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Hippocampal Activation During Transitive Inference in Humans

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ABSTRACT: Studies in rodents have demonstrated that the integration and flexible expression of memories, necessary for transitive inference, depend on an intact hippocampus. To test this hypothesis in humans, we studied brain activation during the discrimination of a series of overlapping and non-overlapping arbitrary visual stimulus pairs. We report that transitive inference about overlapping pairs is associated with right anterior hippocampal activation, whereas recognition of non-overlapping stimulus pairs is associated with bilateral medial temporal lobe activation centered in the anterior parahippocampal gyrus. We conclude that immediate access to simple stimulus-stimulus relationships is mediated via the parahippocampal gyrus, whereas the flexible representation of memory requires the recruitment of the hippocampus. © **2004 Wiley-Liss, Inc.**

KEY WORDS: neuroimaging; fMRI; relational memory; parahippocampal gyrus

INTRODUCTION

The medial temporal lobe is essential for declarative memory, i.e., our ability to recollect facts and events (Squire and Zola-Morgan, 1991). This inference was drawn originally from studies of declarative memory function in patients with medial temporal lobe damage (Scoville and Milner, 1957; Corkin, 2002) and was subsequently confirmed by animal studies (Eichenbaum and Cohen, 2001). The anatomical organization of hippocampus, surrounding cortices, and multimodal association cortex underlying declarative memory has now been well characterized in both the rodent and the primate brain (Lavenex and Amaral, 2000; Witter et al., 2000). Despite this remarkable progress, the precise contributions of the hippocampus and surrounding cortices (i.e., parahippocampal gyrus, entorhinal cortex, and perirhinal cortex) to the formation of declarative memory remain a matter of debate (Brown and Aggleton, 2001; Eichenbaum, 2000; Preston and Gabrieli, 2002).

Experiments in rodents and nonhuman primates provide compelling evidence that the hippocampus and parahippocampal gyrus contribute differentially to recognition memory (Eichenbaum and Cohen, 2001). In short, the parahippocampal gyrus is concerned with familiarity or recency of individual stimulus items, whereas the hippocampus is needed to recollect relations among items (Eichenbaum et al., 1994; Brown and Aggleton, 2001). Several recent functional neuroimaging studies have demonstrated segregated roles of hippocampus and parahippocampal gyrus for declarative memory in humans (Eldridge et al., 2000; Davachi and Wagner, 2002; Strange et al., 2002). However, these neuroimaging studies of declarative memory in humans employ experimental designs that cannot be evaluated further in animals. In the present report, we have followed the reverse strategy, i.e., translating an experimental design, which established a distinct memory function of the hippocampus in animals, into a functional neuroimaging study in humans.

We chose to study transitive inference, i.e., the ability to infer the relationships between indirectly related items that have not been presented together, based on previous learning of a sequence of overlapping premise pairs (e.g., A>C, if A>B and B>C). Using a hierarchically ordered set of five odors (A>B>C>D>E), Dusek and Eichenbaum (1997) demonstrated that disconnection of the hippocampus from either its cortical or subcortical pathway prevents rats from inferring the proper order for odors B and D. This and subsequent experiments in rodents (Fortin et al., 2002; Van Elzakker et al., 2003) support the theory that the hippocampus is necessary to establish a flexible representation of memory.

We used a 2 × 2 factorial design to study the effects of inference (novel vs previously learned pairings) and stimulus sequence (overlapping vs non-overlapping pairs) (Fig. 1). Initially, subjects were trained to discriminate arbitrary visual stimulus pairs. One set of eight stimuli created four non-overlapping pairs (labeled P in Fig. 1), whereas another set of five stimuli created an overlapping sequence (labeled S). We then studied the ability to infer a relationship between items that had not been presented together, based on previous learning of a sequence of overlapping pairs (transitive inference) or non-overlapping pairs (nontransitive inference). We hypothesized that transitive inferences about novel pairings derived from the overlapping stimulus set (labeled IS) would be associated with hippocampal activation, whereas nontransitive inferences about novel pairings derived from the nonoverlapping stimulus set (labeled IP) would not.

