture such associations.

Finally, we predicted that if the hippocampus and/or the anterior MTL support learning new words, their activation would also

be associated with general measures of vocabulary. Correlations between hippocampal volume and productive vocabulary have been reported in 2- to 4-year-olds.³⁹ Here, we also sought to replicate this finding in 2-year-olds.

RESULTS

Toddlers retained newly learned words during the same session as learning and after a 1-week delay

In the object-labeling task, toddlers showed strong retention, as indicated by the rates of selection of the labeled items that were well above chance. Specifically, due to the non-normal distribution of the data, Wilcoxon signed-rank tests were used to examine performance across tasks and delays. Toddlers performed well above chance in their correct selection of the labeled objects both in the immediate, same-session test (mean = 0.73, SD = 0.37; chance: 25%; $n = 37$, $z = 4.86$, $p < 0.001$, $r = 0.80$) and a 1-week delay test (mean = 0.66, SD = 0.40; chance: 25%; $n = 35$, $z = 4.23$, $p < 0.001$, $r = 0.72$) (Figure 2A). Performance in the same-session test was not significantly different from performance at the 1-week delay ($z = -1.14$, $p = 0.25$).

In the puppet-naming task, toddlers also performed well above chance both in the 20-min delay, same-session test (mean = 0.52, SD = 0.41; $n = 32$, $z = 3.00$, $p = 0.003$, $r = 0.53$) and after a 1-week delay (mean = 0.55, SD = 0.43; $n = 32$, $z = 3.17$, $p = 0.002$, $r = 0.56$) (Figure 2B). Performance across the two testing occasions did not differ ($z = 0.50$, $p = 0.62$). Thus, toddlers showed evidence of learning and retaining the new words presented in the study.

MTL activation for newly learned words is associated with memory for these words

We focused on activations in the left hemisphere, given the typical left lateralization of verbal processing and memories^{35,36} and on reducing the number of correlational analyses, given the lack of specific hypotheses concerning the corresponding regions in the right hemisphere. Specifically, we examined activation in a priori structurally defined left hippocampal (Figure 3A) and left anterior MTL regions (including perirhinal and entorhinal cortices), as provided by an age-appropriate template⁴⁰ (Figure 3C). We assessed activation for 2 contrasts: newly learned words (object + puppet > novel) and known words (known > novel). We combined object and puppet words because their presentation followed identical procedures within the functional runs, their use as object labels or puppet names was fully counterbalanced within the runs, and we sought to use a parsimonious approach in the examination of hippocampal and anterior MTL activation, given that we lacked distinct hypotheses regarding neural activation as a function of whether the pseudowords had been learned in association with novel objects or puppets. For illustration purposes and in response to peer review, correlations with each contrast are also reported (Table S1). Nevertheless, we examined associations between neural activation and behavioral performance separately for each behavioral index because these indices were collected separately during our sessions and through different procedures. For the object-labeling task, the accuracy index used for correlational analysis corresponded to the rate of accurate selection of the target object minus the rate of inaccurate selection of known objects. For

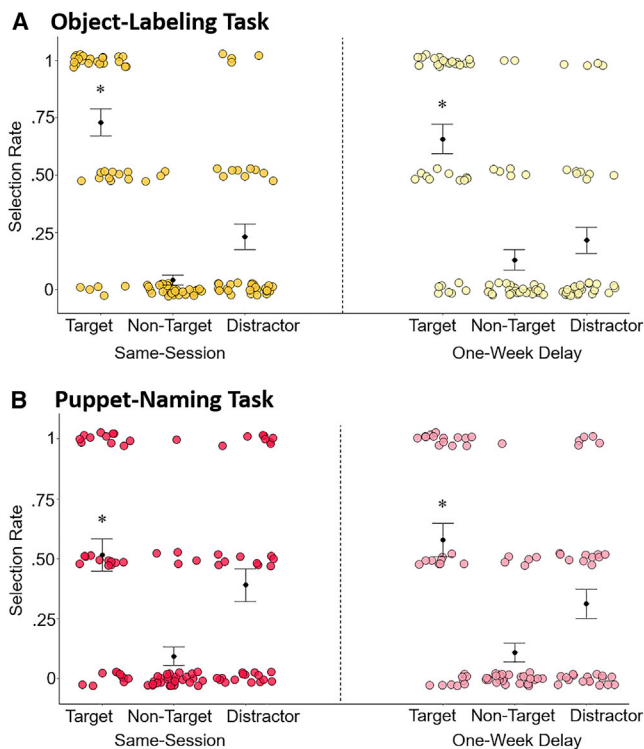


Figure 2. Accuracy on the object-labeling and puppet-naming tasks

On each task, toddlers selected an option from 4: the labeled target item, the unlabeled but presented non-target item, and 2 distractor objects (selection rate averaged in the figure) that represented familiar items. Dots represent the performance of individual participants, averaged across 2 assessments.

(A) Object-labeling task. During the same-session test, toddlers selected the target object at above chance level (25%), $z = 4.86$, $p < 0.001$ (target selection rate: frequency [1] = 22, frequency [0.5] = 10, frequency [0] = 5). After a 1-week delay, toddlers still selected the target at above chance levels, $z = 4.23$, $p < 0.001$ (target selection rate: Freq [1] = 19, Freq [0.5] = 10, Freq [0] = 7).

(B) Puppet-naming task. During the same-session test, toddlers selected the target puppet at above chance levels (25%), $z = 3.00$, $p = 0.003$ (target selection rate: Freq [1] = 11, Freq [0.5] = 11, Freq [0] = 10). After a 1-week delay, toddlers still selected the target at above chance levels, $z = 3.17$, $p = 0.002$ (target selection rate: Freq [1] = 13, Freq [0.5] = 9, Freq [0] = 10). *Selection at greater or lesser than chance level, $p < 0.05$. Error bars represent ± 1 standard error of the mean.

the puppet-name task, the accuracy index used for correlational analyses corresponded to the rate of accurate selection of the named puppet minus the rate of selection of the novel puppets.

