

## Report

# The Privileged Brain Representation of First Olfactory Associations

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## Summary

Authors [1], poets [2], and scientists [3–6] have been fascinated by the strength of childhood olfactory memories. Indeed, in long-term memory, the first odor-to-object association was stronger than subsequent associations of the same odor with other objects [7]. Here we tested the hypothesis that first odor associations enjoy a privileged brain representation. Because emotion impacts memory [8–10], we further asked whether the pleasantness of an odor would influence such a representation. On day 1, we associated the same visual objects initially with one, and subsequently with a second, set of pleasant and unpleasant olfactory and auditory stimuli. One week later, we presented the same visual objects and tested odor-associative memory concurrent with functional magnetic resonance brain imaging. We found that the power (% remembered) of early associations was enhanced when they were unpleasant, regardless of whether they were olfactory or auditory. Brain imaging, however, revealed a unique hippocampal activation for early olfactory but not auditory associations, regardless of whether they were pleasant or unpleasant. Activity within the hippocampus on day 1 predicted the olfactory but not auditory associations that would be remembered one week later. These findings confirmed the hypothesis of a privileged brain representation for first olfactory associations.

## Results

To test the hypothesis of privileged first associations, on day 1 we created two subsequent odor associations (pleasant and unpleasant, ~90 min apart) to the same visual object and tested which of the associations was remembered one week later on day 8. We interleaved an identical auditory task to control for modality and conducted all tests inside a functional magnetic resonance imaging (fMRI) scanner (see [Supplemental Data](#), Section 1, available online, and [Figure 1](#)).

### Behavioral Results Revealed a Privileged Status for First Unpleasant Associations, regardless of Modality

Behavioral results revealed that on day 1 the power of second associations ( $87.5\% \pm 2.9\%$ ) was slightly stronger than first associations ( $81.0\% \pm 3.1\%$ ;  $F_{(1,15)} = 9.2$ ,  $p < 0.0083$ ; [Figure 2A](#)). By day 8, however, visual objects were assigned  $50.5\% \pm 3.8\%$  of the time with the odorant or sound they were first associated with (one week earlier), and only  $34.5\% \pm 2.8\%$  of the time with the odorant or sound they were associated with second (one week earlier) ( $F_{(1,15)} = 6.4$ ,  $p < 0.023$ ). This preferential memory for first associations did

not differ for olfaction or audition ( $F_{(1,15)} = 0.03$ ,  $p = 0.85$ ), but reflected the valence of the first association ( $F_{(1,15)} = 5.97$ ,  $p < 0.027$ ). Specifically, when the first association was pleasant, subjects retrieved first or second associations equally (first  $44.6\% \pm 4.4\%$ , second  $40.3\% \pm 3.9\%$ ,  $t_{(15)} = 0.81$ ,  $p = 0.588$ , [Figure 2B](#)). However, when the first association was unpleasant, subjects retrieved significantly more first than second associations (first  $56.3\% \pm 4.7\%$ , second  $28.8\% \pm 3.7\%$ ,  $t_{(15)} = 3.53$ ,  $p < 0.003$ , [Figure 2C](#)). There was no significant difference between males and females or between the two odor sets used ([Figure S2](#)). In other words, first associations were privileged when they were unpleasant, and this held true in both olfaction and audition.

### Privileged Hippocampal Representation of First Olfactory Associations

To ask whether first associations were represented differently in the brain than second associations, we contrasted fMRI blood oxygenation level-dependent (BOLD) signal related to day 8 correct retrieval of day 1 first associations versus day 8 correct retrieval of day 1 second associations (collapsing across modalities and valence). Note that this contrast did not juxtapose stronger memory with weaker memory, but rather memory that was stronger for an association that occurred first versus memory that was stronger for an association that occurred second (both associations occurred one week earlier). This contrast revealed a markedly sparse overall activity pattern, with pronounced loci in the left hippocampus and right amygdala ([Figure 3A](#)), consistent with the implication of these structures in olfactory and emotional memory [11–19].