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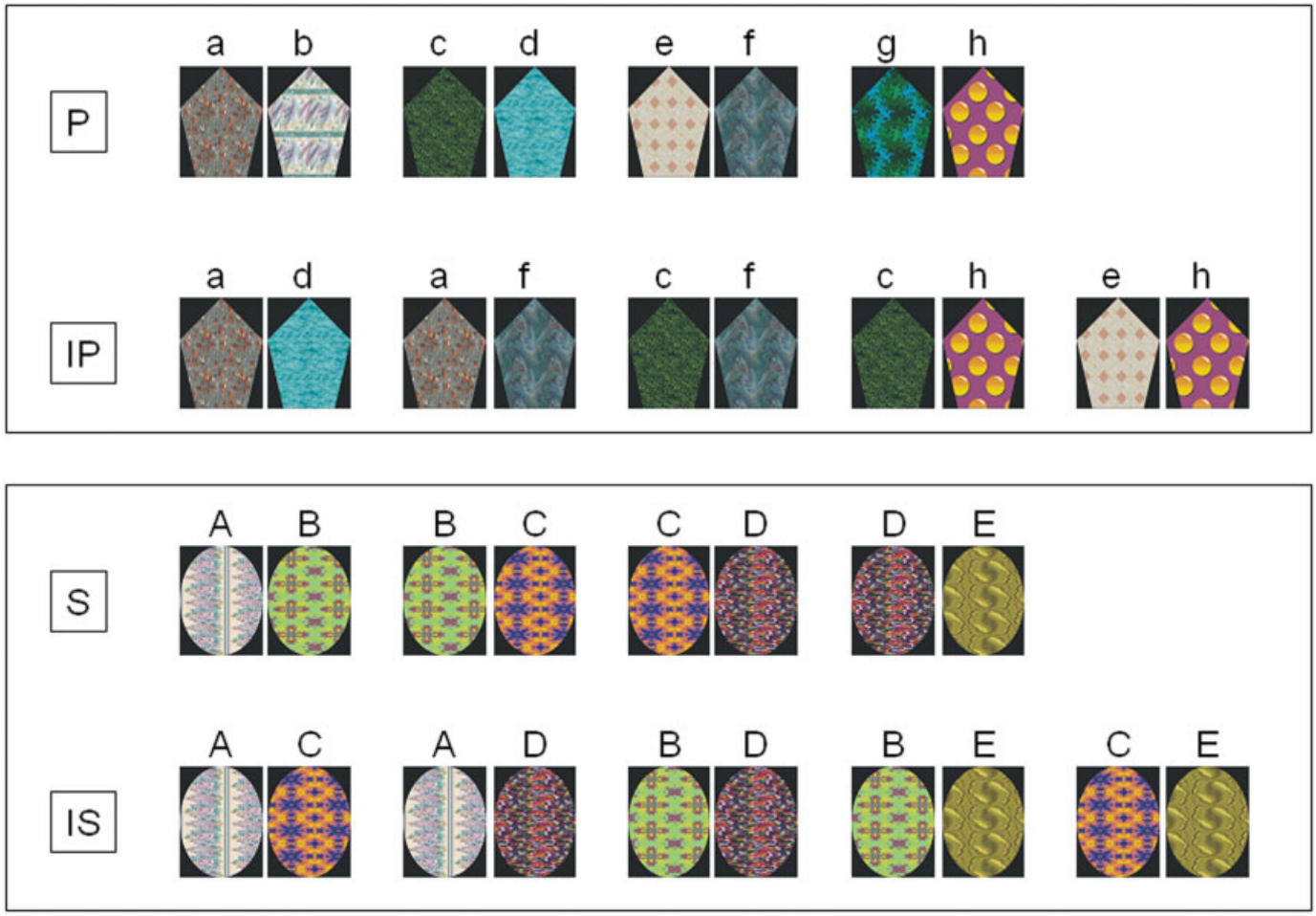

FIGURE 1. Stimulus set and experimental conditions. Prior to scanning, subjects were trained to discriminate non-overlapping pairs (P) and an overlapping sequence of pairs (S). The reinforced item within each pair is shown on the left. During scanning, subjects were asked to recollect the correct response for previously seen pairs (P and S) and to infer the correct response for five novel pairings of non-overlapping pairs (IP) and overlapping pairs (IS). No letters were shown in the experiment, and the presentation of the pairs and the position of the two stimuli within each pair were randomized.

MATERIALS AND METHODS

Subjects

We studied 16 healthy subjects (8 female and 8 male, ages 21-28, mean age 23.9, average estimated IQ (Blair and Spreen, 1989) = 114), who gave informed consent in a manner approved by the institutional review board of the Massachusetts General Hospital. No subject had a history of major medical, neurological, or psychiatric illness.

Stimuli and Paradigm

Stimuli

Thirteen visually distinctive pattern fills were selected from those provided by CorelDraw. Two sets of pattern fills (8 for the non-overlapping pairs, and 5 for the overlapping pairs) were randomly assigned to pairs of pentagon and ellipsoid shapes for each participant.

Training prior to scanning

Participants were informed that they would see pairs of visual patterns on a computer screen, and that one pattern in each pair would always hide a "smiling face" (e.g., ☺). They were then shown each of the pairings, along with the correct answer in each case, and instructed to remember the correct location of the smiling face. Left/right position of individual patterns for each pair was counterbalanced, and participants indicated their response by pressing "1" for the stimulus on the left and "2" for the stimulus on the right. When participants made a correct guess during training, the selected visual pattern would move to reveal the smiling face reinforcement. When participants made an incorrect guess, the selected visual pattern would move, but the smiling face would not appear.

Participants were first trained and tested on the non-overlapping pairs, then on the overlapping pairs, and finally on a mixture of non-overlapping and overlapping pairs. This final testing session was similar in format to that used in the noninference conditions during scanning, with one difference: stimuli pairs were presented

randomly, rather than in blocked sessions, such that on each trial participants were equally likely to be presented with a pair of stimuli from the overlapping set as they were to be presented with a pair of stimuli from the non-overlapping set. The training procedure, identical for non-overlapping and overlapping pairs, was broken into three blocks. The first training block consisted of 60 trials that contained twice as many of two of the four stimulus pairs. For example, during training of the overlapping stimulus set, participants saw 20 instances of AB and BC, and 10 instances of CD and DE. Thus, the sequential stimuli during the first training block were "front loaded." During training of the non-overlapping stimulus set, participants saw 20 instances of AB and CD, and 10 instances of EF and GH. The second training block also consisted of 60 trials that contained twice as many of two of the four stimulus pairs from each set. In this second block, however, the pairs were "back loaded". Thus, participants saw 20 instances of CD and DE, and 10 instances of AB and BC. During the second block of training of the non-overlapping stimulus set, participants saw 20 instances of EF and GH, and 10 instances of AB and CD. The third training block consisted of 24 trials that contained equal numbers of the four stimulus pairs. Thus, for the training of both overlapping and non-overlapping pairs, participants saw six instances of each stimulus.