For the same-session memory test, we found significant associations between memory performance and activations in both the left hippocampus and left anterior MTL. All correlations calculated with the Spearman rho formula and reported as significant survived a Benjamini-Hochberg false discovery rate (FDR = 0.10) correction based on 8 comparisons within same-session behavioral testing. Specifically, activation in the left hippocampus for newly learned words was positively associated with memory for puppet names (target puppet selections minus novel puppet selections; $r_s(30) = 0.38$, $p = 0.03$) (Figure 3B; correlations for target and distractor selections shown separately in Figure S1). Controlling for age did not change this association ($r_s(29) = 0.37$, $p = 0.04$), nor did controlling for the delay between the last exposure to the words and the MRI session ($r_s(29) = 0.40$,

$p = 0.03$). Activation in the left hippocampus for known words and memory for puppet names were not significantly correlated ($r_s(30) = 0.22$, $p = 0.24$).

Activation in the left anterior MTL for newly learned words was also significantly correlated with memory for puppet names ($r_s(30) = 0.45$, $p = 0.009$) (Figure 3D). This association did not change when controlling for age ($r_s(29) = 0.45$, $p = 0.01$) or delay before MRI ($r_s(29) = 0.40$, $p = 0.026$). Unlike in the left hippocampus, in the left anterior MTL, activation for known words and memory for puppet names were also significantly correlated ($r_s(30) = 0.44$, $p = 0.01$). Controlling for age and delay before MRI did not change this association (respectively, $r_s(29) = 0.45$, $p = 0.01$ and $r_s(29) = 0.40$, $p = 0.025$). None of the measured contrasts showed significant correlation with memory for object labels ($r_s < 0.21$, $p_s > 0.21$; Table S1).

At the 1-week delay test, Spearman's rho correlations were also corrected for Benjamini-Hochberg FDR, based on 8 comparisons within this behavioral testing time point. Activation in the left hippocampus for newly learned words was not significantly correlated with memory for puppet names ($r_s(30) = 0.33$, $p = 0.07$) and neither was left hippocampal activation for known words after FDR corrections (p threshold for this comparison = 0.025) ($r_s(30) = 0.37$, $p = 0.04$). Activation in the left anterior MTL for newly learned words was not significantly correlated with memory for puppet names ($r_s(30) = 0.16$, $p = 0.37$); however, activation for known words was ($r_s(30) = 0.47$, $p = 0.007$). Controlling for age did not change this association ($r_s(29) = 0.46$, $p = 0.009$), nor did controlling for delay before MRI ($r_s(29) = 0.45$, $p = 0.01$). The correlation between activation for known words in the left anterior MTL and memory for puppet names was significantly stronger than the correlation between activation for newly learned words in the left anterior MTL and memory for puppet names at this 1-week delay (Steiger's $Z = 2.49$, $p = 0.013$, $n = 32$). We note that the direct comparisons of the correlation between memory for puppets and activation in left anterior MTL at the same-session test and their corresponding correlation at the 1-week delay were not significant (Z hs < 1.02 , p s > 0.31); thus, we cannot draw firm conclusions about the change in correlation strength as a function of time. None of the measured contrasts showed a significant correlation with memory for object labels after a 1-week delay ($r_s < 0.21$, $p_s > 0.27$; Table S2). Finally, although this research is not centered on the direct comparison of activation levels between the left hippocampus and left anterior MTL, these analyses are reported for the sake of completion (Figure S1); similarly, supplemental whole-brain maps (Figure S2C) and correlations with the right brain regions are reported (Table S2).

In summary, activation in the left hippocampus for newly learned words was positively correlated with memory for puppet names only in the same-session test. Results in the left anterior MTL revealed significant associations between activation for newly learned words and memory for puppet names at a same-session test and between activation for known words and memory for puppet names at a 1-week delay.

Activation in control cortical regions is not associated with memory for newly learned words

To establish whether our results were specific to the left hippocampus and anterior MTL, we estimated the newly learned

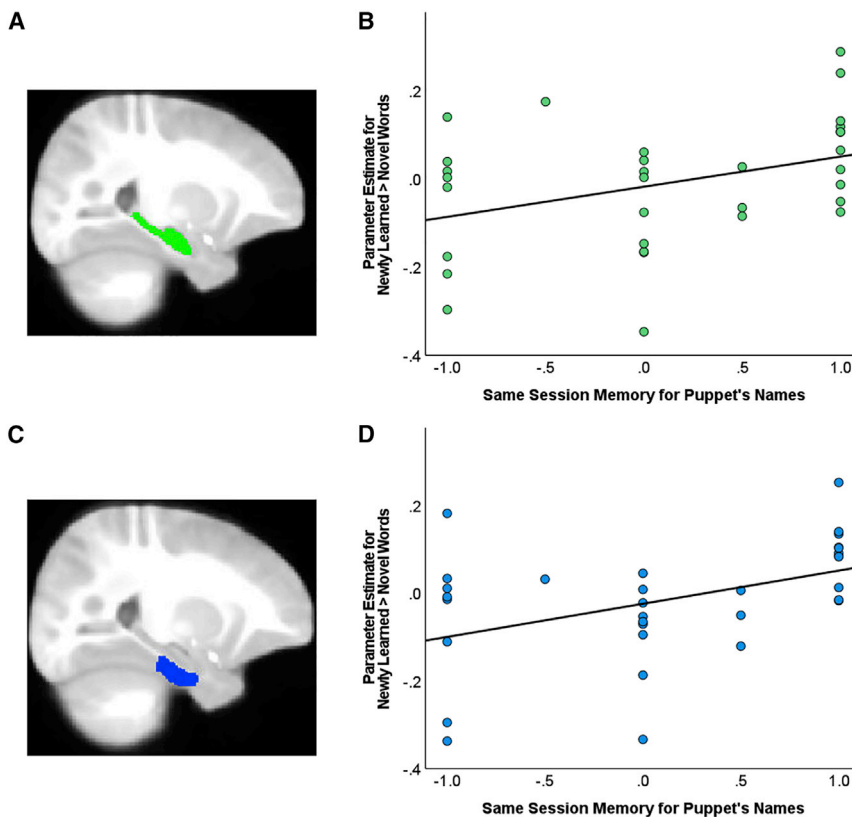


Figure 3. Associations between parameter estimates for newly learned words and same-session memory for puppet names in the left hippocampus and left anterior MTL

The ROIs are visualized on the UNC toddler template, $x = -22$.