One may raise the possibility that this pattern reflected the behaviorally increased salience of first unpleasant associations regardless of modality. To address this, and to examine any modality dependence in this effect, we set out to investigate the activity time course in these regions. In order to avoid a circular analysis [20, 21], we divided the data into independent data sets, using one-third of the data to define the region of interest (ROI) and the remaining two-thirds for data analysis. Because no particular third of the data is privileged, we also repeated this three times and tested the mean.

Re-creating the statistical parametric map of day 8 correct retrieval of day 1 first associations versus day 8 correct retrieval of day 1 second associations on the basis of one-third of the data revealed the previously described hippocampal loci in all three data divisions, as well as in the mean ([Figure 3B](#)). The amygdaloid loci, however, was evident in one of the three divisions and not in the mean.

The independent time-course analysis using the remaining two-thirds of the data negated the possibility that valence underlay the results. In the left hippocampus, there was more activation when subjects retrieved the first than second association ( $F_{(1,14)} = 9.69$ ,  $p < 0.007$ ), and this effect was due to significant differences within olfactory but not auditory associations: there were significant differences between first and second pleasant ( $t_{(14)} = 2.39$ ,  $p < 0.031$ , [Figure 3C](#)) and unpleasant ( $t_{(14)} = 2.31$ ,  $p < 0.036$ , [Figure 3D](#)) odor associations, but not between first and second pleasant ( $t_{(14)} = 0.81$ ,  $p = 0.6$ , [Figure 3C](#)) or unpleasant ( $t_{(14)} = 1.09$ ,  $p = 0.29$ ) sound

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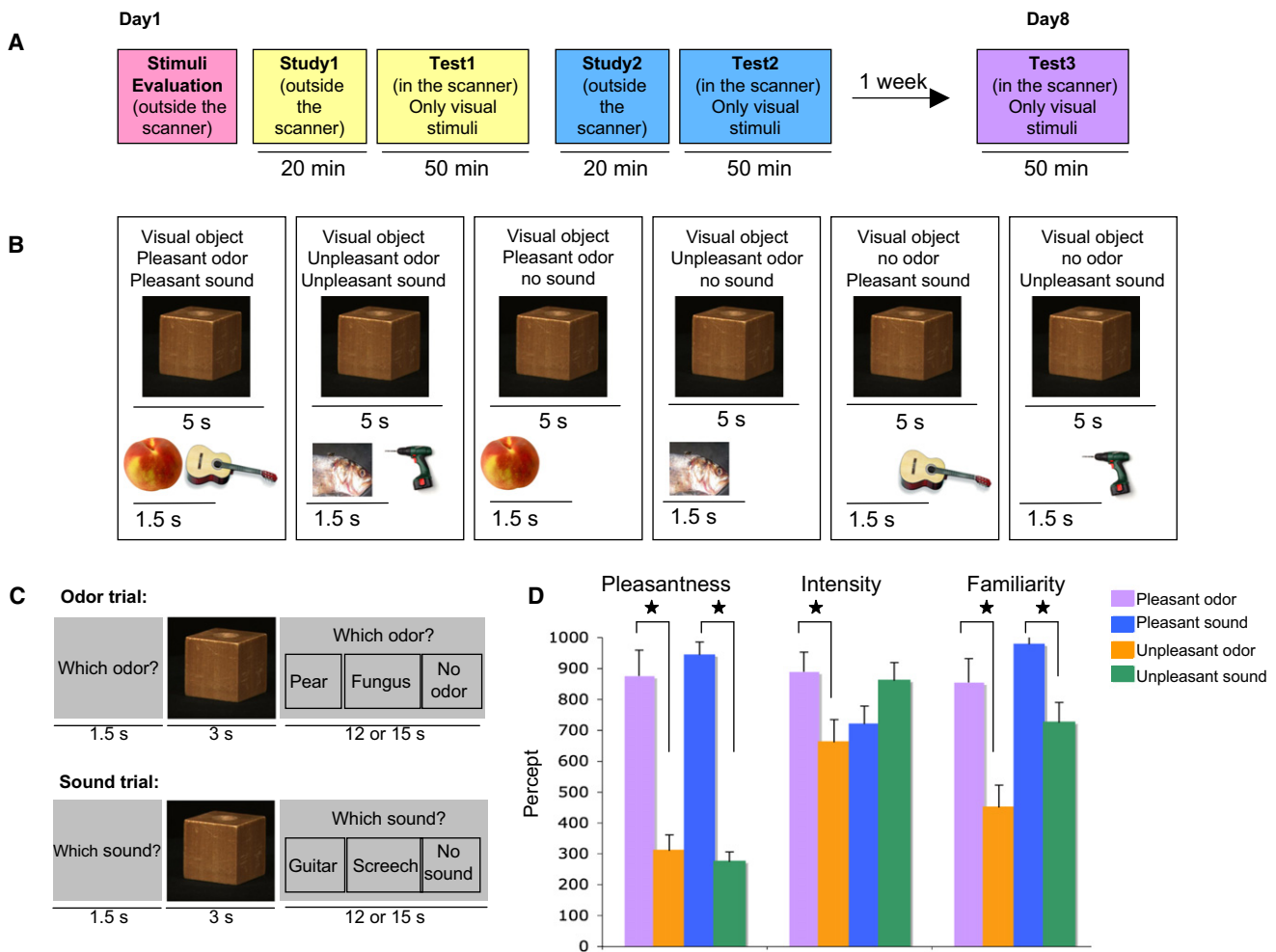