Overall, participants were presented with an equal number of each of the pairs in the overlapping and non-overlapping stimulus sets. This method of training ensured that all participants would not only be able to learn the correct response for each pairing but would also be likely to hierarchically encode the overlapping stimulus set. Our previous work suggested that the initial front loading of pairs was necessary for healthy participants to correctly judge BD during the inference test trials (Titone et al., 2003).

Recognition task during fMRI scan

Subjects participated in two functional magnetic resonance imaging (fMRI) scans, each lasting 5 min. Each scan started and ended with 30 s of fixation trials. In between, blocks of 10 trials of four different types (P, S, IP, IS) were presented in the following sequence: P, S, IP, IS, P, S, IP, IS. For each trial, subjects were instructed to indicate by pressing a button which pattern they associated with reinforcement, based on the previous training session. During scanning, however, the smiling face, used for reinforcement during training, was not presented.

To avoid bias associated with particular object shapes and/or patterns, we rotated the position of the fills within the two sets for each participant (a total of 16 fills was used, each subject saw 13 of the 16 fills) and the two shapes across all subjects (8 subjects saw non-overlapping pairs as pentagons and 8 subjects saw overlapping pairs as pentagons) (Fig. 1).

Functional Imaging

Subjects were scanned in a Siemens 1.5-tesla (T) Sonata high-speed echo-planar imaging device (Munich, Germany). Subjects lay on a padded scanner bed in a dimly illuminated room, wearing ear plugs. Foam padding was used to stabilize the head. Stimuli were generated using Presentation software (Neurobehavioral Systems) on a personal computer, projected onto a screen, and viewed by the subjects via a tilted mirror placed in front of their eyes.

Functional scanning began with an initial sagittal localizer scan. The two functional series lasted 5:10 min each. The first 10 s of each series were discarded, to equilibrate for scanner inhomogeneity. During the remaining time of each series, 120 BOLD functional brain images were collected [TE/TR = 40/2,500 ms; 25 coronal slices, perpendicular to the anterior commissure-posterior commissure (AC-PC) line and starting anterior at the frontal pole, 5-mm thickness, 1-mm skip; voxel size $3.1 \times 3.1 \times 5$ mm, FOV = 200 mm; Flip angle = 90 degrees], to capture 80 trials lasting 3 s each, bracketed by two blocks of fixation trials, lasting 30 s each.

Data Analysis

Behavioral data

The accuracy data were analyzed using a repeated-measures 2 (sequence type: overlapping, non-overlapping) $\times$ 2 (inference type: present, absent) analysis of variance (ANOVA). The latency of correct responses was also analyzed using a repeated-measures 2×2 ANOVA.

Functional neuroimaging data

All functional data were transformed into a common reference space (MNI Talairach brain) and corrected for head motion using SPM99 (Wellcome Department of Cognitive Neurology, London, UK). Functional images were smoothed using an 8-mm full-width/half-maximum (FWHM) Gaussian filter.

Functional images were analyzed in two stages in a mixed-effects model. First, general linear models were created for each subject, which included the effects of session (1,2) and condition ([P], [S], [IP], [IS], and fixation baseline) to explain the variance of BOLD signal change at each voxel. We tested for the main effects of inference ([IP+IS] vs [P+S]) and sequence ([S+IS] vs [P+IP]), as well as their interaction ([IS vs IP] vs ([S vs P]) across the two functional imaging sessions. Second, we pooled all individual contrast images for the main effects and interactions into a one-sample *t*-test for within group effects. Activations were considered significant at a voxel extent threshold of $\geq$50 voxels, with $P < 0.0001$, uncorrected for multiple comparisons. To disambiguate significant results of the main effects analysis, we followed up with analyses that included only two conditions (simple effect analysis). Activations were considered significant at $P < 0.0001$, uncorrected for multiple comparisons.

RESULTS

Behavioral Data

Accuracy

The pattern of accuracy for each of the four conditions (P, S, IP, and IS; Fig. 1) was as follows: 98.0 $\pm$ 4.0% (mean $\pm$SD) for P; 95.9 $\pm$ 6.9% for S; 94.5 $\pm$ 15.0% for IP; and 91.7 $\pm$ 10.4% for

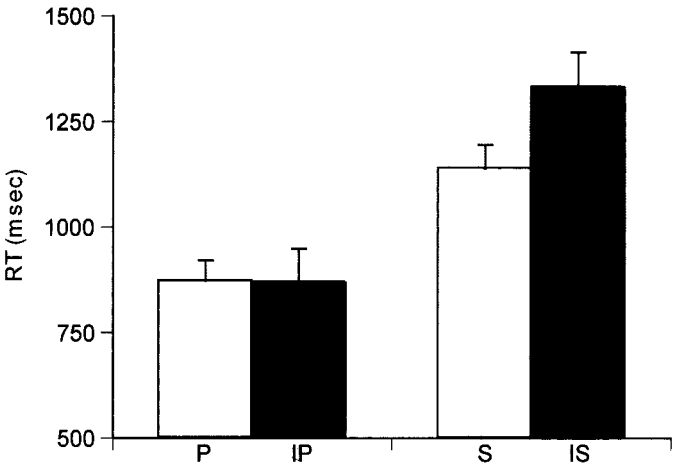

FIGURE 2. Recall accuracy and reaction time. Bar graphs display mean (±SD) reaction time for the four conditions: previously seen (P) and novel (IP) non-overlapping pairs and previously seen (S) and novel (IS) overlapping pairs.

IS. Responses to non-overlapping pairs of items were not significantly different from responses to overlapping pairs (main effect of sequence: $F(1, 15) = 3.3$, $P = 0.09$). Responses to previously learned pairs were not significantly different from responses to novel pairings (main effect of inference: $F(1, 15) = 1.8$, $P = 0.20$). The interaction between the two main effects, i.e., sequence and inference, was not significant ($F(1, 15) = 0.8$, $P = 0.78$).