(A) The structurally defined left hippocampal ROI is shown in green.

(B) Correlation between activation for newly learned words (object + puppet > novel) in the left hippocampus and memory accuracy for puppets (hits minus false alarms) ($r_s(30) = 0.38$, $p = 0.03$).

(C) The structurally defined left anterior MTL ROI is shown in blue.

(D) Correlation between activation for newly learned words in the left anterior MTL and memory accuracy for puppets (hits minus false alarms) ($r_s(30) = 0.45$, $p = 0.009$).

See also [Figure S1](#) and [Tables S1](#) and [S2](#).

Hippocampal function and volume independently predict concurrent productive vocabulary

To replicate previous research,³⁹ we showed that left hippocampal volume was significantly positively correlated with productive vocabulary ([Figure S3](#)). To examine whether hippocampal function was associated with vocabulary accounting for hippocampal structure, we

words contrast in the left superior temporal gyrus, as a control region relevant for language but not necessarily memory, and in the left lateral occipital cortex, which we reasoned would be unrelated to memory for words. These regions of interest (ROIs) were developed to be a similar size to the left hippocampal ROI used ([STAR Methods](#)). None of the correlations between activation in either control region and behavioral measures were significant ([Table S2](#)).

MTL activation for newly learned words is associated with productive vocabulary

We found that productive vocabulary (raw score) as assessed by parental report in the first session was significantly associated with activation for newly learned words in the left hippocampus ($r(35) = 0.45$, $p = 0.005$; [Figure 4A](#)) and in the left anterior MTL ($r(35) = 0.48$, $p = 0.003$; [Figure 4B](#)). These correlations held when controlling for age ($r_s(34) \geq 0.44$, $p_s \leq 0.007$). Productive vocabulary was not significantly associated with activation for known words in the left hippocampus ($r(35) = 0.23$, $p = 0.18$) or the left anterior MTL ($r(35) = 0.27$, $p = 0.11$). These correlations account for a Benjamini-Hochberg FDR correction for the four correlations related to vocabulary. An ancillary analysis presented in the [supplemental information](#) showed that activation in two regions of the prefrontal cortex⁴¹ was also associated with productive vocabulary ([Figure S2](#)). Finally, an exploratory longitudinal analysis showed that left anterior MTL activation for known words predicted productive vocabulary after a 10-month delay ([Table S2](#)).

simultaneously entered age, activation in the left hippocampus for the newly learned words contrast, and left hippocampal volume in a multiple regression predicting productive vocabulary. The model was significant, $F(3,32) = 9.30$, $p < 0.001$; adjusted R^2 of 0.42. Age ($\beta = 0.37$, $p = 0.008$), left hippocampal activation for newly learned words ($\beta = 0.37$, $p = 0.008$), and left hippocampal volume ($\beta = 0.40$, $p = 0.005$) independently predicted productive vocabulary. Given that parents completed the vocabulary measure during the first behavioral session, this measure reflects language acquired before participation. Thus, toddlers with a greater vocabulary may be advantaged at recruiting the hippocampus. An exploratory longitudinal analysis shows that left anterior MTL activation also predicts vocabulary growth ([Table S2](#)).

DISCUSSION

The present study was designed to investigate whether the left hippocampus and left anterior MTL, which includes the perirhinal cortex, contribute to memory for newly learned words in 2-year-olds. We addressed this question by having toddlers learn and retain pseudoword labels for objects and puppets, and later undergo an fMRI paradigm, while asleep, during which we delivered these words, well-known words, and completely novel pseudowords. We focused on activation in the left hippocampus and left anterior MTL because of their documented role in associative memory,^{9,28,42} including word-referent associations^{24,26} and face-name associations.^{33,34} We found that toddlers exhibited strong retention of the associations between

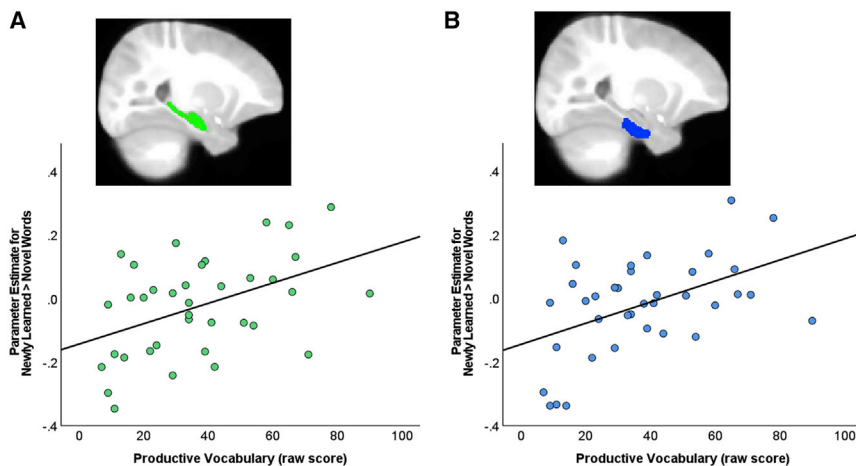


Figure 4. Parameter estimate values for newly learned words in the left hippocampus and left anterior MTL and productive vocabulary

(A) Parameter estimate values for newly learned words in the left hippocampus were positively correlated with productive vocabulary as measured by raw score on the MacArthur-Bates CDI III ($r(35) = 0.45$, $p = 0.005$). This correlation persisted when controlling for age ($r(34) = 0.42$, $p = 0.01$).

(B) Parameter estimate values for the same contrast in the left anterior MTL were also positively correlated with productive vocabulary ($r(35) = 0.48$, $p = 0.003$). This correlation also persisted when controlling for age ($r(34) = 0.46$, $p = 0.004$).

See also [Figure S3](#) and [Table S2](#).

pseudowords and their object or puppet referents both shortly after learning and at a 1-week delay. We found that functional activations in both the left hippocampus and left anterior MTL across all newly learned words were associated with memory for puppet names and productive vocabulary.