Figure 1. Experimental Design

(A) On day 1, stimuli evaluation was followed by study 1, test 1, study 2, and test 2. On day 8, 1 week later, there was a final memory test, test 3. (B) In study 1 and study 2, subjects memorized the association between a particular visual object and a particular smell and/or sound. One of the 60 visual objects was presented for 5 s, concurrently with a smell presented for 1.5 s, a sound presented for 1.5 s, or both, resulting in the six types of associations shown in (B). (C) In test 1 and test 2, a given trial started with an indicator as to whether this would be a sound trial or an odor trial. A visual object was then displayed, and three text options were presented as possible past associations. Each test contained 120 trials, 60 odor trials, and 60 sound trials, with an interstimulus interval of 15 to 18 s. In test 3, the key part of this study, was identical to tests 1 and 2, yet in terms of analysis there was no “right” or “wrong” association; there was only an order of “first” or “second” association. (D) Mean subjective ratings of odorant and sound pleasantness, familiarity, and intensity. Error bars represent the standard error of the mean (SEM).

associations (Figure 3D). In other words, there was a unique hippocampal representation for first olfactory associations only, regardless of valence. This was highlighted by the comparison that revealed greater activity for first pleasant olfactory associations than for second unpleasant olfactory associations (Tukey post-hoc test,  $p < 0.015$ ) (see Figure S1 for similar results in the amygdala).

#### Hippocampal Activity Differentiated Later Remembered from Unremembered First Olfactory Associations

The above analysis revealed a unique brain representation for first olfactory associations regardless of valence. Was this representation a reflection of a long-term process that occurred throughout the week of day 1 up to and including retrieval on day 8, or did this reflect a process that was already in place at the time of retrieval at day 1? To address this, we projected the functional ROIs obtained at test 3 back into the scans

at test 1 and 2 and asked whether activity at that time could predict which association would prevail a week later, the first or the second. In other words, we used the functional activity on day 8 to generate ROIs in which we tested activity measured separately on day 1 (details in Supplemental Data, Section 2).

We found that already at day 1, there was higher activation during retrieval episodes for first odor associations than retrieval episodes for second odor associations ( $t_{(14)} = 2.21$ ,  $p < 0.044$ ), and activation for first odor, but not sound, associations could predict whether associations would be retrieved one week later. This effect, however, was significant only for pleasant odor associations in the hippocampal ROI ( $t_{(14)} = 2.3$ ,  $p < 0.037$ ) and for unpleasant odors in the amygdala ROI ( $t_{(14)} = 2.5$ ,  $p < 0.025$ ). To determine whether this difference between the hippocampus and amygdala reflected a genuine difference in functionality between these regions, or in turn a restricted depiction of function as a reflection of the ROI