Response latency

Response latency of correct responses (in milliseconds) for each of the four conditions (P, S, IP, and IS; see Fig. 1) was as follows: 871.2 ± 194.7 (mean ±SD) for P; 1138.3 ± 234.6 for S; 868.4 ± 289.0 for IP; and 1330.4 ± 334.2 for IS (Fig. 2). Responses to overlapping pairs were significantly slower than responses to non-overlapping pairs (main effect of sequence: $F(1, 15) = 67.6$, $P < 0.0001$). Responses to novel pairings were significantly slower than responses to previously learned pairs (main effect of inference: $F(1, 15) = 8.9$, $P = 0.009$). Furthermore, the increase in reaction time associated with inferential judgments was significantly greater for the overlapping pairs than the non-overlapping pairs (sequence-by-inference interaction: ($F(1, 15) = 19.9$, $P < 0.0001$). Thus, whereas judgments not requiring transitive processing resulted in no increase in reaction time, transitive inferences required substantial additional processing (Fig. 2). Similar main effects and interaction were found when latencies of all responses were analyzed (main effect of sequence: $F(1, 15) = 64.2$, $P < 0.0001$; main effect of inference: $F(1, 15) = 8.4$, $P = 0.01$; sequence-by-inference interaction: $F(1, 15) = 21.0$, $P < 0.0001$). We will refer to this significant sequence-by-inference interaction as the transitive inference effect. The transitive inference effect was more pronounced in the BD trials of the IS condition, which resulted in significantly longer reaction time compared to the other four trial types of the same condition (i.e., AC, AD, CE, BE) (paired *t*-tests, all $P < 0.01$).

fMRI Data

Two behavioral effects, the transitive inference effect and the recognition of non-overlapping pairs, were associated with significant medial temporal lobe activation.

Transitive inference

We investigated the transitive inference effect by testing which voxels showed a significant sequence-by-inference interaction (contrast: [IS > IP] > [S > P]). This analysis revealed significant right anterior hippocampal activation during transitive inference (Table 1 and Fig. 3). Activation of the same right anterior hippocampal region during transitive inference was confirmed in the simple comparison of transitive versus nontransitive inference [IS>IP] and the comparison of transitive inference versus judgments on overlapping items [IS>S] (Table 1). Hippocampal acti-

TABLE 1.

Medial Temporal Lobe Activation*

Effect	Brain region	z score	MNI Talairach
Inference-by-Sequence Interaction			
Contrast: [IS > IP] > [S > P]	R anterior hippocampus	4.44	34, −14, −16
Simple effects of transitive inference			
Contrast: [IS] > [IP]	R anterior Hippocampus	4.22	36, −6, −22
		4.13	34, −10, −16
Contrast: [IS] > [S]	R anterior Hippocampus	3.89	34, −4, −14
Recognition of non-overlapping pairs			
Contrast: [P + IP] > [S + IS]	R anterior Parahippocampal gyrus	4.97	24, −18, −20
	L anterior Parahippocampal gyrus	4.90	−28, −16, −20
Recognition of previously learned non-overlapping pairs			
Contrast: [P] > [S]	R anterior Parahippocampal gyrus	4.99	24, −16, −24
	L anterior Parahippocampal gyrus	4.88	−28, −16, −22

*Letters in square brackets refer to the four conditions as indicated in Figure 1. *P* = pairs, *S* = sequence, *IP* = inferred pairs, *IS* = inferred sequence. Coordinates refer to the MNI305 stereotactic space, an approximation of Talairach space (Talairach and Tournoux, 1988).

