A category-specific response to animals in the right human amygdala

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The amygdala is important in emotion, but it remains unknown whether it is specialized for certain stimulus categories. We analyzed responses recorded from 489 single neurons in the amygdalae of 41 neurosurgical patients and found a categorical selectivity for pictures of animals in the right amygdala. This selectivity appeared to be independent of emotional valence or arousal and may reflect the importance that animals held throughout our evolutionary past.

The amygdala is involved in processing both aversive and appetitive stimuli[1,2]. Earlier notions that the amygdala might be specialized to mediate fear responses have been supplemented by accounts in which the amygdala processes more abstract attributes, such as stimulus unpredictability[3]. Electrophysiological recordings in monkeys have found single neurons that respond to faces[4,5], as well as to the reward value of conditioned and unconditioned stimuli[6,7]. In humans, neurons have been reported that are selective for a variety of visual stimuli[8], and neuroimaging studies of the amygdala argue for a broad role in processing stimuli that are strongly rewarding or punishing[9]. This diversity of findings has left it unclear exactly what stimulus categories or dimensions the amygdala might process.

To test whether neurons in the human amygdala have preferential responses to particular stimulus categories, we recorded from the medial temporal lobe (MTL) in 41 neurosurgical patients undergoing epilepsy monitoring. Informed written consent was obtained from each subject. Subjects were sitting in bed while they viewed approximately 100 images per session on an LCD monitor (1 s each with six repetitions in pseudorandom order as described previously[10]). Stimulus sets contained images of persons, animals, landmarks or objects. During 111 experimental sessions, we recorded from a total of 1,445 single neurons in the amygdala (489 neurons), hippocampus (549 neurons) and entorhinal cortex (407 neurons) (**Supplementary Fig. 1**). Of these, 183 single units (57 in amygdala, 86 in hippocampus and 40 in entorhinal cortex) responded significantly to one or more of the presented stimuli (see below).

Neurons in the amygdala responded preferentially to pictures of animals rather than to pictures of other stimulus categories (**Supplementary Figs. 2** and **3**). To statistically compare neuronal selectivity across regions and categories, we calculated the probability that any neuron from a given MTL region responds to any stimulus from a given category. Comparison of response probabilities for different stimulus categories in the three MTL regions showed a highly significant selectivity ($P < 10^{-15}$) in the responses of amygdala neurons for animal images, whereas responsiveness in hippocampus exhibited no significant difference ($P = 0.9$) between any stimulus categories and entorhinal cortex showed a significantly decreased ($P < 0.03$) response to persons compared with other categories (**Fig. 1a**). For all of the neurons that showed a significant response to at least one stimulus in the entire set, we compared the average response magnitudes to each of the four stimulus categories; this analysis confirmed selectivity for animals in the amygdala ($P < 10^{-5}$)
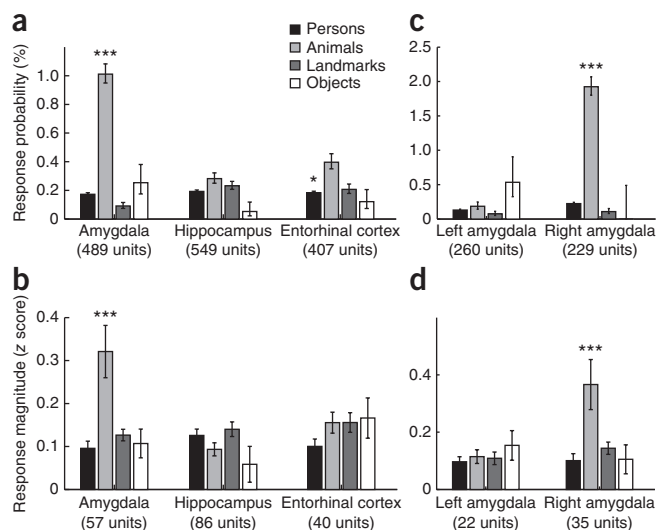

Figure 1 Amygdala neurons respond preferentially to animal pictures. (**a**) Response probabilities of neurons in different MTL regions to different stimulus categories revealed significant preferences in the amygdala ($P < 10^{-15}$, main effect of increased responses to animals at ~1%) and entorhinal cortex ($P < 0.03$, main effect of decreased responses to persons), but not in the hippocampus. (**b**) Mean response magnitudes of all responsive neurons showed increased response activity of amygdala neurons to animals ($P < 10^{-5}$). (**c**,**d**) The animal preference in both response probability and magnitude was seen only in the right amygdala ($P < 10^{-15}$ and $P < 0.0005$, respectively). Error bars denote binomial 68% confidence intervals (**a**,**b**) and s.e.m. (**c**,**d**). $*P < 0.05$, $***P < 0.001$.

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BRIEF COMMUNICATIONS

Figure 2 A specific category response to animals in the right amygdala at the population level. (**a**) For a set of 201 amygdala units (96 left, 105 right) that were all presented with the same 57 stimuli (23 persons, 16 animals, 18 landmarks), we constructed representational dissimilarity matrices by determining the dissimilarity in evoked response patterns for each pair of stimuli (as $1 - r$ from the Pearson correlation across units). (**b**) Hierarchical cluster analysis automatically grouped stimuli with similar response patterns together into clusters. In the right amygdala, this unsupervised procedure yielded a cluster that contained all animal stimuli, whereas no such category effect was found in the left amygdala.
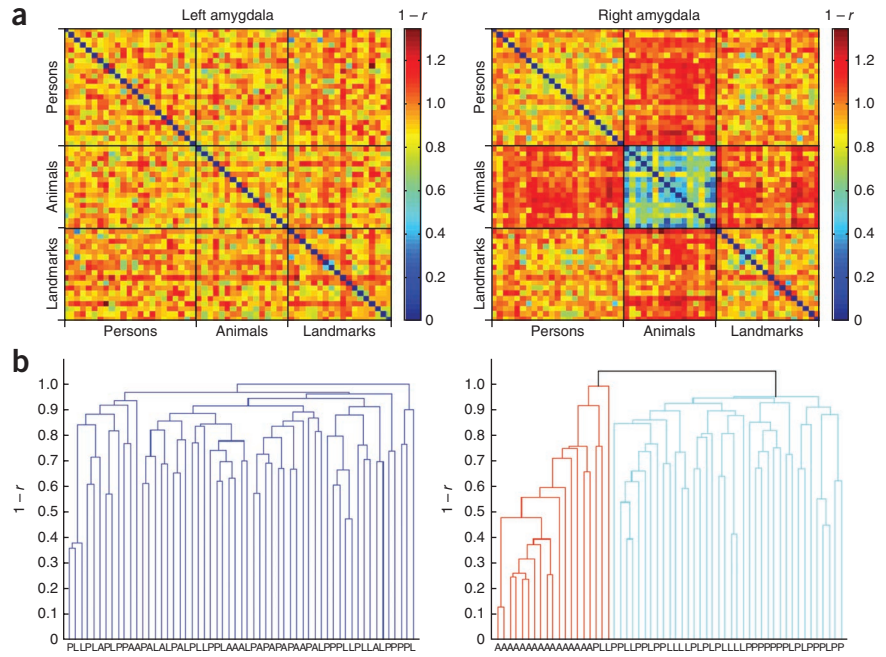
and revealed that there was no significant preference ($P > 0.05$) in the other two regions (**Fig. 1b**). To test for laterality effects, we repeated the above analysis separately for the left and right amygdala. We found that neuronal responses selective for animals arose exclusively from the right amygdala (**Fig. 1c,d**). This pattern of results remained unchanged when we included multi-unit data in the analysis (**Supplementary Figs. 4** and **5**; also see **Supplementary Results**).

To test whether specific response patterns of amygdala neurons to animals are also present at the population level, we analyzed how images are segregated by response patterns using a categorization technique that has been applied to neurons in monkey inferotemporal cortex[11]. To apply this technique, we used an objective algorithm to select the largest subset of units from a total of 1,239 single and multi-units in the amygdala that had all been presented with the largest subset of stimuli. This subset consisted of 201 amygdala units (96 left, 105 right) that had all been presented with an identical subset of 57 stimuli from three different categories (**Supplementary Fig. 6**; see **Supplementary Methods** for details about the selection procedure). Representational dissimilarity matrices reflecting the dissimilarity in response between all pairs of these 57 stimuli revealed a specific response pattern to animals in the right amygdala that differed from the response patterns to persons and landmarks (**Fig. 2a** and **Supplementary Fig. 7a**). Hierarchical clustering analysis, which groups the stimuli objectively based on similar response patterns, further confirmed that animals and non-animals formed distinguishable clusters in the population code of the right, but not left, amygdala (**Fig. 2b** and **Supplementary Fig. 7b**). This pattern of results remained unchanged when we restricted the analysis to single units (data not shown), and no such category effects were found in the hippocampus or entorhinal cortex (**Supplementary Fig. 8**).

The mean response latency of amygdala units that responded to animal pictures (324 ms) was significantly shorter ($P = 0.006$) than the latency to stimuli from all other categories (398 ms; **Supplementary Fig. 9**). This effect was statistically significant in the amygdala, but not in the hippocampus or entorhinal cortex. Although this expedited processing of animal pictures may reflect their biological importance and category selectivity, the observed amygdala latencies are nevertheless similar to those found in other regions in the temporal lobe[10], and thus seem more likely to be generated along the cortical object recognition pathway than via a rapid subcortical route[12].