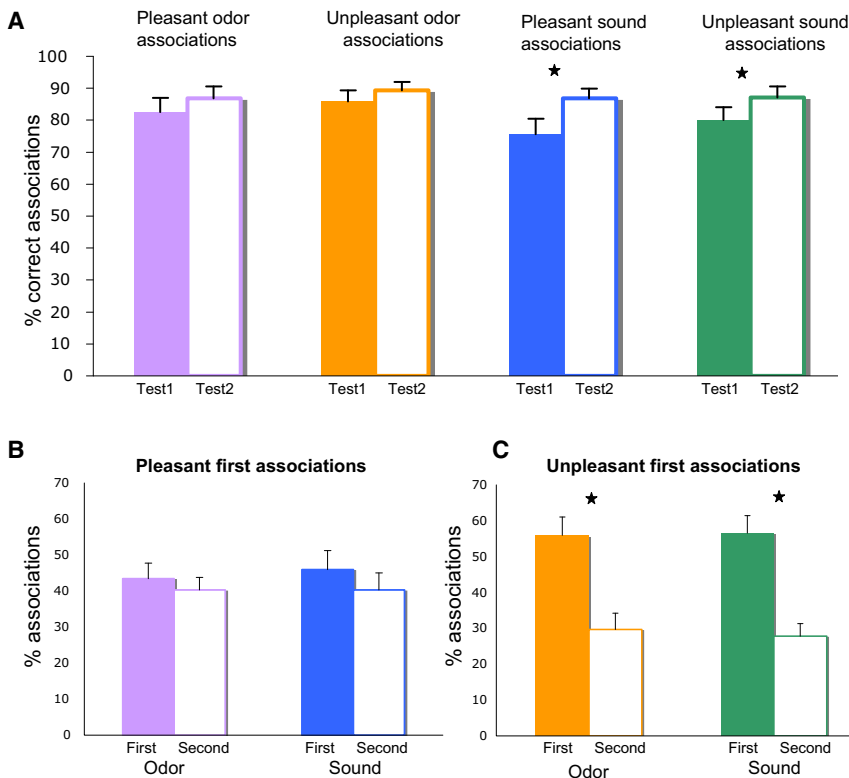


Figure 2. Better Memory for First Unpleasant Associations

(A) Proportion of first and second pleasant odor and unpleasant odor and sound associations correctly remembered on day 1. Memory accuracy was slightly but significantly better for second sound associations than for first sound associations.

(B) Proportion of first and second pleasant odor and sound associations remembered on day 8. There was no difference in the proportion of first and second pleasant associations retrieved. The left bars show the value for a pleasant odor when it was presented first compared to when it was presented second.

(C) Proportion of first and second unpleasant odor and sound associations remembered on day 8. Significantly more first than second unpleasant associations were retrieved. The left bar shows the value for an unpleasant odor when it was presented first compared to when it was presented second.

Error bars represent the SEM.

that was back-projected from test 3 activity, we conducted a contrast of test 1 activity for first associations later remembered at test 3 versus those not later remembered ( $p < 0.01$  uncorrected). This contrast revealed several ROIs (Table S1), including a left hippocampal region extending posterior to the region previously extracted from day 8 activity (Figure 4A). An examination of activity in this region, which used independent data sets again with one-third of the data for ROI generation and the remaining two-thirds for analysis, revealed activity patterns that were predictive for both pleasant and unpleasant olfactory associations ( $F_{(1,14)} = 14.6$ ,  $p < 0.002$ , Figure 4B). In other words, by examining activity in this region during the first retrieval session of day 1, we could predict whether an olfactory association would be remembered one week later. This was not the case for auditory associations, nor for the second retrieval session of day 1. In sum, there was a privileged brain representation for first olfactory associations. This representation was independent of stimulus valence, was not evident in audition, and was predictive of memory performance one week later.

## Discussion

The behavioral results indicated that the first association, whether olfactory or auditory, was stronger only if it was unpleasant. To the best of our knowledge, this robust (Figure 2C) behavioral effect has not been previously reported for long-term memory. Behavioral salience of first unpleasant over pleasant associations may represent a potential adaptive mechanism considering the potential cost of failing to learn a first negative association and the potential benefit of a malleable first positive association.