Recent research suggests that both the hippocampus and the anterior MTL may support memory for words,^{13,26,43} but the examination of their functional contribution in early childhood is still lacking. Relational memory capabilities, which depend on hippocampal processes, have been shown to support word learning.^{16,17} The hippocampus may be particularly important when infants and toddlers encounter the initially arbitrary association between a word and its referent.¹⁵ Once consolidation occurs, cortical structures may play a stronger role as individuals use words to make reference to their meaning that holds true across situations. There is also reason to believe that sleep-related consolidation occurs more rapidly in children than adults.⁴¹

The first 2 years of life appear to be a time of robust hippocampal development,⁴⁴ which coincides with substantial vocabulary expansion beginning in infancy.⁴⁵ This correspondence leads to questions of whether the associative capabilities bequeathed by a rapidly developing hippocampus in toddlers may support learning new words. To date, evidence in favor of this possibility comes from one study reporting a positive correlation between hippocampal volume and productive vocabulary in 2- to 4-year-old children,³⁹ a finding that was replicated in our study in a more age-homogeneous sample of 2-year-olds alone.

Critically, we found associations with hippocampal function. Memory for puppet names tested after a 20-min delay was associated with left hippocampal activation for newly learned words. This provides more direct evidence for the operation of associative mechanisms linking initially arbitrary co-occurrences between words and objects. These activations were observed during sleep; as such, we cannot establish whether they fully correspond to patterns of activation during active memory retrieval. Moreover, studies in rodents have indicated that memories formed without hippocampal engagement still activated the hippocampus during slow-wave sleep,⁴⁶ suggesting that activity during sleep may reflect processes through which various forms of knowledge are generalized or consolidated. However,

others^{22,23} have also demonstrated correlations between hippocampal activation in response to auditory content associated with past experiences, presented during sleep to toddlers, and memory. Moreover, research in adults has shown that aspects of memory representations are reactivated during sleep to stabilize them.^{47–49} Overall, hippocampal activation can be interpreted as memory reactivation.

Future research should examine whether the reactivation of newly learned words during sleep prompts consolidation of the memory for these words.¹⁵ Whereas the activation for these newly learned words may not reflect the organization of the lexicon, associative content has been successfully targeted with audio in sleeping adults³⁸ as well as toddlers.^{29,30} In a recent study in which Dutch adolescents learned new Japanese words, greater hippocampal activity was found for these new words immediately after learning compared to at a 1-week delay.⁴³ Decreased hippocampal activation over time despite strong retention of the words suggests consolidation processes. The extent and timing of this neural process in toddlers is unknown. Future research should track the evolution of hippocampal function as new words become integrated in the toddlers' lexicon, and presumably undergo consolidation. Manipulating the time at which neuroimaging data are obtained (e.g., after initial learning, after long delays) is essential to begin to characterize how patterns of neural activation in the hippocampus may change due to forgetting, consolidation, and knowledge accumulation during early development.

Activations associated with newly learned words extended to the anterior MTL. Encompassed within this region is the perirhinal cortex, which supports the rapid fusion of object features into a unitized representation.^{25,26} Although the perirhinal cortex is often examined in relation to processing of object representations,³⁷ it has also been connected to the encoding of auditory and visual associations.²⁶ Unitization processes in the perirhinal cortex may be particularly relevant for the formation of fused word-referent associations, in which the parts cannot be separated from the whole. Activations in the left anterior MTL and their associations with memory for puppet names were found as consistently as those in the left hippocampus, countering the idea that extrahippocampal cortices are entirely responsible for early word learning (albeit the argument primarily refers to infants

and younger toddlers²¹). Instead, it is more likely that the hippocampus supports arbitrary associations and surrounding anterior MTL regions contribute to unitization-based associative memory.³⁴ Future research should examine the role of these MTL regions further while also integrating an account of the contribution of additional brain regions that have been shown to support sustained hippocampal activation for newly learned words over extended time windows (e.g., dopaminergic-reward regions⁵⁰).

The activation for newly learned words in the left hippocampus and left anterior MTL (as well as the medial prefrontal cortex⁴¹) was positively associated with productive vocabulary. Our measure is based on parental report and thus captures the extent of vocabulary learned before and up to participating in our study. Therefore, our results suggest that toddlers with more extensive vocabularies have a better ability to recruit regions involved in processing the initially new associations between words and their referents. Previous research has provided behavioral evidence that young children with more extensive vocabularies exhibit faster and more extensive vocabulary expansions.⁵¹ Many factors likely contribute to this vocabulary expansion, including the development of new learning capacities,⁵² and the frequency of exposure to language.⁵³ Our findings suggest the additional contribution of relational processes.

Unlike activation for newly learned words, activation for known words in the left anterior MTL was significantly correlated with memory for puppet names after a 1-week delay. Direct comparison between the correlation between 1-week delay puppet memory and known versus newly learned words showed a significantly stronger correlation with known words. However, these findings should be taken with a grain of salt, given that all other direct comparisons across brain-behavior correlations were not significant. Moreover, our exploratory longitudinal analyses show that it was this activation for known words that predicted vocabulary growth during a 10-month delay. It would be tempting to suggest that these exploratory longitudinal findings may capture aspects of the consolidation processes and integration of the words into semantic memory. However, we cannot rule out the possibility that activation for known words reflects instead a recent experience with the item associated with the label, especially since both labels referred to toys that were available during the laboratory visit. If this is the case, then the association between activation for known words and change in vocabulary may be due to relationships between general long-term memory processes and vocabulary. Future research should focus on the longitudinal connection between medial temporal lobe recruitment, relational memory capabilities, and the development of a lexicon. This may include manipulations in which known objects are or are not named and/or played with during the session to establish the extent to which medial temporal activations do represent word processing, recent memories, or both. To our knowledge, memory representations of specific items from the MacArthur-Bates Communicative Development Inventory III (CDI III) (known or not) have not been examined using fMRI methods in this age group, but the comparison of representations associated with more recently or less recently learned words could be illuminating. One may expect, consistent with memory research with adults, that more remotely learned words may have a weaker hippocampal signature reflecting

consolidation processes^{54,55} due to semanticization,¹⁵ although others may disagree with this time-dependent hypothesis.⁵⁶