Previous studies have implicated the human amygdala in fear- and threat-related processing[9]. The animal images that elicited neuronal responses in the amygdala contained both aversive and cute animals, and we found no relationship between amygdala responses and either the valence or arousal of the animal stimuli (**Supplementary Fig. 10**). Furthermore, we reproduced an animal versus non-animal category effect in the right amygdala in an additional functional magnetic resonance imaging (fMRI) control experiment using stimuli that were controlled for emotional valence and arousal (**Supplementary Fig. 11**). In addition, we ruled out an influence of confounding stimulus features (**Supplementary Control Analyses** and **Supplementary Fig. 12**) such as sharpness of contours that have been shown to affect amygdala activity[13].

Taken together, our results demonstrate that the right amygdala is specialized for processing visual information about animals. The selectivity appears to be truly categorical and argues in favor of a domain-specific mechanism for processing this biologically important class of stimuli. A plausible evolutionary explanation is that the phylogenetic importance of animals, which could represent either predators or prey, has resulted in neural adaptations for the dedicated processing of these biologically salient stimuli. This idea is consistent with recent findings that animals can be detected preferentially in change-blindness tasks[14]. The right-lateralized effect that we found is consistent with previous findings that support the notion that, early on in vertebrate evolution, the right hemisphere became specialized for detecting and responding to unexpected and behaviorally relevant stimuli[15]. In the future, it will be important to replicate our finding in species other than humans and to investigate the mechanisms that generate this functional laterality (see **Supplementary Discussion**). It will be particularly interesting to see whether such a hemispheric asymmetry can also be found at earlier stages of the cortical object recognition pathway that constitutes the visual input to the amygdala.

Note: Supplementary information is available on the Nature Neuroscience website.

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Supplementary Information

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Supplementary Methods

Electrophysiological Recordings

All studies conformed to the guidelines of the Medical Institutional Review Board at UCLA. Electrode locations were based exclusively on clinical criteria and were verified by MRI or by computer tomography co-registered to preoperative MRI. Based on MRI analysis and stereotaxic electrode placement, we estimate that most of the neurons recorded in the amygdala were located in the basolateral nuclear complex. Each electrode probe had nine micro-wires protruding from its tip, eight high-impedance recording channels (typically 200-400 kΩ) and one low-impedance reference with stripped insulation. The differential signal from the micro-wires was amplified using a 64-channel Neuralynx system, filtered between 1 and 9000 Hz, and sampled at 28 kHz. Spike detection, and sorting was performed after band-pass filtering the signals between 300 and 3000 Hz[1]. Sorted units were classified as single units (SU), multi-units (MU), or artifacts based on spike shape and variance, ratio between spike peak value and noise level, the inter-spike interval distribution of each cluster, and presence of a refractory period for the single units[2].

We recorded from MTL neurons in 41 patients with pharmacologically intractable epilepsy (33 right handed; 23 male; 18–54 years old), implanted with chronic electrodes to localize the seizure focus for possible surgical resection[3]. Our original data set comprised 119 sessions in which we recorded from micro-wires in the amygdala, hippocampus, and entorhinal cortex. 8 sessions which yielded no units in any of these three regions were excluded. The remaining

111 sessions showed a variable yield of units (**Supplementary Fig. 1a**). The median number of sessions per patient was 2 (range 1 to 7). Each session lasted approximately 30 min. Subjects were sitting in bed, facing a laptop computer on which pictures of persons, animals, landmarks, or objects were shown. A median number of 99 (range 60 to 202) different images per session were shown, centered on a laptop screen and covering about 1.5°, and displayed six times each for 1 s in pseudorandom order. After image offset, subjects had to indicate whether the picture contained a human face or something else by pressing the 'Y' and 'N' keys, respectively. This simple task, on which performance was virtually flawless, required them to attend to the pictures. Every stimulus presentation was preceded by a fixation cross for 500 ms to assess baseline firing activity. In a slight variant of the paradigm (23 of 111 sessions), images were presented for 500 ms (20 sessions) or 750 ms (3 sessions), and the attention task was omitted. Since the different types of sessions were previously shown to exhibit the same neuronal response dynamics[4], we included all in our analyses. For *post hoc* verification we repeated every analysis of the electrophysiological data after removing these sessions and confirmed that this did not alter any of the reported findings.

Stimuli consisted of images of persons (grand average 72%), animals (10%), landmarks (15%), and objects (3%) that had been downloaded from the internet. The composition of the stimulus set was tailored to the individual patient and thus varied across sessions (**Supplementary Fig. 1b**). The person category mostly contained images of celebrities that were familiar to the patients, such as actors, sport stars, politicians etc. These typically had neutral or friendly facial expressions. Landmarks included famous or familiar as well as unknown buildings and landscapes. The animal category generally contained pictures of random animals that the subjects did not know personally. Objects included food items, vehicles, tools and others. Throughout the course of the 111 experimental sessions, we used a total of 1183 different stimuli (933 images of persons, 68 images of animals, 104 images of landmarks, and 78 images of objects).

We recorded from a total of 3598 neurons (2153 MU / 1445 SU) in the amygdala (1239 units; 750 MU / 489 SU), hippocampus (1239 units; 690 MU / 549 SU) and entorhinal cortex (962

units; 555 MU / 407 SU) (**Supplementary Fig. 1a**). Of these, 460 units (277 MU / 183 SU) responded significantly to one or more of the presented stimuli (131 amygdala units [74 MU / 57 SU], 218 hippocampal units [132 MU / 86 SU], 111 entorhinal units [71 MU / 40 SU]).

Analysis of Electrophysiological Data

To determine whether a unit responded to one or more of the presented stimuli, we used the response criterion described in previous work[4]. We divided the 1000 ms after stimulus onset into 19 overlapping 100 ms bins, and for each bin we compared the spike rates for the 6 presentations of each stimulus to the baseline intervals of 500 ms before all the stimulus onsets in a session (approx. 100 x 6) by means of a two-tailed Mann-Whitney U test, using the Simes procedure[5] to correct for multiple comparisons and applying a conservative significance threshold of P=0.001 to reduce false positive detections.

Over all 111 sessions, we counted the number of instances when a neuron from a given MTL region r showed a significant response to an image from a given stimulus category c and divided this number by the total number of instances when an image from that category was presented to a neuron from that region. The resulting response probabilities $RP_{r,c}$ are based on the cumulative exposure of neurons in different regions r to images from different stimulus categories c and can therefore be compared across regions and categories.

$$RP_{r,c} = \frac{\sum_{s=1}^{111} responses_{s,r,c}}{\sum_{s=1}^{111} units_{s,r} \cdot stimuli_{s,c}}$$

E.g., if 10% of amygdala neurons responded to 10% of all animal pictures in each session, the resulting response percentage would be 1%.

To test for preferential responses to any of the four stimulus categories in each of the three MTL regions analyzed in our 41 patients, we applied the Mantel-Haenszel chi square test, a generalized version of Pearson's chi square test for analysis of 2 x 4 x 41 contingency tables, stratified for different subjects.[6,7]

In addition, we compared the average response magnitudes between the four stimulus categories for every neuron that showed a significant response to any stimulus at all. For each of these 460 units (277 MU, 183 SU), we calculated the normalized response activity to each of the ca. 100 presented stimuli as a z-score by subtracting the mean baseline activity (during the 500 ms fixation interval before each stimulus presentation) across all stimulus presentations from the average response activity during the 6 presentations (1000 ms each) of a particular stimulus and dividing this difference by the standard deviation of response activities across the ca. 100 stimuli. We then averaged over the stimuli from each category to obtain the response magnitude to each category for each neuron and statistically compared these using a repeated-measures ANOVA (analysis of variance) for each MTL region.

To analyze population response patterns to different stimuli in the amygdala on the basis of their representational similarity[8,9], we first used an automated, objective algorithm to select an optimal set of stimuli that were presented to a larger number of units (MU and SU) from different patients. This was necessary since some of our stimulus material varied across sessions and patients. To determine the maximal set of amygdala units and stimuli presented to them, we used a greedy algorithm. We first selected the complete picture set from the session with the highest yield of amygdala units. We then added the session whose picture set produced the largest intersection of stimulus sets, replaced our previous stimulus set with this intersection, and repeated this step until all 111 sessions were included in the intersection set. During each step we recorded the number of stimuli in the intersection set and the cumulative number of units across sessions that these stimuli were presented to. After plotting the number of stimuli against the cumulative number of units, we selected an operating point on this curve by maximizing the product of units times stimuli (**Supplementary Fig. 6a**), resulting in a set of 201 units (96 in the left, 105 in the right amygdala) that were all presented with the same 57 stimuli (23 persons, 16 animals, 18 landmarks, see **Supplementary Fig. 6b**). No additional selection criterion was applied. In particular, units were not selected based on their responsiveness as in the previous analysis. The units determined by this procedure had been recorded from 8 patients during 10 experimental sessions.

Representational dissimilarity matrices for the left and right amygdala were generated by determining for each pair of stimuli the dissimilarity between the associated response patterns[8,9]. Dissimilarity was quantified as 1 minus the linear correlation of activity across units. Response activity for each stimulus was averaged across the 6 presentations (1000 ms after stimulus onset) and then z-score transformed based on the mean and standard deviation across all baseline intervals (i.e., 500 ms before stimulus onset) in a session.