That unpleasant associations took precedence over pleasant associations is consistent with the robustness of aversive conditioning in protocols such as fear conditioning and

conditioned taste aversion (CTA) [22]. In conditioning to avoid closely spaced tastes in the CTA paradigm, it is, however, the last taste that becomes preferably associated with subsequent malaise [23], yet in our long-term-memory paradigm an unpleasant

association obtained salience in memory only if it was the first association (second unpleasant association did not take precedence). Thus, it is not only the valence but also the primacy that gives unpleasant associations in our paradigm their long-term-memory salience.

Our behavioral data did not indicate privileged status for either sensory modality, yet the brain data did. Hence a unique brain representation was evident for first olfactory associations, regardless of their valence (Figures 3 and 4). This unique pattern was already evident at initial retrieval on day 1, where it predicted retrieval one week later, and was evident at retrieval on day 8, where it predicted which association was first one week earlier. Critically, this brain representation was not evident for second olfactory associations, even if they were those to be later remembered, nor was it evident for auditory associations. A unique brain representation for first olfactory associations is consistent with experience, as described in both the scientific [3–6, 24, 25] and the nonscientific literature, most notably by Marcel Proust in *Remembrance of Things Past* [1], where the taste of a madeleine (and its inevitable retronasal smell [26]) instantly revived the childhood memory of its encounter. Whereas in the latter renowned example it was the smell that triggered a visual image, here we probed the inverse situation, whereby the visual image triggered the memory of a smell. That said, we submit that these are two sides of the same coin, reflecting the unique neural representation of the initial pairing between an odor and experience.

Collapsing across modalities and valence, we found differences between first and second associations. In behavior, these differences reflected valence, and in brain activity these differences reflected modality. On the one hand, we observed a behavioral effect (privileged first unpleasant associations) that was not manifested in our brain imaging results. This, however, reflected the omnibus contrast we conducted. Had