We acknowledge several limitations in our study. First, we did not assess sleep stage because of concerns that this would interfere with our ability to collect neuroimaging data. Slow-wave sleep and memory consolidation have been associated in toddlers, children, and adults.^{57,58} Moreover, previous work has demonstrated sleep-related consolidation (by way of napping after learning) in toddlers.⁵⁹ Thus, future research should attempt to integrate sleep assessments in these fMRI paradigms. Second, the correlations between hippocampal and anterior MTL activation and behavior on experimental tasks were restricted to the puppet-naming task. It is possible that the higher levels of performance on the object-labeling task, with a sizeable proportion of toddlers performing perfectly on it, reduced our chances to identify correlations with behavior. The fact that activation across pseudowords learned with either puppets or objects is related to performance on the puppet-naming task and to productive vocabulary suggests that memory for words in both tasks seemingly engage common neural mechanisms. Future studies with adjustments to the object-labeling task to promote larger performance variability may substantiate this possibility. It is possible, however, that the puppet-naming task was more strongly correlated with hippocampal and anterior MTL activation because this task, being more naturalistic, may have encouraged the association with additional aspects of the learning episode. Moreover, the memory for puppet names was tested after a 20-min delay, whereas memory for objects was tested immediately after learning. Thus, memory for puppet names in the same session may be more akin to episodic retrieval. Regardless, fMRI experiments in adults have shown the MTL, and especially the hippocampus, are involved in the encoding and retrieval of arbitrary name-face associations.^{33,34,60} Future research should ascertain these possibilities as well as characterize how different MTL areas may support distinct word learning processes (e.g., fast mapping, which may implicate the perirhinal cortex²⁴; novelty detection may engage the hippocampus⁶¹). It is also noteworthy that activation for newly learned words in the superior temporal gyrus was not significantly associated with any behavioral measures in this study, bolstering the case that our behavioral tasks are better suited for capturing memory processes than processing of linguistic features of the stimuli; still, future research should examine the role of the superior temporal gyrus in word learning in early life.

Third, toddlers were assessed with the same tests at the 1-week delay that were used in the same-session test. Reduced novelty of the distractor may have facilitated toddlers' target selection at the 1-week delay, given the strong novelty preference at this age.⁶² Although we prioritized eliminating variability in the appearance of the test array across sessions for the present study, future studies should examine whether replacing the distractors affects performance. Finally, the present study only recruited toddlers who could speak and understand English and did not examine the effects of exposure to multiple languages in the home. The examination of multilingualism may be a promising avenue for future investigations.

In conclusion, this study showed novel associations between activation in regions of the MTL relevant to associative memory, performance in a novel word-learning task, and productive

vocabulary. These findings may prompt future investigations of the dynamic interplay between neurocognitive processes supporting memory and word learning in early childhood, a time of great expansion of verbal skills.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
 - Lead contact
 - Materials availability
 - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
 - Human subjects
 - Materials and equipment
- **METHOD DETAILS**
 - Procedure overview
 - MacArthur-Bates Communicative Development Inventory III
 - Object-labeling task
 - Puppet-naming task
 - fMRI word task: imaging acquisition and design
 - fMRI word task: procedure
 - Exploratory longitudinal follow-up
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
 - Quantification of behavioral performance
 - fMRI data processing and parameter estimation
 - Assessment of hippocampal volume
 - Statistical analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2021.09.058>.

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AUTHOR CONTRIBUTIONS

E.G.J. and S.G. designed the research; K.G.E. provided feedback on the design; E.G.J., L.M., C.W.N., and S.G. developed the procedure for fMRI data collection during sleep; E.G.J. and L.M. collected the data; E.G.J. analyzed the data; E.G.J. and S.G. wrote the manuscript; and C.W.N., K.G.E., and L.M. provided feedback on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects. We worked to ensure that the study questionnaires were prepared in an inclusive way. While citing references scientifically relevant for this work, we

also actively worked to promote gender balance in our reference list. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw data	This paper	OSF: d8f5y
Software and algorithms		
Presentation stimulus delivery software	Neurobehavioral Systems ⁶³	https://www.neurobs.com/
FSL 5.0.8	⁶⁴	https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/
Freesurfer 5.0	⁶⁵	https://surfer.nmr.mgh.harvard.edu/
ASAT	⁶⁶	https://www.nitrc.org/projects/segadapter
SPSS v.27	⁶⁷	https://www.ibm.com/analytics/spss-statistics-software
Other		
Macarthur Bates CDI III	⁶⁸	https://mb-cdi.stanford.edu/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Elliott G. Johnson (egjohnson@temple.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- De-identified data have been deposited through the Open Science Framework at (<https://osf.io/d8f5y/>) and are publicly available as of the date of publication
- This paper does not report original code
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human subjects

A sample of 38 typically developing toddlers (M = 28.2 months; SD = 2.05 months; Range = 24.9 - 31.9 months; 23 female) participated in this research. Toddlers were recruited from several different communities located in the greater Sacramento area. Recruitment was restricted to English speaking (and understanding) toddlers without a history of neurodevelopmental disorders or prematurity as reported by parents. The sample included 20 toddlers reported as Caucasian, 10 as more than one race, 4 as Asian, and 4 as African American. The parents of 11 toddlers reported that their toddlers were Hispanic/Latino, and 26 toddlers were not, with one toddler's parents not reporting. Families' reported incomes varied from greater than \$90,000 (n = 14), to \$60,000-90,000 (n = 10), \$40,000-60,000 (n = 7), \$25,000-40,000 (n = 3), \$15,000-25,000 (n = 2), and 2 did not report income. We were able to conduct an exploratory longitudinal analysis on a subset of these toddlers (n = 27), with an assessment of productive vocabulary after 10 months (SD = 6.7 months; M age = 38.7 months; range 30.8 - 57 months; 16 female); the results of these analyses are reported in [Table S3](#).