The dissimilarity values for the 57 stimuli were then subjected to a hierarchical clustering analysis[10]. This analysis proceeds from single-stimulus clusters and successively combines the two clusters closest to each other in terms of the average response-pattern dissimilarity, so as to form a hierarchy of clusters that can be displayed as a dendrogram. The vertical height of each horizontal link in our dendrograms indicates the average response-pattern dissimilarity (1-r) between the stimuli of the two linked sub-clusters ('group average linkage algorithm'). To evaluate whether population responses indicate category preferences in other MTL regions, we generated representational dissimilarity matrices for the entorhinal cortex and hippocampus in the same way as described for the amygdala (**Supplementary Fig. 8**).

The latencies of amygdala units (MU and SU) responding to animal or non-animal pictures were calculated using Poisson spike train analysis as described in our earlier work[4]. For this procedure, the interspike intervals (ISIs) of a given unit are processed continuously over the entire recording session, and the onset of a spike train is detected based on its deviation from a baseline exponential distribution of ISIs. For each response-eliciting stimulus, we determined the time between stimulus onset and the onset of the first spike train in all six image presentations. Only spike train onsets within the first 1000 ms after stimulus onset were considered. The median length of these six time intervals was taken as response latency. For sparsely firing units with mean baseline firing activity of <2 Hz, Poisson spike train analysis generally failed to pick up a reliable onset; thus, we used the median latency of the first spike during stimulus presentation instead. For units responding to more than one stimulus, the median of the different stimulus latencies was taken.

The 23 animal pictures that were found to elicit amygdala responses in any of the 111 analyzed sessions (**Supplementary Fig. 10a**) were rated for emotional valence and arousal by 14 control subjects (**Supplementary Fig. 10b**). To test for a relationship between the response percentages and the affective ratings of these pictures, we applied various statistical tests, including t-test, Mann-Whitney-U test, and Pearson's and Spearman's correlation test. To test furthermore for an influence of emotional saliency (i.e., both positive and negative valence), we transformed valence ratings to a scale centered around zero and took absolute values before rerunning the statistical tests.

Supplementary Results

Amygdala neurons responded more frequently and, on average, more strongly to animal stimuli than to stimuli from the other tested categories (**Fig. 1, Supplementary Fig. 4**). In principle, this preferential response could be caused by at least two different effects. On the one hand, the number of units that respond to animals could be particularly high. On the other hand, those units that respond to animals could do so with lower within-category selectivity, i.e., they could respond to a higher portion of animal stimuli than what is observed for units responding to other categories. In our data we found a combination of both effects.

Of the 131 responsive units (MU and SU) in the amygdala (61 left, 70 right), 32 units (7 left, 25 right) showed significant responses to a total of 93 presented animal stimuli while 102 units (55 left, 47 right) responded to a total 190 presented stimuli from other categories. Thus, although animal pictures constituted only 10% of the stimulus material, 24% of all responsive amygdala units responded to animals ($P<0.00001$; binomial test). Furthermore, amygdala units that responded to animals responded on average to 2.9 pictures (corresponding to an average of 31% of the presented animal pictures), whereas units that responded to any of the other categories responded to an average of 1.9 stimuli (corresponding to an average of 7% of the pictures from the respective non-animal category) ($P<10^{-9}$; Mann-Whitney U test; see also **Supplementary Fig. 5**). Only 3 amygdala units responded both to animals and non-animals.

After excluding 74 multi-units from the analysis, of the remaining 57 responsive single amygdala units 17 units (4 left, 13 right, recorded from 9 different subjects) responsive to animals were found to respond to an average of 3.1 pictures, while 41 units responsive to other categories (18 left, 23 right) responded to an average of 1.7 stimuli ($P<10^{-7}$; Mann-Whitney U test).

To verify statistically that the observed category preference could not have arisen from a random noisy population of neurons, we used a bootstrapping procedure. For each of the 131 responsive amygdala units, we generated an ROC (Receiver Operating Characteristics) curve by varying the response threshold and plotting true positive detections (animal stimuli with response firing rates above threshold) against false positive detections (non-animal stimuli with response firing rates above threshold). The area under this curve (AUC) was used as a selectivity index for each neuron. From these indices, we generated the empirical cumulative distribution function along with 95% confidence intervals using the Kaplan-Meier estimator and Greenwood's formula. A chance distribution was calculated from 1000 surrogate realizations of a stimulus set with randomly relabeled categories. Comparison of the two distributions showed that significantly more units had a high selectivity index than expected by chance (**Supplementary Fig. 3**).

The laterality in responsiveness to animals between the left and right amygdala was statistically significant both in terms of response probability and response magnitude. For the response magnitude we performed a repeated measures two-way ANOVA with category (animals / non-animals) as within-sample factor and hemisphere (left / right) as inter-sample factor that yielded a significant interaction between hemisphere and category ($P<0.016$). Posthoc testing confirmed that the response magnitude to animals in the right amygdala was indeed significantly higher than in the left amygdala ($P<0.01$; t-test; cf. **Fig. 1d**) . Since the Mantel-Haenszel test is not designed for more than one dependent variable, we performed a direct comparison of response probabilities to animals between left and right amygdala neurons based on 2-by-2 contingency tables which yielded a significantly higher response probability in the right amygdala ($P<10^{-9}$; Mantel-Haenszel chi square test; cf. **Fig. 1c**).

Supplementary Control Analyses

Influence of epileptic focus and handedness on laterality

To rule out an effect of the underlying epileptic process in these recordings, we repeated the entire analysis after excluding data from the hemisphere that was found to contain the seizure-generating area. Patients with unclear focus lateralization or bilateral epileptic foci were entirely excluded from this analysis. Results for this 'non-epileptic' data set confirmed a significant category effect in the right ($P<0.001$ both for response probability and response magnitude), but not left amygdala.

To test for an effect of handedness on the observed laterality, we performed the Mantel-Haenszel test separately for the 33 right-handed and the 8 left-handed patients. Both groups showed a significant category effect for response probability to animals in the right, but not in the left amygdala ($P<10^{-10}$ and $P=0.01$, respectively), indicating that the effect we report is not driven by the handedness of the participants.

Potentially confounding stimulus features

To control for potential confounding effects, we evaluated several additional factors in our stimulus set. Previous studies have described distinct category-selective regions for faces and bodies in the human occipito-temporal pathway[11]. Since the animals in our stimulus set were usually depicted with body parts whereas many of the persons were shown as face only, we tested whether the difference in responses to animals and persons was actually a difference in response to faces versus bodies by contrasting the response probability for persons with or without body parts but found no significant effect ($P=0.95$). Secondly, we tested for effects of familiarity of the stimuli since the animals were not personally known to the subjects whereas 95% of the persons were familiar. In agreement with previous findings[12], responsiveness to unknown and familiar persons did not differ statistically in the amygdala ($P=0.98$). We also evaluated food items as a subcategory of objects potentially related to reward-processing, but found no significant preference of amygdala neurons for this stimulus category as compared to persons or landmarks ($P=0.47$).

As a fourth potential confound, we investigated whether previous fMRI findings that the human amygdala responds more strongly to objects with sharp/pointed contours than with smooth contours[13] might bear an impact on our results. Since sharp and pointed contours are reflected by high spatial frequencies (smoothing a picture essentially eliminates these high spectral frequencies), we tested whether the preference for animal stimuli may be due to higher spectral power in the high frequencies of these pictures. For this purpose we calculated the spatial power spectrum for every presented image and compared these spectra for animals and non-animals. Neither for the stimuli used in the electrophysiology experiments nor for the IAPS pictures[14] used in **Supplementary Fig. 11** did we find a significant difference between stimulus categories in spectral power at higher frequencies.

Effects of habituation

In functional imaging studies, amygdala activation has been shown to habituate during repeated presentation of the same type of stimuli. In our electrophysiology study, such a habituation could weaken responses to persons (which make up 72% of the stimulus material) more strongly than responses to animals (which make up only 10% of the stimulus material). To test for habituation, we investigated whether the response to a given stimulus category depends on the temporal saliency of a presentation, i.e., on the time passed since the last presentation of a stimulus from the same category. For this aim we evaluated the order of presentations in each of the 111 experimental sessions. For all the 131 responsive amygdala units we then plotted the normalized response activity (relative to baseline) during each stimulus presentation against the distance from the previous stimulus from the same category (e.g., this distance would be 1 for 2 consecutive stimuli from the same category, or 7 in the case of 6 interjacent presentations of stimuli from other categories). The presence of habituation would be reflected by a significant positive correlation between response magnitude and distance from previous stimulus of the same category. Nonparametric correlation analysis (Spearman's rho) showed no significant correlation for any of the four stimulus categories (**Supplementary Fig. 12**), indicating no within-category habituation at the neuron level.

Supplementary Discussion

Comparison to findings from non-human primates

The finding of a neuronal response preference for animals over people in the human amygdala is remarkable given that the monkey amygdala responds to faces of both humans and monkeys. Most electrophysiology studies in macaques, however, have not explicitly tested responses to animals and generally report a much greater proportion of cells that are selective for other images than for monkey faces[15].

As there are well-documented brain regions specialized in face processing in humans, in particular the fusiform face area (FFA)[16,17], it is conceivable that explicit neuronal representations of human faces are located in regions outside the amygdala. Interestingly, fMRI activation of the FFA by faces is also lateralized in favor of the right hemisphere[16]. In non-human primates there are likewise areas dedicated to face processing outside the amygdala[18]. Differences in face processing between humans and non-human primates may be the result of an evolutionary adaptation reflecting the fact that present-day humans are able to distinguish thousands of individuals unlike monkeys that live in troops of a few dozen individuals.