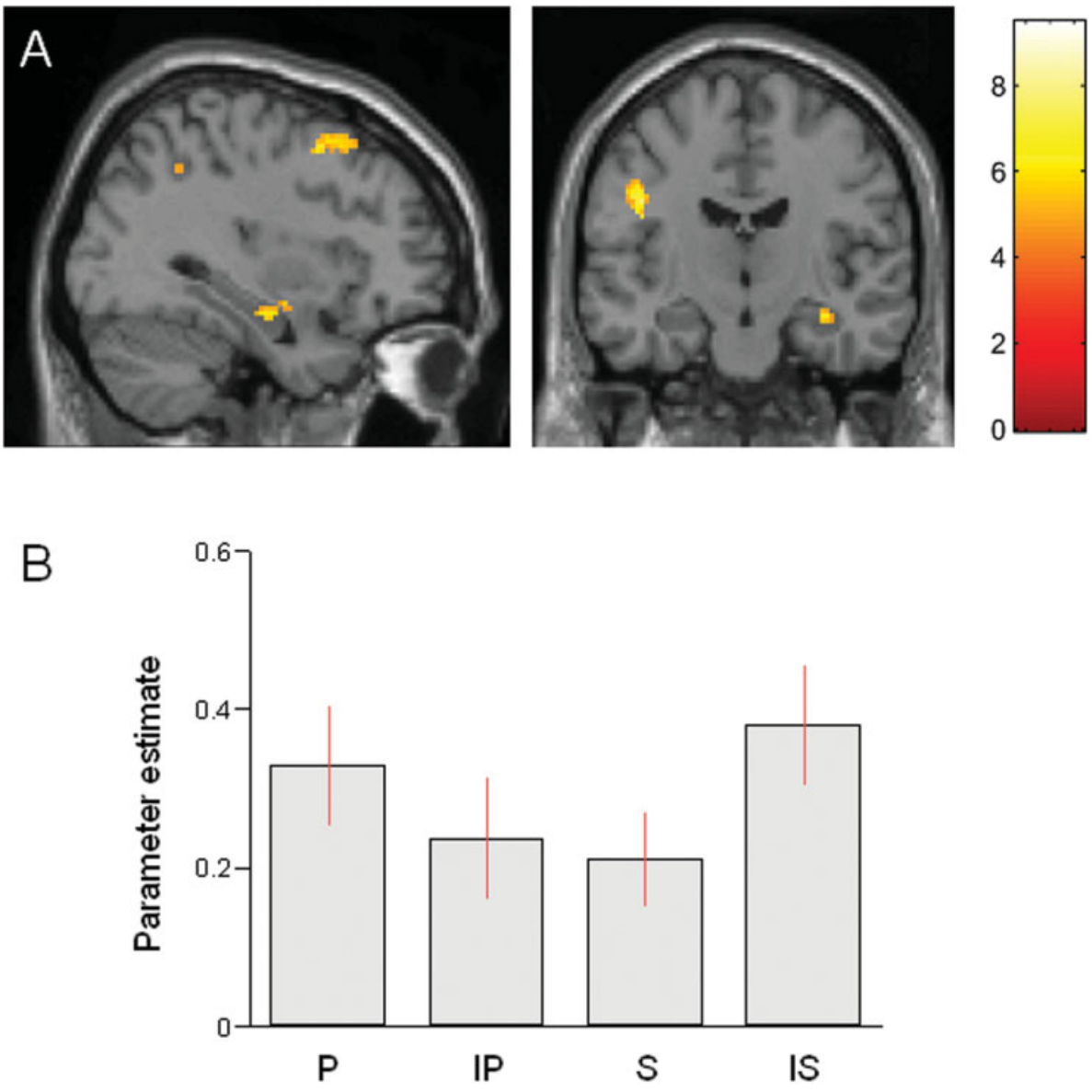

FIGURE 3. Right anterior hippocampal activation during transitive inference. A: Areas of significant activity in 16 subjects ($P < 0.0001$, uncorrected) are mapped onto a template structural image of a single subject. B: Contrast of parameter estimates (± 1 SE) for the magnitude of the hemodynamic response in right anterior hippocampus for the four experimental conditions (i.e., previously seen (P) and novel (IP) non-overlapping pairs and previously seen (S) and novel (IS) overlapping pairs) compared with the cross-hair fixation baseline condition. The parameter estimates are collapsed across sessions within subjects and averaged across subjects.

vation during transitive inference was also seen in individual subjects (Fig. 4).

We explored the neural circuitry underlying transitive inference by reviewing all voxels with a significant sequence-by-inference interaction. A distributed network of brain regions, including the pre-supplementary motor area (pre-SMA), bilateral frontal cortex, bilateral parietal cortex, bilateral posterior temporal cortex, and the pulvinar showed significant activation associated with transitive inference (Table 2 and Fig. 5). To disambiguate the two effects that contributed to the transitive inference effect in these brain regions, we studied the main effects of inference (novel pairs > previously learned pairs) and sequence (overlapping pairs > non-overlapping pairs). We found significant main effects of inference and sequence in pre-SMA (peak activation at coordinates −4, −20, −52; z = 5.64 and 2, 12, 56; z = 4.41 respectively), left prefrontal cortex (−52, 18, 34; z = 5.76 and −46, 28, 32; z = 4.58, respectively), and left parietal cortex (−46, −54, 50; z = 4.86 and −38, −52, 58; z = 5.47, respectively). In addition, a main effect of sequence was observed in right prefrontal cortex (46, 4, 54; z = 4.33), right parietal cortex (38, −48, 44; z = 5.16), and bilateral temporal cortex (−20, −58, −12; z = 5.22 and 26, −52, −20; z = 4.87). In contrast, neither of these two main effects was present in the hippocampus.

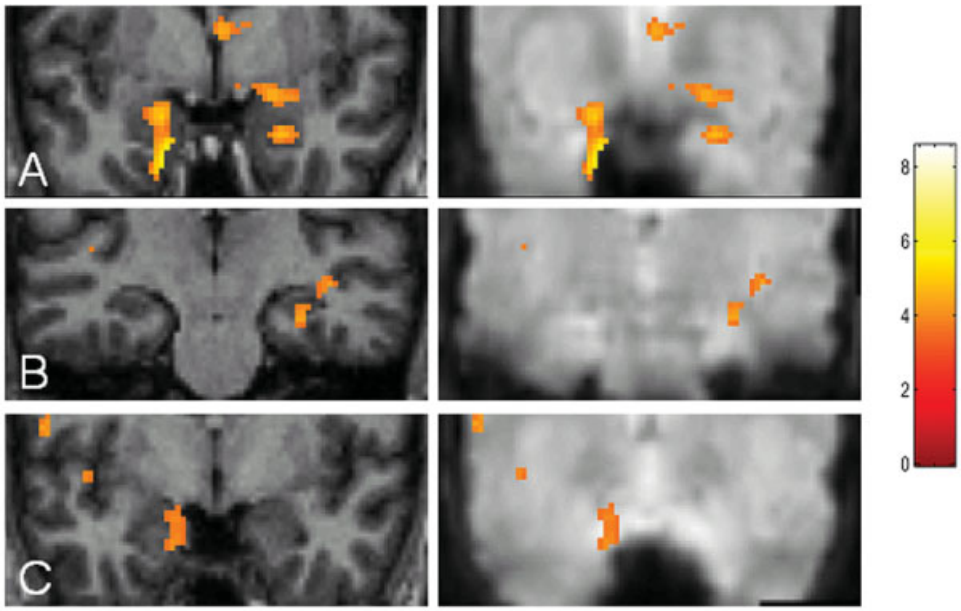

FIGURE 4. Hippocampal activation during transitive inference. Areas of significant activity ($P < 0.0001$, uncorrected) in three individual subjects (A–C) are displayed on each subject's structural (left column) and functional (right column) image.