Finally, an additional 45 toddlers were tested behaviorally, but did not fall asleep (n = 31) or could not stay asleep during the MRI session (n = 14). We verified that these 45 toddlers were comparable to those who completed the neuroimaging session in terms of age, sex, race, ethnicity, family income, and performance on our behavioral tasks as well as vocabulary ([Table S4](#)). All procedures were approved by the University of California, Davis, Institutional Review Board Administration. Parents or guardians provided written consent to participation. Toddlers were given a small book after each of their experimental sessions and families were given \$50 dollars and a 3D-print of their toddler's brain as compensation for their participation.

Materials and equipment

All participants were administered the behavioral tasks during sessions held in child friendly laboratory rooms at the UC Davis Center for Mind and Brain. Participants were administered the functional Magnetic Resonance Imaging (fMRI) task using neurobehavioral

systems' Presentation software (V 20.1) using scripts generated for this experiment. The fMRI task was delivered with a 3T Siemens Tim Trio at the UC Davis Imaging Research Center.

METHOD DETAILS

Procedure overview

Toddlers participated in three laboratory visits. Visit 2 occurred roughly 3 days after Visit 1 ($M = 2.79$ days; $SD = 3.88$; Range = 0-13 days) and Visit 3 occurred roughly one week after Visit 2 ($M = 6.47$ days; $SD = 1.34$; Range = 2-9 days). During these visits, toddlers received a general assessment of productive language (Visit 1 using the MacArthur Bates Communicative Development Inventory III) and were assessed for their learning of 4 new words (Visits 2 and 3) using the two tasks described below (two words with the Object-Labeling Task and two words with the Puppet-Naming Task). These three visits were followed by one nighttime visit to the MRI during which the fMRI Word Task was completed during sleep; this last visit was scheduled as soon as possible after the laboratory visits ($M = 0.91$ days after third visit, $SD = 0.98$ days, Range = 0-4 days).

MacArthur-Bates Communicative Development Inventory III

The 100-word checklist of productive vocabulary was administered to parents during the first visit to the laboratory. Parents indicated which words in the list of words were part of their toddlers' productive vocabulary. One parent did not fill out this form.

Object-labeling task

This task was designed to be consistent with a typical word learning paradigm in which the label for an object is taught through direct instruction.³² Labels were associated with 3-dimensional single-color foam objects designed to be novel and distinct. Possible labels included 12 monosyllabic pseudowords (burb, croy, luff, yunk, stip, jeg, flam, kly, vone, grom, zoop, woat), which were consistent with English phonotactics. Toddlers completed two object labeling trials, one in each of two separate rooms. In each trial, toddlers were first exposed to 4 objects. Two objects were new-shaped objects and two represented common object distractors. Toddlers were first familiarized with all 4 objects for one minute and were encouraged to manipulate each of them, to reduce the possibility that a simple novelty preference would drive their later selections.⁶² Toddlers were taught a label for one of the new-shaped objects ("Look at the Grom... This is a Grom"), which the experimenter repeated 3-5 times over the course of 30 s, while the toddlers were encouraged to manipulate it. Whereas the targeted number of repetitions was three, occasionally experimenters would repeat the name up to two more times if toddlers appeared distracted. Toddlers' attention was then directed to the non-labeled new-shaped object for another 30 s ("Look at this!"). Toddlers were directed to manipulate the object, but no pseudo-word label was given for this second object. After a final 30 s delay, toddlers were re-presented with all 4 of the objects, including the labeled new-shaped object (*target object*), the non-labeled new-shaped object (*non-target object*), and two common item *distractor objects*, and were asked to hand the labeled (target) new-shaped object (referred to by name) to the experimenter. Toddlers were requested to hand the object over in order to ensure that they had chosen one object over the other options. The language utilized by the experimenter was "Please give me the Grom." If the toddlers did not select an item, this language was repeated, or if they attempted to hand multiple items to the experimenter they were told "please give me only the Grom." A maximum of 4 opportunities were given to the toddlers to hand (indicate by making a sustained touch/push of) a single item to the experimenter. After toddlers performed the task, the experimenter repeated the name of the object, but no reminders were given after that in anticipation of the one-week delay test. This process was repeated in a different room with a different set of objects and pseudoword labels, which were counterbalanced across participants. After *one week*, toddlers were administered the same test in each of the rooms without reminders. Specifically, toddlers were re-exposed to all 4 items, and were given the opportunity to play with all of them for one minute in order to avoid selection of the item that they simply wanted to play with the most. They were then once again requested to physically hand the labeled new-shaped object (i.e., "Please give me the Grom").

During the same-session assessment, 1 toddler refused to make selections due to fussiness. During the one-week delay assessment, 1 toddler's responses could not be used due to experimental error, and 2 toddlers did not provide responses due to lack of cooperation on this task.

Puppet-naming task

This task was designed to present a naturalistic learning opportunity. In each room toddlers were repeatedly told the proper name (i.e., pseudoword) of a fabric hand puppet ("This is my friend Croy") which accompanied the toddlers during a naturalistic play session. As in the Object Labeling Task, 12 possible pseudowords were used from the same list (burb, croy, luff, yunk, stip, jeg, flam, kly, vone, grom, zoop, woat). Toddlers were introduced to the animal puppets upon entering a room in which they played a series of memory games including a task on a tablet followed by a puzzle task. The names of the puppets were repeated 3-5 times over the course of a 5-minute period. Once again, the targeted number of repetitions was three, however if toddlers were judged to have not paid attention the names were repeated up to two additional times. Because the puppet was present while children were engaged in puzzle games, the experimenter would refer to the puppet by stating that it liked the games (i.e., "Croy likes this game"). When the puppet's name was mentioned, the experimenter waved the puppet slightly to assist the toddler in orienting to the puppet. Meanwhile, a second unnamed puppet was clearly visible on a table in the room and was not named but was instead briefly indicated to the toddler ("look at this puppet"). After 20 minutes, during which puzzle games were played and the object-labeling task administered, the