Amygdala, object recognition, and memory

Neurons in monkey inferior temporal cortex encode the category and even identity of a visually presented object within less than 150 ms after stimulus presentation[19]. Likewise, studies with intracranial electroencephalographic (EEG) recordings in humans[20,21] reported category-specific event-related potentials with latencies of 150 – 200 ms, and behavioral responses (eye saccades) in a categorization task in humans have been observed as early as 120 ms after stimulus onset[22].

The neuronal responses in the amygdala described in our study show typical response latencies between 300 and 400 ms. At this time the detection or classification of presented objects should already have taken place. These types of responses have previously been

shown to be associated with conscious rather than unconscious perception[23-25]. It is therefore conceivable that they subserve the long-term encoding of declarative episodic memories related to this important class of stimuli rather than mere object detection.

Selectivity and baseline firing rates

Over 85% of our recorded units, both in the amygdala and in the other MTL regions, showed no significant response to any of the presented stimuli. While this could, in part, be due to the rather conservative response criterion used in our analysis, we attribute most of this lack of response to the very high selectivity observed in our units together with their low firing rates, consistent with what we and others have reported in prior studies. In a previous study[4], we have shown that responsive neurons in the MTL, particularly in the hippocampus and amygdala, exhibit lower baseline firing rates than reported for visual cortex, e.g., for monkey inferotemporal cortex. The baseline activity of these human units was found to be inversely correlated to their selectivity. While it is common in many single electrode studies in animals to move the electrode until the tip is close to an active cell, the electrode positions in our human subjects are fixed. This may result in a greater proportion of units with low spontaneous firing rates in our study as compared with animal studies, and a concomitant greater response selectivity.
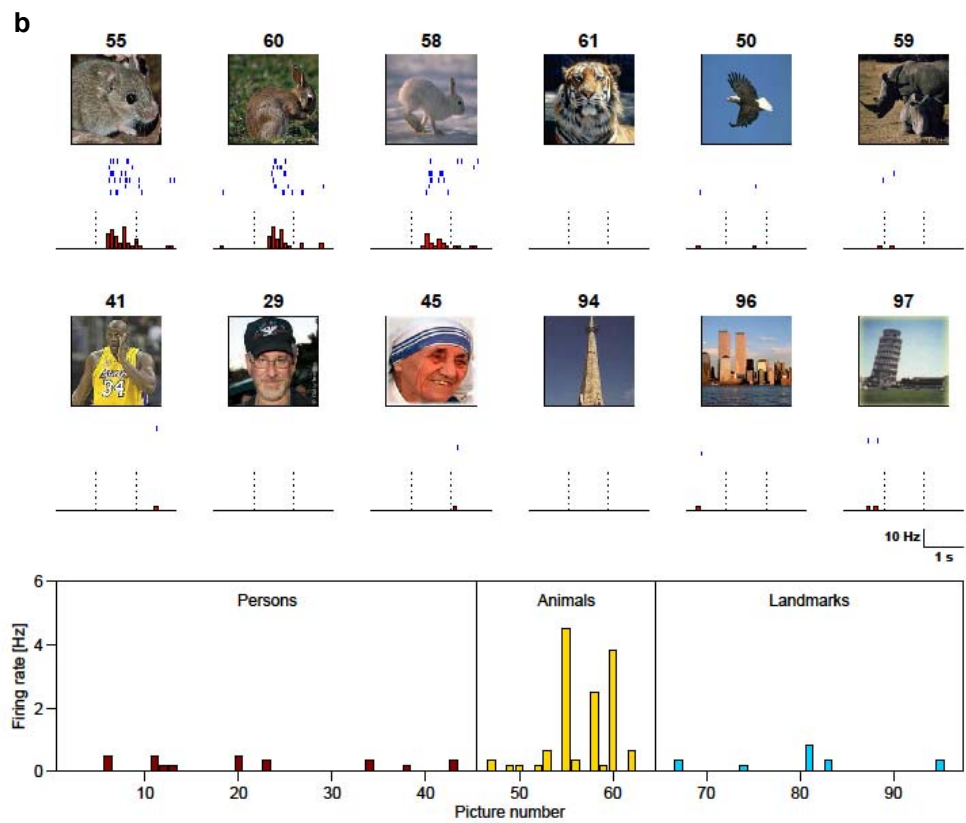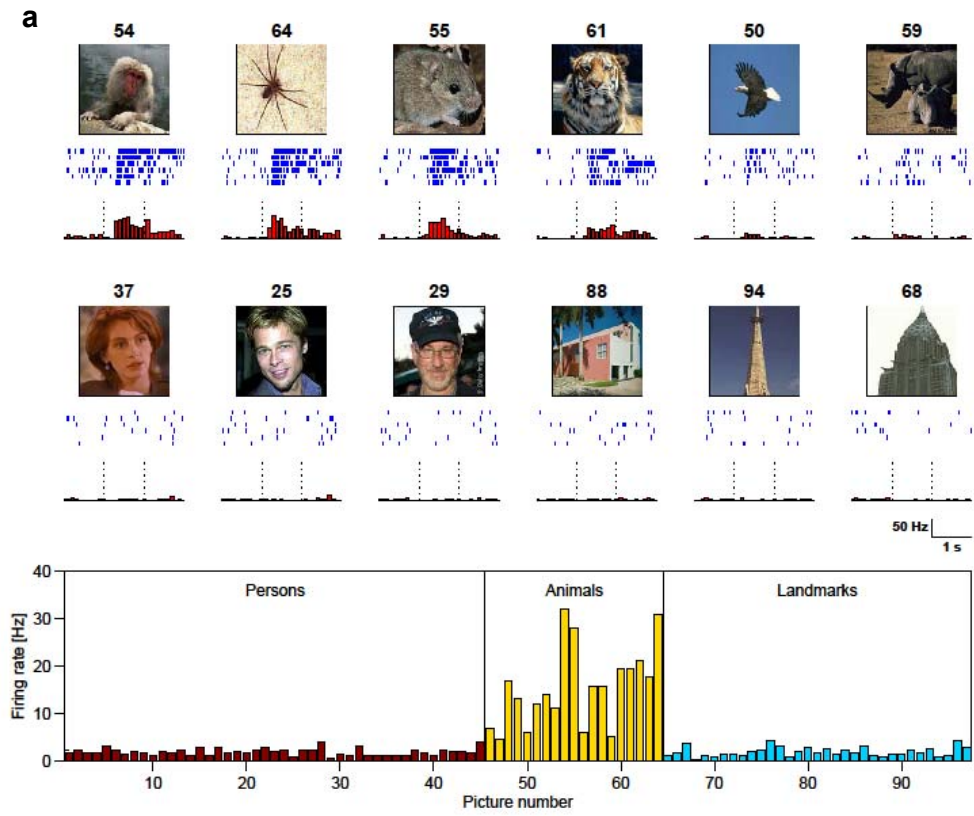
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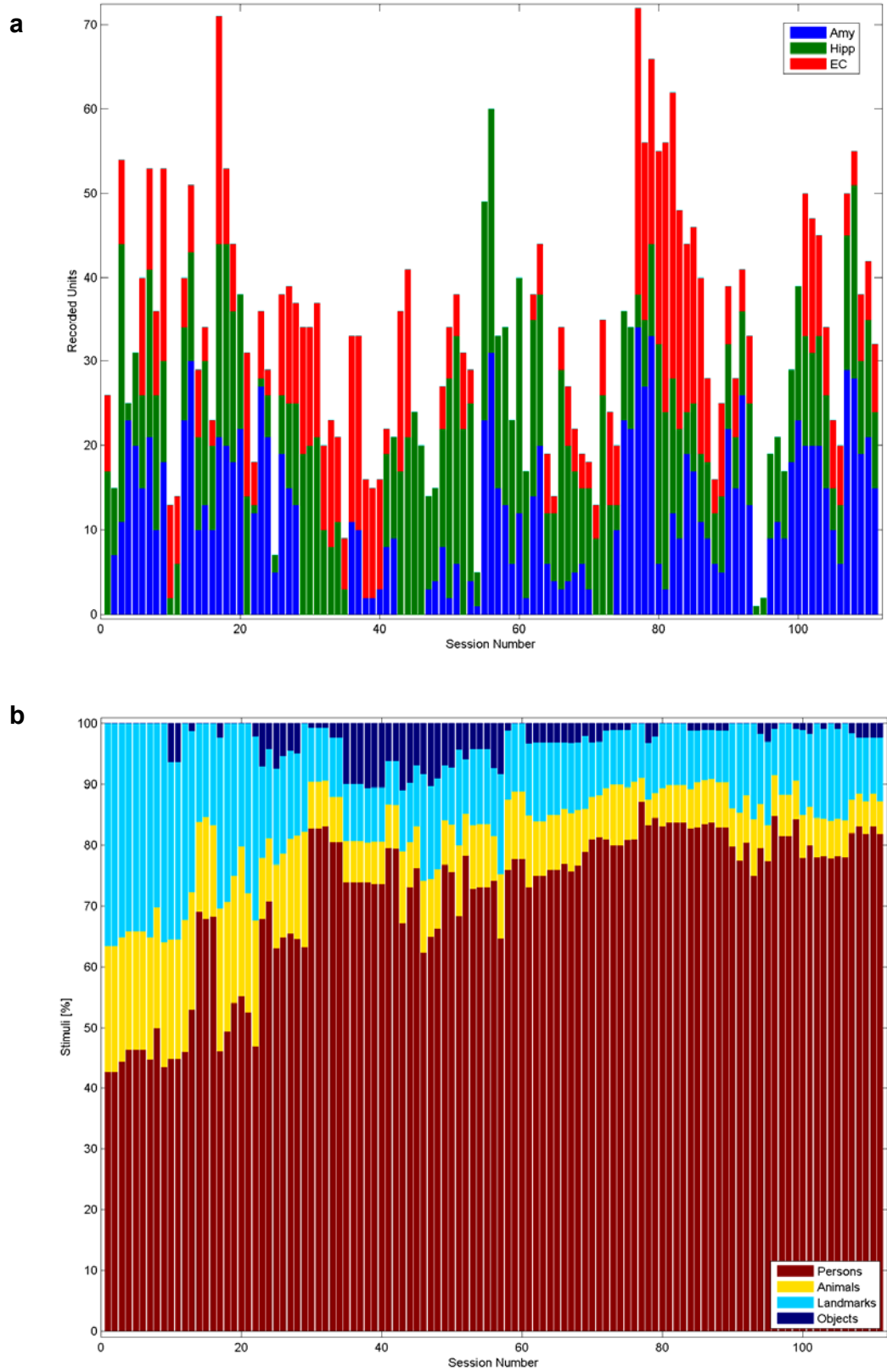
Supplementary Figure 1 Recorded units and stimulus composition.
(a) Number of units (single and multi-units) recorded from the three different MTL regions for each of the 111 sessions analyzed. **(b)** Composition of the image sets for the 111 sessions analyzed. Image sets were optimized to detect neurons firing to specific familiar individuals (Quiroga et al., 2005).