Recognition of non-overlapping pairs

The discrimination of non-overlapping pairs was associated with significantly greater medial temporal lobe activation centered in the parahippocampal gryus when compared with the discrimination of overlapping pairs (Table 1 and Fig. 6). This effect was primarily due to significantly greater activation during the recognition of previously learned non-overlapping pairs when compared with previously learned overlapping pairs (Table 1). A similar (but subthreshold) effect in the same region was observed for the contrast of the novel non-overlapping and overlapping pairs (peak activation at coordinates −28, −14, −24; z = 3.31 and 22, −10, −16; z = 3.20).

To explore further the neural circuitry underlying the simple recognition of previously learned non-overlapping pairs, we reviewed all voxels that displayed greater activation when compared to the recognition of previously learned overlapping pairs. The only other region with significant activation was in the medial prefrontal cortex (coordinates −4, 50-, −6; z = 4.45).

DISCUSSION

We report that the right anterior hippocampus is part of the neural circuitry underlying transitive inference for arbitrary visual stimulus patterns. In contrast, the simple recognition of previously learned pairs of non-overlapping visual stimuli results in significantly faster reaction times and greater medial temporal lobe activation centered in the parahippocampal gyrus. We interpret these

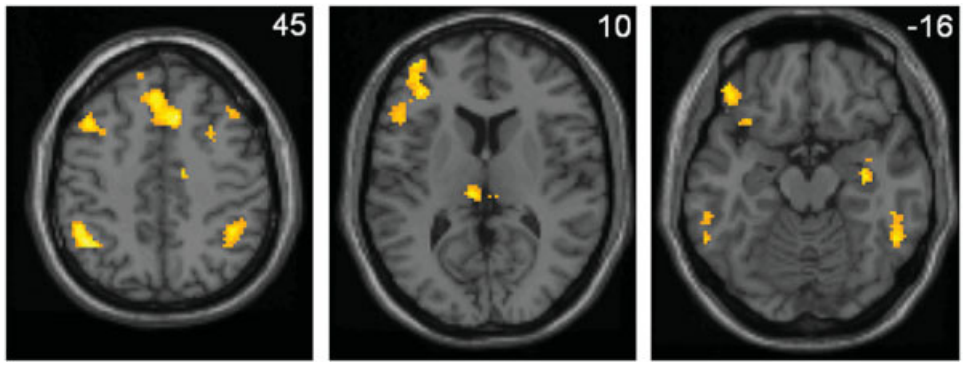

FIGURE 5. Transitive inference network. Horizontal slices through a template structural image of a single subject are shown to display areas of significant activity in 16 subjects ($P < 0.0001$, uncorrected) in bilateral frontal cortex, pre-SMA, and bilateral parietal cortex (at 45 mm above the anterior commissure-posterior commissure [AC-PC] axis), left prefrontal cortex and pulvinar (+10 mm), and left inferior frontal cortex, bilateral inferior temporal cortex, and right hippocampus (−10 mm).

TABLE 2.

*Transitive Inference Network**

Brain region	H	z score	MNI Talairach coordinates		
Pre-SMA	R	5.32	8	18	50
Inferior temporal gyrus (BA 37)	L	5.14	−44	−52	−6
	R	4.80	54	−50	−12
Middle temporal gyrus (BA 21)	L	5.04	−58	−30	−8
Premotor cortex (BA 6)	L	4.99	−32	2	62
	R	4.98	30	10	50
Inferior frontal gyrus (BA 47)	L	4.95	−44	36	−8
	L	4.42	−30	20	−12
Inferior parietal cortex (BA 40)	L	4.87	−42	−54	46
	R	4.72	46	−50	42
Anterior cingulate cortex (BA 24)	L	4.79	−4	6	26
Pulvinar	L	4.44	−8	−24	10
Hippocampus	R	4.44	34	−14	−16

*H indicates hemisphere. Coordinates refer to the MNI305 stereotactic space, an approximation of Talairach space (Talairach and Tournoux, 1988).

data as evidence for an immediate access to previously learned information via the parahippocampal gyrus, whereas the flexible representation of memory requires the recruitment of the hippocampus. Thus, this study confirms in humans the importance of the hippocampus for the orderly organization of stimulus relations, a finding previously demonstrated in rodents (Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003), and extends recent fMRI studies of relational memory in humans (Rombouts et al., 1997; Sperling et al., 2001; Davachi and Wagner, 2002).

Neural Circuitry of Transitive Inference

Inferential thinking is found in both humans and animals. Transitive inference, the paradigmatic example of inferential thinking, has been described in humans as young as 4 years old (Bryant and Trabasso, 1971), in nonhuman primates (McGonigle and Chalmers, 1977), in rats (Davis, 1992; Roberts and Phelps, 1994; Dusek and Eichenbaum, 1997), and in pigeons (von Fersen et al., 1991). It appears that transitive inference has evolved throughout phylogenesis as a beneficial strategy, for example, to infer the rank order of animals in the habitat (Delius and Siemann, 1998).