testing trial was administered. The test array included the named puppet (*target puppet*), the visible but unnamed puppet (*non-target puppet*), and two new animal puppets from the same animal puppet set (*distractor puppets*). Toddlers were requested to physically hand the item over, and only a single item, in order to ensure that they had chosen one item over the other options. The language utilized by the experimenter was “Please give me Croy.” If toddlers did not select an item, this language was repeated, or if they attempted to hand multiple items to the experimenter they were told “please give me only Croy.” A maximum of 4 opportunities were given to the toddler to hand (or indicate by clearly touching or pushing) a single item, barring this their answer was coded as missing data. After toddlers performed the task, the experimenter repeated the name of the puppet, but no reminders were given after that in anticipation of the one-week delay test. This process was repeated a second time in another room with different puppets and pseudoword labels, which were counterbalanced across participants. The pseudowords were counterbalanced for their use as object labels, puppet names, or novel words in the neuroimaging session described in a later section. Approximately one-week later, toddlers were tested for their memory for the puppet’s name. Toddlers were presented with the named puppet, the unnamed puppet, and the two distractor puppets used in the room during the first session. Toddlers were once again requested to physically hand over the puppet (“Please give me Croy”)

During the same-session assessment, 2 toddlers did not provide responses due to inattention, 3 toddlers refused to respond due to fussiness, and 1 toddler’s responses could not be used due to experimental error. During the one-week delay assessment, 6 toddlers did not provide responses due to lack of cooperation on this task.

fMRI word task: imaging acquisition and design

Images were acquired using a 3-T Siemens TIM Trio MRI system at the University of California, Davis, Imaging Research Center using a 32-channel head coil. Functional images were acquired using a gradient echo-planar imaging pulse sequence [repetition time (TR), 1,500 ms; echo time (TE), 24 ms; field of view (FOV), 216 mm; number of slices, 46; voxel size, 3 mm isotropic; 330 volumes acquired]. During the functional run, toddlers heard 20 s blocks of four conditions, namely *puppet*, presenting the names for the two named puppets, *object*, presenting the names for the two labeled foam objects, *novel* presenting the two pseudowords not taught to the participant, and *known* presenting two well-known words (“ball” and “train”; all parents verbally confirmed that toddlers knew these words) for a total of 12 active blocks (Figure 1D). On each block condition, words in the pair (e.g., two puppet words) alternated every 1.5 s. All words were delivered at 100 dB, and so with the noise attenuation of 28 dB from earplugs the sound was roughly 72 dB. Blocks of each condition were presented three times totaling 12 active blocks. Twelve 20 s blocks of silence separated the 12 active blocks. All participants received the same basic order of stimuli, however the words that corresponded to the puppet, object, and novel conditions varied and were counterbalanced across participants. Neurobs’ Presentation stimulus delivery software (<https://www.neurobs.com/>) and MR Confon auditory delivery system (<https://www.crsitd.com/tools-for-functional-imaging/audio-for-fmri/mr-confon/>) were used to deliver auditory information during the fMRI sequence.

Additionally, a T1-weighted high-resolution magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence in the sagittal plane (TR, 2,500 ms; TE, 3.12 ms; FOV, 210 mm; voxel size, 0.80 mm isotropic) which captured the entire brain, was used for functional MRI image registration as well as for estimation of overall hippocampal volume. The entire sequence including the T1-weighted image (5min 48sec), the functional sequence (8min 4sec), and localizers (22sec) took approximately 15 minutes.

fMRI word task: procedure

During Visit 1 to the laboratory, parents or guardians were provided with an introductory booklet describing the neuroimaging component of the research. They were also asked questions about their toddler’s sleep schedule, bedtime routine, and nighttime habits in order to maximize the participant’s comfort and the chances of a successful nighttime scan. Based on this information, we developed a plan for the day and night of the scan, and parents were instructed on how to practice at home to acclimate their toddlers using earplugs, headphones, and delivering MRI scanner sounds during sleep. During Visits 2 and 3, experimenters discussed these night practices with the parents/guardians and reminded them to continue in preparation of the scanning session, which was scheduled to coincide with the habitual bedtime of the toddler.

During the nighttime scanning session, the MRI scanning room was arranged to be comfortable, with a standard setup that included memory foam mattresses, pillows, blankets, large stuffed animals etc. If toddlers had their own comfort items that passed safety screening, these were also included in the setup. After toddlers arrived at the Imaging Research Center, we prepared toddlers for the session which required them to be asleep on the MRI bed wearing safety and sound delivery materials. Some parents arrived with their toddlers already asleep, whereas others used either the MRI room or a side room with an air mattress to get their toddlers to fall asleep. Sleep time was recorded, and once toddlers had been asleep in the supine position for roughly 20 minutes, earplugs and headphones were put on, and they were placed into the head coil of the MRI. If toddlers awoke at any point in this process (e.g., when earplugs were put on), experimenters waited for toddlers to fall asleep and another 20 minutes before repeating the necessary steps. The minimum time from arrival to start of imaging was about 30 minutes, however due to differences in toddlers’ ability to fall asleep and stay asleep during preparation for the scan, it could take longer to start the scanning session (e.g., an hour or more). This time included careful monitoring of the toddler to ensure that they were asleep, making intermittent checks whether they were stirring, and checks to ensure that hearing protection and auditory delivery materials were assuredly secure before imaging commenced.

After toddlers were asleep in the proper position, parents were given the option to either stay in the MRI room, or to watch on a screen from the control room. Regardless, a research assistant carefully observed the sleeping toddlers from the MRI room for the duration of the scan in order to signal to immediately stop the scan upon noticing signs of awakening. If toddlers showed heavy

stirring or signs of awakening ($n = 14$), the session was stopped. For comparisons between toddlers that did versus did not complete the neuroimaging session see [Table S4](#).

Exploratory longitudinal follow-up

After approximately 10 months from participating, a subset of parents completed The MacArthur Bates CDI III form over the phone or during a subsequent visit to the laboratory to explore predictors of vocabulary growth ([Table S3](#)).

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of behavioral performance

Two trials of the Object-Labeling task were completed; this yielded two memory indices averaging performance across the two rooms: a same-session memory discrimination index and a one-week delay index, each calculated as the rate of correctly selected objects minus rate of incorrectly selected known objects.