a

54   64   55   61   50   59

37   25   29   88   94   68

50 Hz |
1 s

Persons   Animals   Landmarks

Firing rate [Hz]

Picture number

b

55   60   58   61   50   59

41   29   45   94   96   97

10 Hz |
1 s

Persons   Animals   Landmarks

Firing rate [Hz]

Picture number

Supplementary Figure 2 Two examples of a single unit in the amygdala activated by animal pictures. **(a)** *Upper rows*: Responses of a neuron in the right amygdala to pictures from different stimulus categories, presented in randomized order. For each picture, the corresponding raster plots (order of trials from top to bottom) and peri-stimulus time histograms are given. Vertical dashed lines indicate image onset and offset (1 s apart). *Lower row*: The mean response firing rates of this neuron between image onset and offset across 6 presentations for all individual pictures. Pictures of persons, animals and landmarks are denoted by brown, yellow and cyan bars, respectively. **(b)** Same as **(a)**, but for a neuron that responded only to the three rodents in the animal picture set.
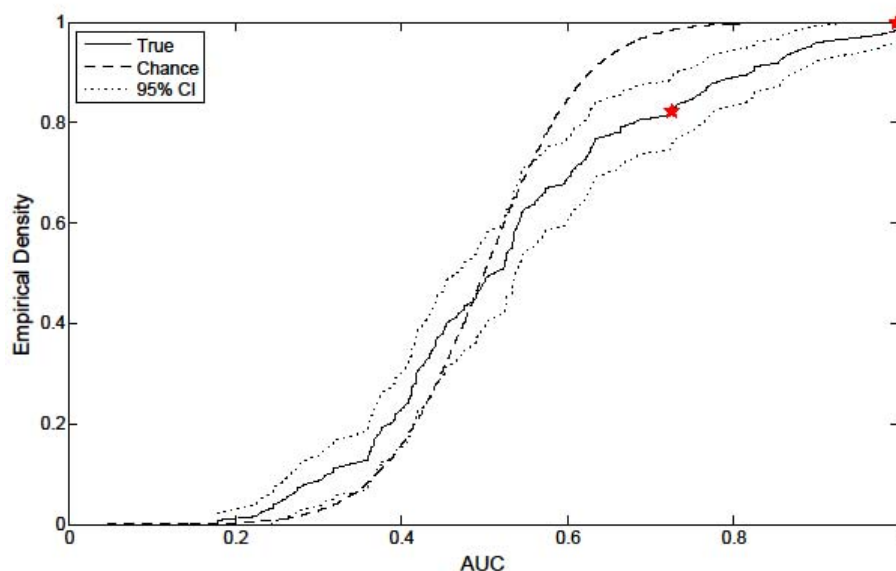
Supplementary Figure 3 Cumulative distribution function of the area under the curve (AUC) as a selectivity index for amygdala units.
For each of the 131 responsive amygdala units (single and multi-units), an ROC (Receiver Operating Characteristics) curve was generated by varying the response threshold and plotting true positive detections (animal stimuli with response firing rates above threshold) against false positive detections (non-animal stimuli with response firing rates above threshold). The area under this curve (AUC) for each neuron was used to generate the cumulative distribution function along with 95% confidence intervals (CI). A chance distribution was calculation from 1000 surrogate realizations after random relabeling of stimulus categories. The plot shows that significantly more units had a high AUC value (>0.6) than expected by chance. The two neurons shown in **Supplementary Fig. 2** are denoted as red stars (right and left, respectively) in this distribution.
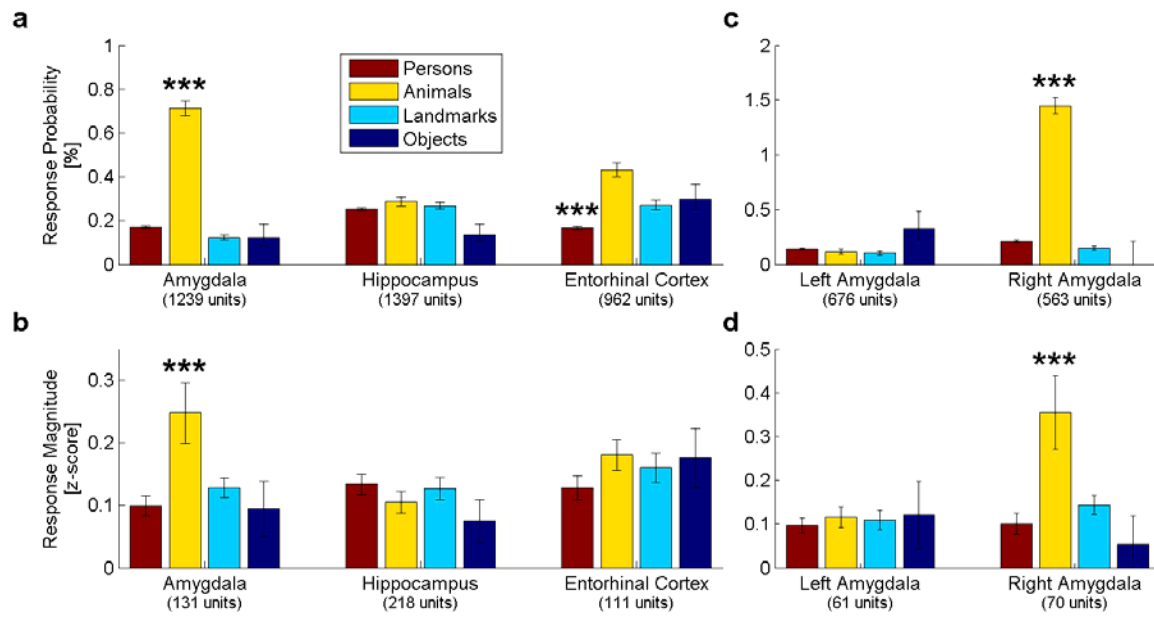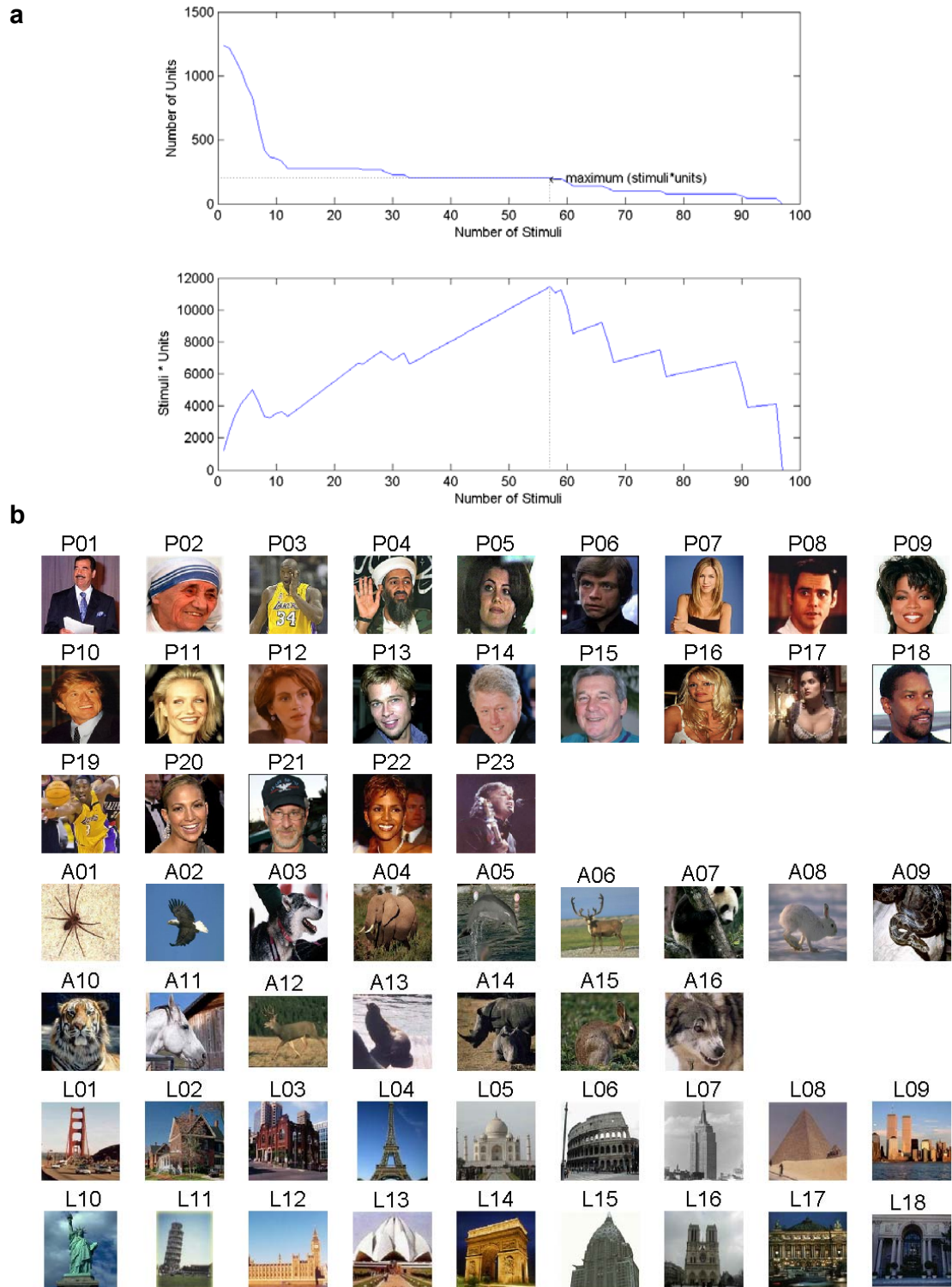
Supplementary Figure 4 Amygdala units respond preferentially to animal pictures.
Same as **Fig. 1** but including single units as well as multi-units. **(a)** Response probabilities of units in different MTL regions to different stimulus categories show significant preferences in the amygdala ($P<10^{-15}$; main effect of increased responses to animals) and entorhinal cortex ($P<10^{-9}$; main effect of decreased responses to persons), but not in the hippocampus. **(b)** Mean response magnitudes of all responsive units show increased response activity of amygdala units to animals ($P<0.0005$). **(c, d)** The animal preference in both response probability and magnitude is seen only in the right amygdala ($P<10^{-15}$, $P<0.0002$, respectively). Error bars denote binomial 68%-confidence intervals (a,b) and s.e.m. (c,d), respectively; ***, $P<0.001$.