What are the cognitive mechanisms underlying transitive inference (Harris and McGonigle, 1994; Wynne, 1998; Bryson, 2001), and what are their neural correlates? When subjects learn to discriminate a series of overlapping stimulus pairs, reaction times increase during the learning of pairs that do not include end items, but do not change for pairs with end items (Acuna et al., 2002b). Furthermore, reaction times are shorter during subsequent recognition of novel pairs with more intervening items (symbolic distance effect) (Moyer and Landauer, 1967; Acuna et al., 2002b). These two observations indicate that series of overlapping stimulus pairs are stored as a unified and flexible representation, which provides strong evidence for transitive inference as a general relational memory capacity (Eichenbaum and Cohen, 2001). In contrast, some of the results of instrumental transitive inference testing in animals can be explained by the value transfer theory, which predicts that the ability of a stimulus to attract a response is determined by positive/negative values acquired during reinforcement training (Frank et al., 2003; Van Elzakker et al., 2003; von Fersen et al., 1991).

The present results suggest that a distributed but specific network of brain regions underlies transitive inference in humans. We found significant activation associated with transitive inference in the pre-SMA (Brodmann area 6) and bilateral parietal, prefrontal, and inferior temporal cortices. This pattern is similar to that in the recent study by Acuna et al. (2002a), who trained healthy control subjects on a hierarchically ordered set of 11 different unicolored shapes. The authors report significant brain activation in bilateral prefrontal cortex, pre-SMA, premotor area, insula, precuneus, and lateral posterior parietal cortex during the recognition of novel pairs of visual stimulus items. The slice selection used by Acuna et al. (2002a) did not cover the medial temporal lobe. Other recent studies of transitive inference using verbal premise pairs (Goel and Dolan, 2001) or iconic stimuli (Dickins et al., 2001) also reported significant prefrontal and parietal cortex activation. Of interest, patients with prefrontal cortex damage exhibit deficits in the integration of relations (Waltz et al., 1999), as do patients with schizophrenia (Titone et al., in press). Taken together, studies in humans have established an important role of frontal and parietal multimodal association cortices, both of which are closely connected with the hippocampus via the entorhinal cortex, for transitive inference.

Our main effects analysis demonstrated that pre-SMA, left parietal cortex, and left prefrontal cortex contributed not only to the transitive inference effect, but to all trials that involved inferences (transitive as well as nontransitive) and overlapping pairs (previously seen as well as novel). Furthermore, right parietal, right prefrontal, and bilateral temporal cortex activation was greater for all overlapping pairs. In contrast, right hippocampal activation was not seen in the main effects analyses, but only in the interaction and the simple effects of transitive inference. This indicates that the hippocampus contributes uniquely to the ability to infer relationships between a sequence of items, while pre-SMA, parietal, and prefrontal cortex contribute more broadly to inferential judgments and the recognition of overlapping pairs of visual stimuli.

Distinct Patterns of MTL Activation for Transitive Inference and Recognition

Rodents trained to discriminate the overlapping stimulus set A>B>C>D>E cannot correctly discriminate the novel stimulus pair BD after hippocampal lesion, but they are still able to discriminate correctly the novel pairing of the two end items A and E