Two trials of the Puppet Naming Task were completed; this yielded two memory indices averaging performance across the two rooms: a same-session as learning memory discrimination index and a one-week delay index, each calculated as the rate of correctly selected puppets minus rate of incorrectly selected novel puppets.

Our measure of productive vocabulary corresponded to parental report of the number of words produced out of a total of 100 on the CDI III form. We used the raw score (rate of used words out of 100) as our vocabulary measure.

fMRI data processing and parameter estimation

Data were preprocessed using FSL routine pipelines and analyzed using FEAT in FSL 5.0.8 (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>). Preprocessing included brain extraction (BET), motion correction (MCFLIRT), spatial smoothing at 6mm FWHM, slice timing correction, and a high pass filter of 40 s to address low frequency signals present longer than the combination of one active (20 s) and one passive (20 s) block. Additionally, FSL's motion outliers function identified motion outliers utilizing relative intensity differences which were included as covariates of non-interest. Two participants were excluded from the 40 total who were scanned due to excessive motion as defined by greater than 2mm absolute motion in the functional image (remaining mean absolute motion = 0.35mm, range 0.09 – 1.9mm). Functional images were registered first to the participant's MPRAGE image using the BBR linear registration algorithm in FLIRT, and then to a toddler image template developed at the University of North Carolina (<https://www.nitrc.org/projects/pediatricatlas/>) utilizing a 12 DOF linear registration in FLIRT for group space analysis. This toddler template was developed from a sample of 95 toddlers. General linear models were conducted in FSL FEAT modeling activity for the puppet, object, novel, and known words during their presentation. This time series was convolved with a double-gamma hemodynamic response function to yield the two memory-related contrasts of focus, puppet + object > novel, and, known > novel.

Analyses at the group level for these contrasts were calculated in the UNC atlas space⁴⁰ and were restricted to an anatomically defined mask encompassing the left hippocampus, as well as one encompassing the left anterior MTL (corresponding to the perirhinal and entorhinal cortices). Specifically, anterior MTL was derived from the parahippocampal gyrus mask included in the atlas and obtained by keeping the portion of this mask anterior to the Y coordinate value corresponding to point in which the hippocampal head ends, and the hippocampal body begins.

Control analyses (see [Table S1](#)) were also carried out in the left superior temporal gyrus (a language area of the brain but not necessarily associated with memory for words) and the left lateral occipital cortex (expected to be wholly unrelated to language content). The control ROIs were developed from probabilistic maps obtained from the Harvard-Oxford Cortical Atlas included within the FSL software FSL VIEW, which were registered into the UNC toddler atlas space. The whole number probabilistic value (1–100) that most closely approximated the size of the left hippocampal mask (3033 voxels) was used, and both masks were very close in volume (superior temporal: 3087 voxels; lateral occipital: 3044 voxels). The left anterior MTL ROI was slightly smaller at 2708 voxels.

Ancillary analyses in response to peer review were also carried out in the medial prefrontal cortex and inferior temporal gyrus, in regions implicated in nap-related memory consolidation⁴¹ (see [Figure S2](#)). Masks for these two ROIs were made by creating a 5mm sphere centered on each of the two peak voxels (although the IFG mask needed a shifted x-coordinate due to the different size of the toddler cortex). These masks were then registered into the UNC toddler atlas space for final analysis. The registration process caused the medial prefrontal sphere ($x = -16, y = 38, z = -6$; 1791 voxels) to be slightly larger than the inferior frontal gyrus sphere ($x = -32, y = 30, z = -2$; 1523 voxels) which is consistent with a slightly larger medial prefrontal than inferior frontal mask in the original publication.⁴¹ Once these structurally-defined regions of interest were identified, analyses proceeded as for the other regions examined here.

Finally, a supplemental whole brain analysis (thresholded at $Z > 1.96$ and cluster $p < 0.05$) including an all pseudoword contrast was included for visualization purposes (See [Figure S2C](#)).

Assessment of hippocampal volume

Total hippocampal volume was obtained using Freesurfer 5.0 with a semi-automated correction. The MPRAGE scan was reoriented into an axial orientation, and then standard automatic hippocampal parcellation procedures were utilized. While the FreeSurfer analysis is already based on a manual training set, we incorporated a second step of bias correction developed from images collected in a previous early childhood MRI study,²² segmented by a trained experimenter. This correction was implemented using the automatic

segmentation adaptor tool (ASAT; <https://www.nitrc.org/projects/segadapter>) which is developed to address systematic errors in automatic segmentation protocols, and has been recently validated in a MRI sample including children of a similar age to the sample in the current experiment.³⁹ The images from three toddlers were of insufficient quality to be segmented by FreeSurfer, however two could be segmented manually. While reported statistics include these two manually traced scans, when these are removed from analysis the results hold.

Statistical analysis

Due to the non-normal distribution of selections, Wilcoxon Signed Rank Tests were utilized to examine average levels of behavioral performance across tasks and delays. Rosenthal correlation coefficients⁶⁹ were used to examine the effect size of the one sample Wilcoxon Tests of behavioral performance.

Spearman's rho correlation coefficients were used for correlations between behavioral performance and parameter estimate values extracted from the fMRI task, due also to the non-normal distribution of behavioral task selections. These correlations at 20 Minute Delay Test (8) and One-Week Delay Test (8) were corrected for multiple comparisons using the Benjamini-Hochberg adjustment for false discovery rate.⁷⁰ Correlations between productive vocabulary and parameter estimate values utilized a Pearson's correlation coefficient. These correlations between productive vocabulary and parameter estimate values (4) were also corrected for multiple comparisons using the Benjamini-Hochberg adjustment for false discovery rate. Simultaneous multiple linear regressions were used to examine the association between hippocampal function and vocabulary, accounting for hippocampal structure.

All of the statistical tests used for these analyses used two-tailed significance tests and nearly all were conducted with SPSS (IBM version 27). Benjamini-Hochberg FDR values were calculated by hand, and Steiger's Z⁷¹ comparisons utilized an online calculator (http://www.psychmike.com/dependent_correlations.php).