single and multi-units   **single units only**
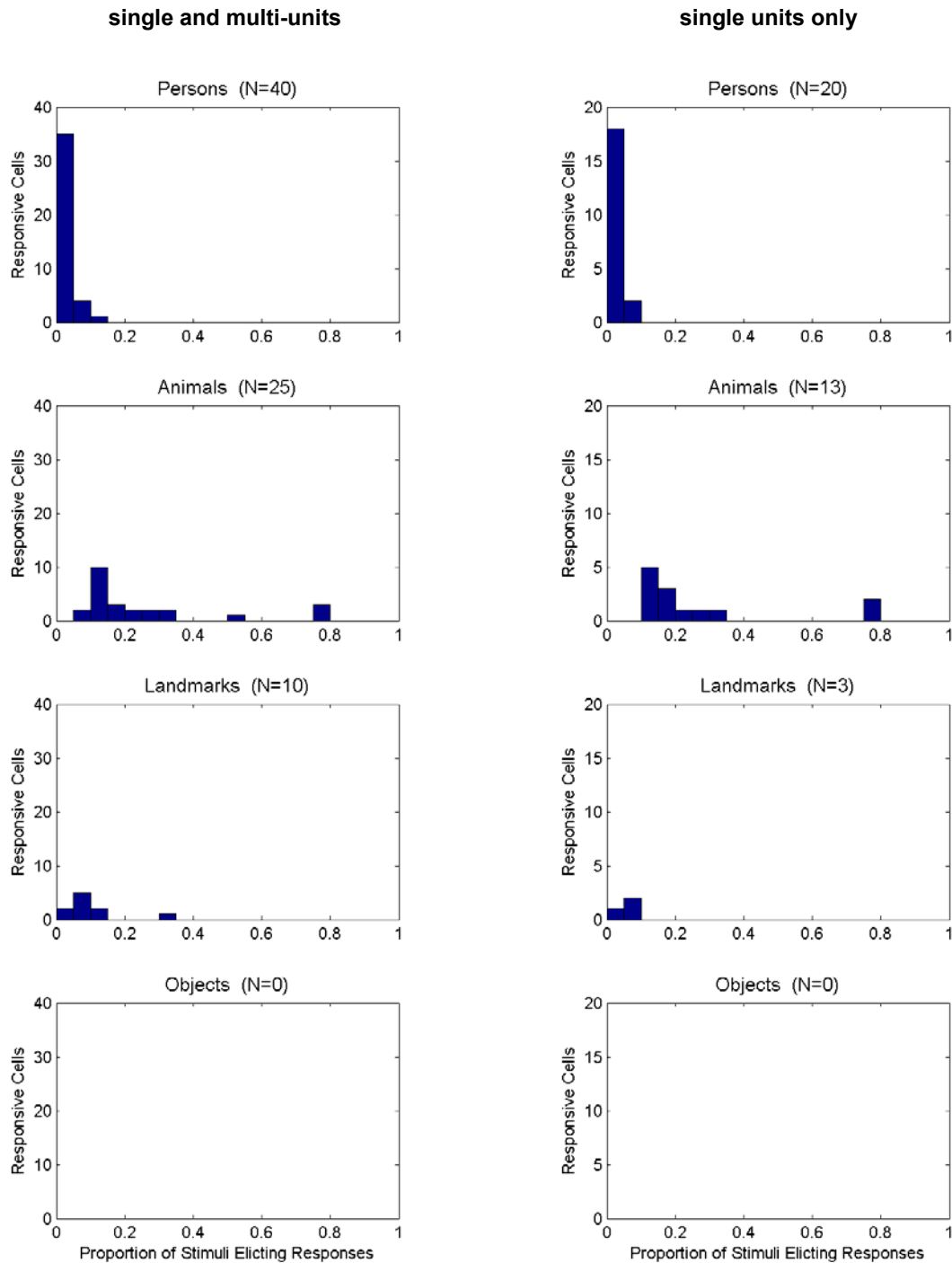
**Supplementary Figure 5** Within-category selectivity of units in the right amygdala.
Selectivity distributions of the 70 responsive units in the right amygdala shows (left panel), among them 57 single units (right panel), show that responses to animals are more categorical than, e.g., the more individualized responses to persons. Note that the higher number of responses to persons is due to the fact that person stimuli were shown more often than stimuli from other categories. The mean proportion of response-eliciting stimuli differed significantly between animals and other categories ($P < 10^{-9}$ for single and multi-units; $P < 10^{-6}$ for single units only; Mann-Whitney U test; see also Supplementary Results).