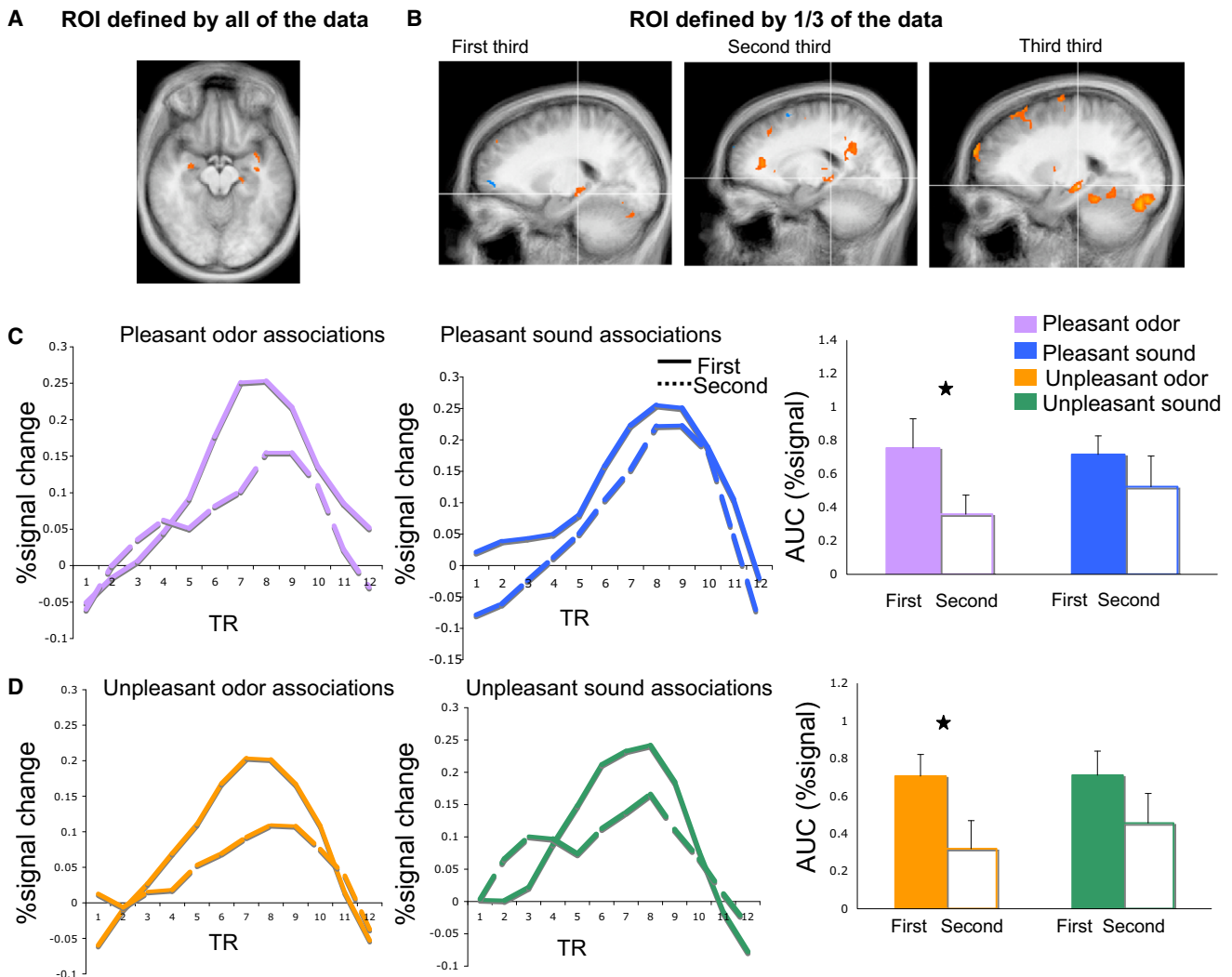


Figure 3. Increased Hippocampal Activity for First Odor Associations

(A) The contrast of day 8 remembered first association versus day 8 remembered second association (collapsing across valences and modalities) revealed sparse activation, with significant activity in the left hippocampus and right amygdala.  
 (B) The contrast of day 8 remembered first association versus day 8 remembered second association (collapsing across valences and modalities) revealed sparse activation, with significant activity in the left hippocampus in each of the three subdivisions of the data.  
 (C) Average data, across all three data divisions, extracted from left hippocampus. Percent signal change and area under the curve for pleasant first and second odor and sound associations are shown. Significant increased activity for first versus second pleasant odor associations was evident.  
 (D) Percent signal change and area under the curve for unpleasant first and second odor and sound associations. Significant increased activity for first versus second unpleasant odor associations was observed.  
 Error bars represent the SEM.

we generated specific contrasts aimed at revealing pleasant versus unpleasant associations, we expect to have uncovered the neural substrate previously implicated in this process [27–29]. On the other hand, we observed a brain imaging effect (privileged first olfactory associations) that was not manifested in our behavioral results. This privileged representation that was not uncovered by simple behavior was a key finding of this study, yet this is not to say that behavioral tests that go beyond testing performance accuracy alone would fail to uncover such differences.

The current study joins several others in which event-related activity in hippocampal and parahippocampal areas predicted later memory [30–33]. However, here we measured activity during episodes of successful initial retrieval (and not encoding) and used it to predict retrieval at a later time. In other

words, we observed unique brain activation during successful retrieval of an item that will be remembered one week later as compared to activation during equally successful retrieval of an item that will be forgotten.

Although our result supported our hypothesis, we would like to clearly state its limitations. First, it is possible that a subject's strategy for learning the second association would be to remember that it is "not the first association," and it is possible that this is why first associations were retrieved more often than second associations on day 8. If that was the case, however, we would expect this to hold true both for pleasant and unpleasant associations. In contrast to this expectation, only unpleasant first associations were retrieved more often. We deem it unlikely that such a strategy would have been applied in a valence-specific manner. Second, studying long-term