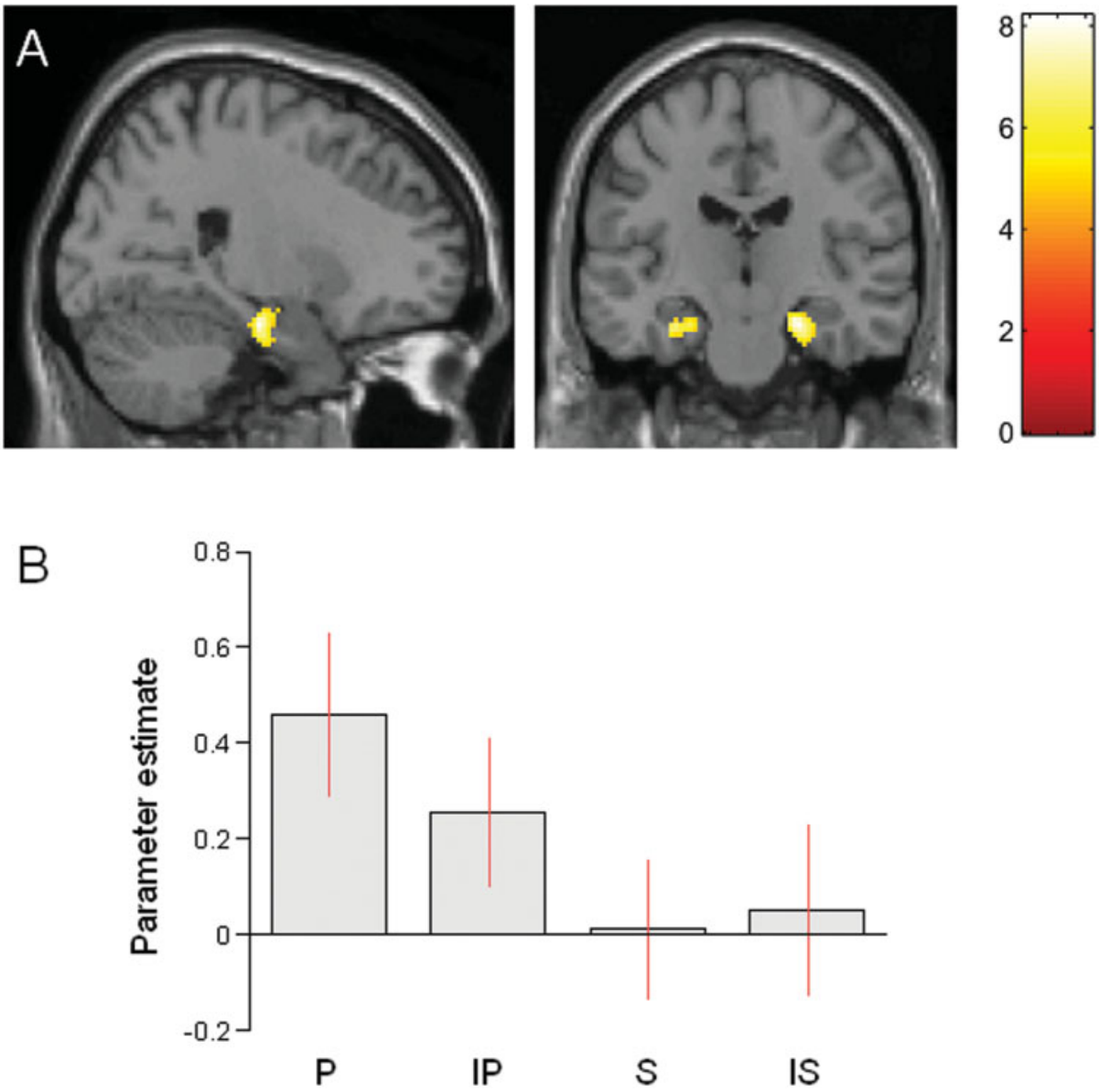

FIGURE 6. Recognition of non-overlapping pairs. A: Areas of significant activity in 16 subjects ($P < 0.0001$, uncorrected) in bilateral anterior parahippocampal gyrus are mapped onto a template structural image of a single subject. B: Contrast of parameter estimates (±1 SE) for the magnitude of the hemodynamic response in right anterior parahippocampal gyrus for the four experimental conditions (i.e., previously seen [P] and novel [IP] non-overlapping pairs and previously seen [S] and novel [IS] overlapping pairs) compared with the cross-hair fixation baseline condition. The parameter estimates are collapsed across sessions within subjects and are averaged across subjects.

(Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003). The present study provides novel evidence that, in humans, the flexible representation of a hierarchically ordered sequence of overlapping stimulus items recruits the hippocampus. Our study design differs from the animal studies in so far as we contrasted pairs that require different degrees of transitive inference (the four pairs AC, AD, BE, CE include one end item, whereas the BD trial does not) with novel pairs of non-overlapping items (which are equivalent to the AE trials in the rat experiment). It is likely that individual trials or blocks of trials without end items would reveal even greater hippocampal activation.

A recent positron emission tomography (PET) study reported hippocampal activation during learning of overlapping pairs and subiculum activation during the recognition of novel non-overlapping pairs (Nagode and Pardo, 2002). Furthermore, previous fMRI studies of relational memory in humans have demonstrated hippocampal activation during the encoding (Rombouts et al., 1997; Sperling et al., 2001; Davachi and Wagner, 2002) and recognition (Stark and Squire, 2001) of inter-item associations. Our results extend these reports by showing that associations that require a flexible representation of a hierarchically ordered set of stimuli are associated with significantly greater hippocampal acti-

vation compared to a simple association between two items. This provides support for the notion that the hippocampus supports the encoding and retrieving of sequences of events that compose episodic memories (Eichenbaum and Cohen, 2001; Lisman, 1999).

The transitive inference trials resulted in significantly longer reaction time, but it is unlikely that simple task difficulty could explain the right anterior hippocampal activation. First, the reaction time for the previously seen overlapping pairs (condition S) was significantly longer than the one for the previously seen non-overlapping pairs (condition P), but the contrast (S-P) was not associated with any hippocampal activation. Second, conditions with shorter reaction time (P and IP) were associated with more significant medial temporal lobe activation than the conditions with longer RT (S and IS). Both observations argue against the notion that longer reaction time as an index of increased task difficulty translates into increased hippocampal activation.

While some have proposed that associative and single item recognition memory segregate along the anatomical border of hippocampus and surrounding cortices (Brown and Aggleton, 2001), others have argued that such division of labor cannot be absolute (Suzuki and Eichenbaum, 2000; Stark and Squire, 2001; Stark et al., 2002). Our study provides evidence that the parahippocampal gyrus contributes to the recognition of previously learned non-overlapping pairs of visual stimulus patterns. Previous studies have established that the parahippocampal gyrus is critical for paired-associate learning (Bunsey and Eichenbaum, 1993) and some forms of concurrent discrimination learning (Hood et al., 1999). Furthermore, it appears that the anterolateral aspects of the parahippocampal gyrus (perirhinal cortex, Brodmann areas 35 and 36) contribute to recognition memory in an automatic fashion, to allow for the association of different sensory features of an object (Murray and Bussey, 1999; Murray and Richmond, 2001). It is in this context that we interpret the greater medial temporal lobe activation centered in the parahippocampal gyrus during the recognition of previously learned non-overlapping pairs compared with the recognition of overlapping pairs. The number of pairs to be remembered and the degree of item familiarity/novelty did not differ between the two conditions, but the number of items to be remembered did (eight items in the four non-overlapping and five items in the four overlapping pairs). Despite the greater memory load, the recognition of the non-overlapping pairs resulted in significantly faster reaction time. This suggests that simple stimulus-stimulus relationships can be represented by the parahippocampal gyrus (as evidenced by the greater activation), allowing for immediate access (as evidenced by the faster reaction time).

In conclusion, we have demonstrated hippocampal activation during transitive inference in humans. Future experiments are needed to dissect the different components of brain activation during transitive inference in healthy and in patient populations (Titone et al., 2003). Such studies will provide valuable information about the functional connectivity underlying transitive inference, whereas animal experiments allow us to study the cellular and molecular mechanism in well-defined brain regions. Ultimately, experimental paradigms that can be explored in both animals and humans will allow us to uncover the mechanisms of declarative memory.

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