a

b

Supplementary Figure 6 Selection of units and stimuli for the population response analysis.
(a) Starting with the picture set from the session with the highest yield of amygdala units (single and multi-units), sessions are added one by one to produce the largest intersection of stimulus sets (see Methods) After plotting the number of stimuli against the cumulative number of units that these stimuli were presented to across sessions(*upper row*), an operating point is selected that maximizes the product of units times stimuli (*lower row*), resulting in a set of 201 units (96 in the left, 105 in the right amygdala) that were all presented with the same 57 stimuli (23 persons, 16 animals, 18 landmarks).
(b) The set of 57 stimuli used for the population analysis.
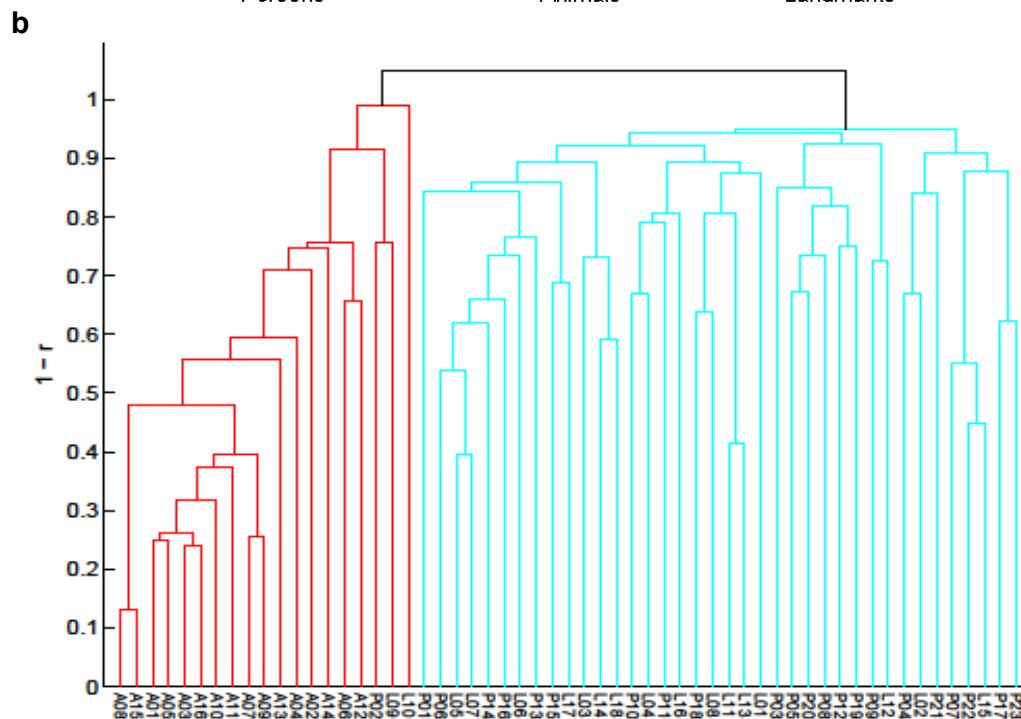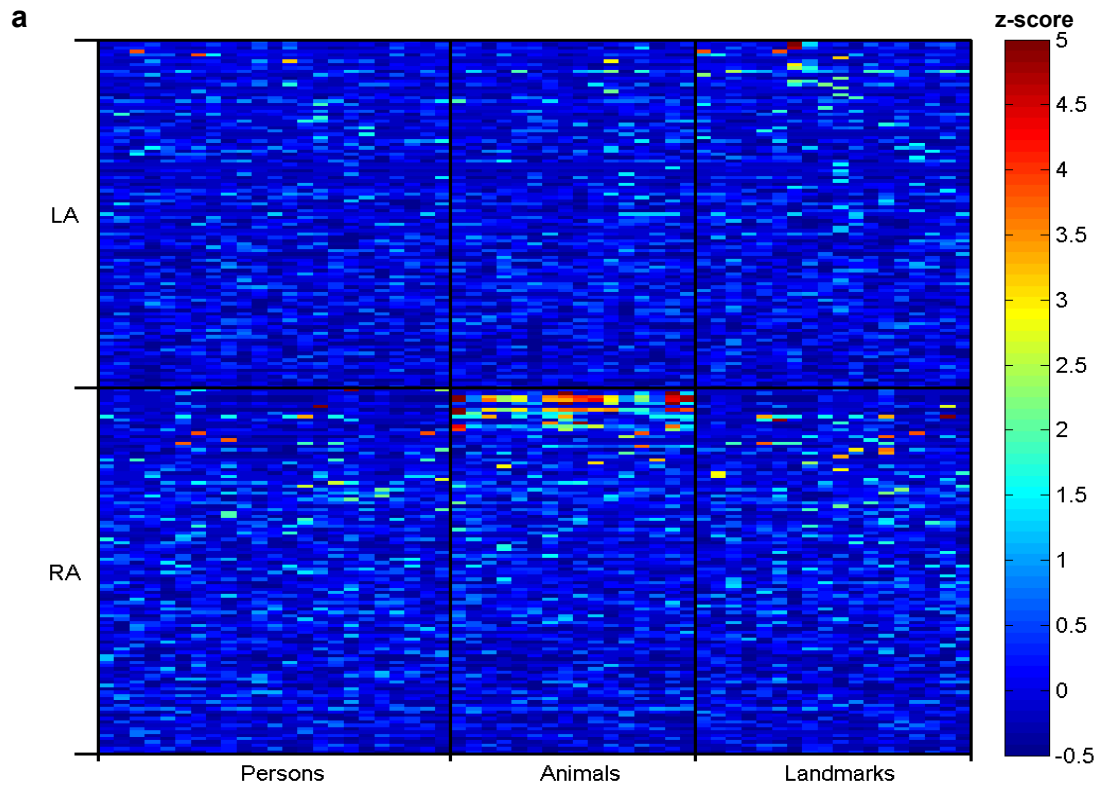
Supplementary Figure 7 Stimulus-response behavior of units used in the population analysis.
(a) Stimulus-response matrix for the 201 units in the left and right amygdala which were all exposed to the same set of 57 stimuli (see **Supplementary Fig. 6**; from left to right P01, ..., P23, A01, ..., A16, L01, ... L18). Response magnitudes were z-score transformed relative to baseline and color-coded. Units in both regions are ranked from top to bottom according to their maximum response activity. The dissimilarity matrices in **Fig. 2a** were generated from these response magnitudes (i.e., from the columns of the matrices in this plot). **(b)** Same hierarchical clustering dendrogram as in **Fig. 2b, right panel**, but with exact labelling of the stimuli displayed in **Supplementary Fig. 6**.
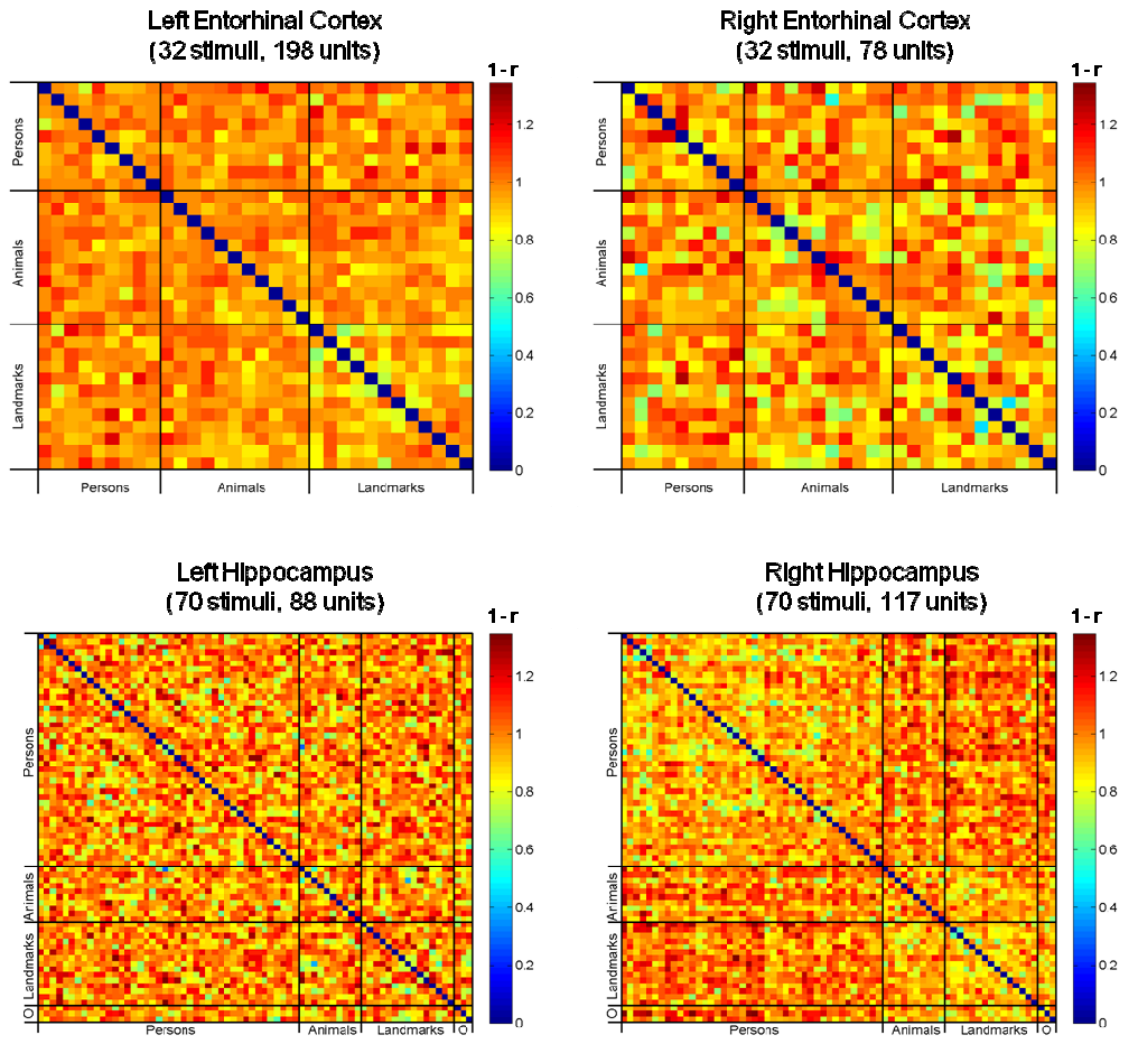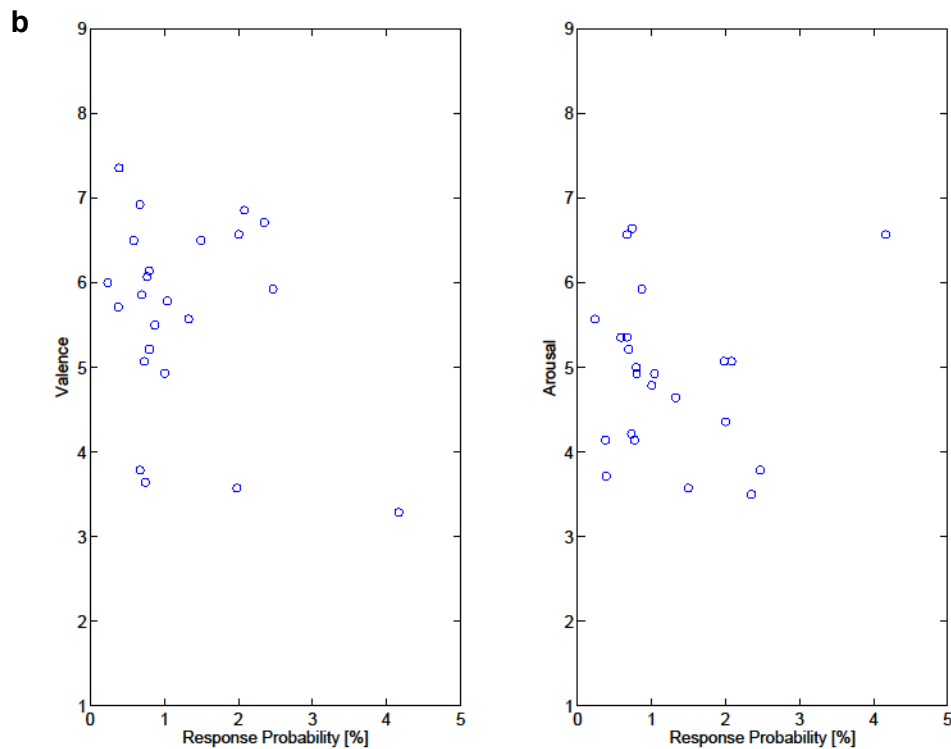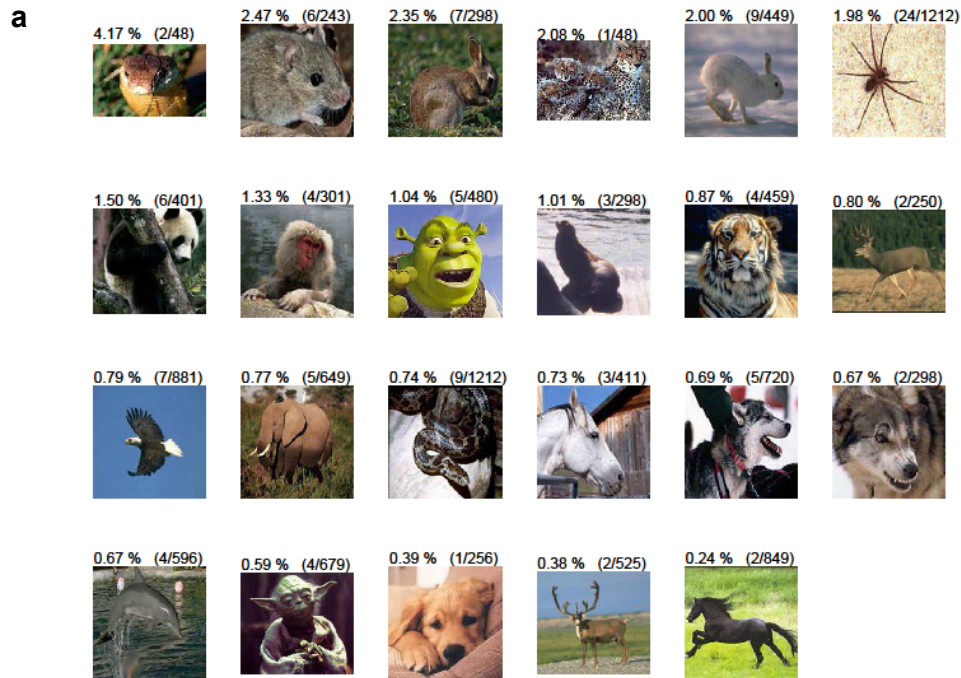
Supplementary Figure 8 Population responses in other MTL areas.
Representational Dissimilarity Matrices for the entorhinal cortex and hippocampus show no specific category effect for animals in the population code in these regions. O=Objects.
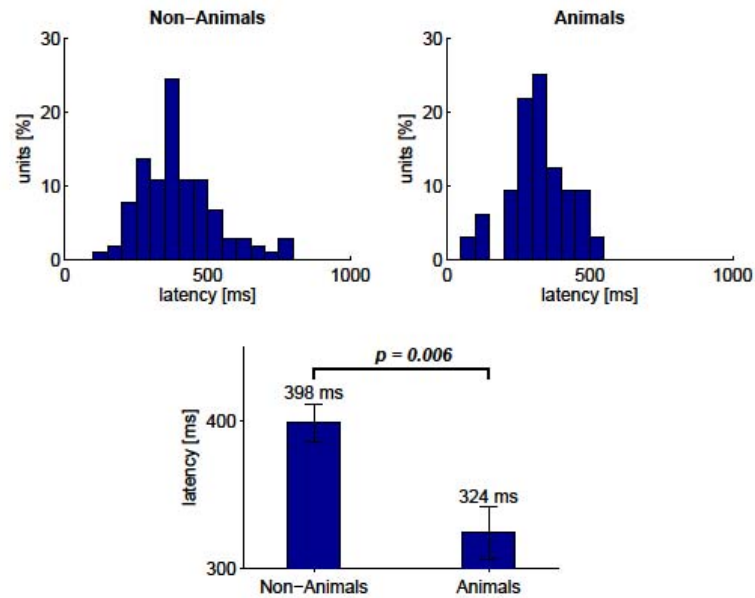
Supplementary Figure 9 Amygdala units respond faster to animal pictures than to non-animal pictures.