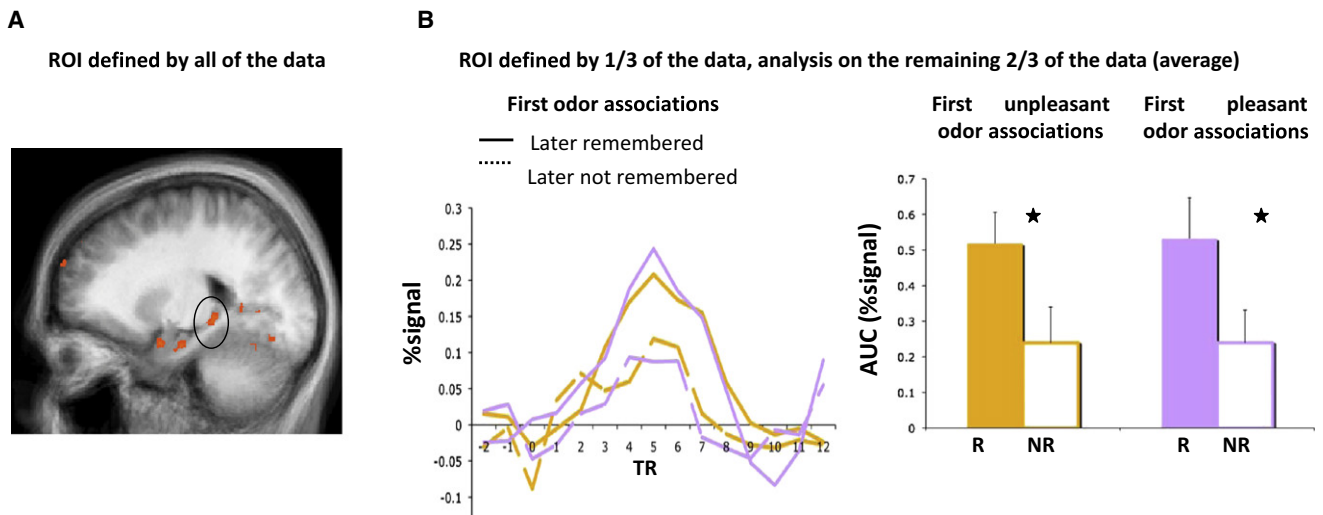


Figure 4. Hippocampal Activity Predictive of Memory One Week Later

(A) The contrast of first olfactory association remembered one week later versus first olfactory association not remembered one week later revealed significant activity in several regions (Table S1) including the left hippocampus.

(B) Average data across all three data divisions, extracted from left hippocampus. Percent signal change and area under the curve for pleasant first and second odor associations are shown. Significant increased activity for first remembered (R) one week later versus first not remembered (NR) one week later odor association is shown. Error bars represent the SEM.

memory in a laboratory setting has its limitations, and one often has to choose between a more naturalistic versus a more controlled setting [34]. In opting for the former, Herz et al. probed subjects' biographies in order to present them with the scent and image (picture of the bottle) of a perfume that elicited a specific, pleasant, personal memory and, as a control, the scent and image of a perfume that did not elicit an emotional memory [35]. In opting for the latter, we gained precise control over the initial odor exposure, but limited the naturalistic nature of our setting. Nevertheless, our findings dovetailed with Herz et al. in uncovering an amygdala and hippocampal representation for specific odor memories.

To conclude, our findings of a unique brain representation for first odor associations allows us to dub olfaction as a "sense of first impressions." We hypothesize that activity in a network that includes the amygdala and hippocampus together underlies the observations that were the impetus for this study, namely, the phenomenon wherein a memory evoked by an odor originates from early childhood, given that it was in early childhood that one first associated between a specific odorant and experience.

#### Experimental Procedures

Detailed procedures are in the [Supplemental Experimental Procedures](#) (sections 1 and 3). In brief, 16 healthy right-handed normosmics (8 women), aged 24 to 36, participated after providing informed consent. Out of the scanner, odorants were delivered by olfactometer [36] sounds [37, 38] through earphones, and 60 emotionally neutral object photos [39] (Figure S3) were presented on a computer monitor. In the scanner, photos were back-projected into the scanner bore. The experimental timeline is in Figure 1 and in more detail in the [Supplemental Data](#) (section 1). All the raw MR data is publicly available for download at <http://www.weizmann.ac.il/neurobiology/worg/materials.html> and the MR analysis scheme is detailed in the [Supplemental Data](#) (section 3).

#### Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, three figures, and two tables and can be found with this article online at [http://www.cell.com/current-biology/supplemental/S0960-9822\(09\)01857-0](http://www.cell.com/current-biology/supplemental/S0960-9822(09)01857-0).

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