Top: Latency histograms of the 131 responsive amygdala units (single and multi-units) to animal (N=32) and non-animal images (N=102) in 50 ms bins. *Bottom*: Mean latencies ± SE and statistical comparison of latencies (two-sided Mann-Whitney U test).

a

b

Supplementary Figure 10 Animal responses and emotional content.
(a) The entire set of 23 animal pictures that elicited a response from amygdala neurons, ranked by amygdala response probability. Note that we classified the fictional characters 'Shrek' and 'Yoda' as animals, since amygdala cells frequently responded to them in combination with other animals. Classifying these two stimuli as objects instead of animals does not alter any of the reported findings.
(b) Ratings of emotional valence (left) and arousal (right) versus response probability for the 23 animal pictures shown in panel (a). Statistical analysis showed no significant relationship between response probability and emotional valence or arousal ($P>0.2$ for all tests). In particular, there was no significant correlation between emotional dimensions and responsiveness (Pearson's rho=-0.26; P=0.23 and rho=0.01; P=0.96, respectively).
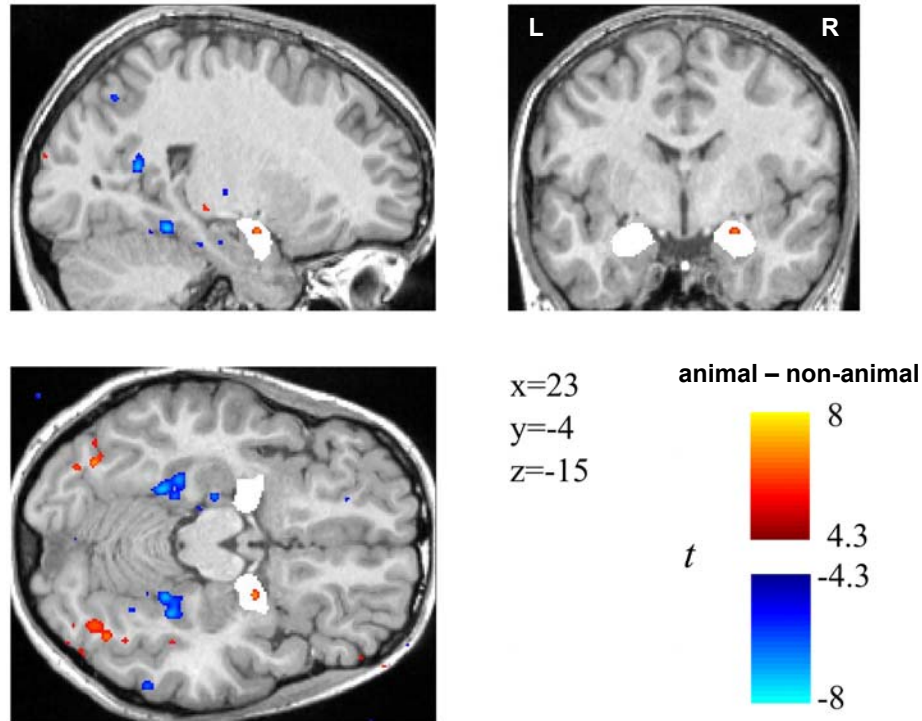
Supplementary Figure 11 fMRI activation of the amygdala by animal pictures is not an epiphenomenon of emotional valence and arousal.

60 animal pictures from the International Affective Picture System (IAPS) were individually matched with 60 non-animal IAPS pictures for emotional valence and arousal, and presented to 10 healthy subjects (right handed; 7 male; 18-31 years old) in a 3T Siemens MRI scanner. Animal and non-animal pictures were each divided into 4 groups of 15 pictures and presented in a blocked design (200 ms fixation cross followed by 800 ms stimulus presentation) using a 1-back memory task with 3 randomly chosen repetitions per block. Blocks were thus 18 s long and were shown 8 times in random order with animal and non-animal blocks alternating.

Group analysis of 10 subjects using a standard general linear model (GLM) showed a cluster of voxels in the right amygdala (MNI coordinates x=23; y=-4; z=-15) that responded more strongly to animal than to non-animal pictures (*P*<0.001, uncorrected; *P*=0.02 after small-volume correction based on the total volume of both amygdalae).

This animal vs. non-animal contrast is independent of emotional valence and arousal since stimuli from both categories were matched for these emotional dimensions.
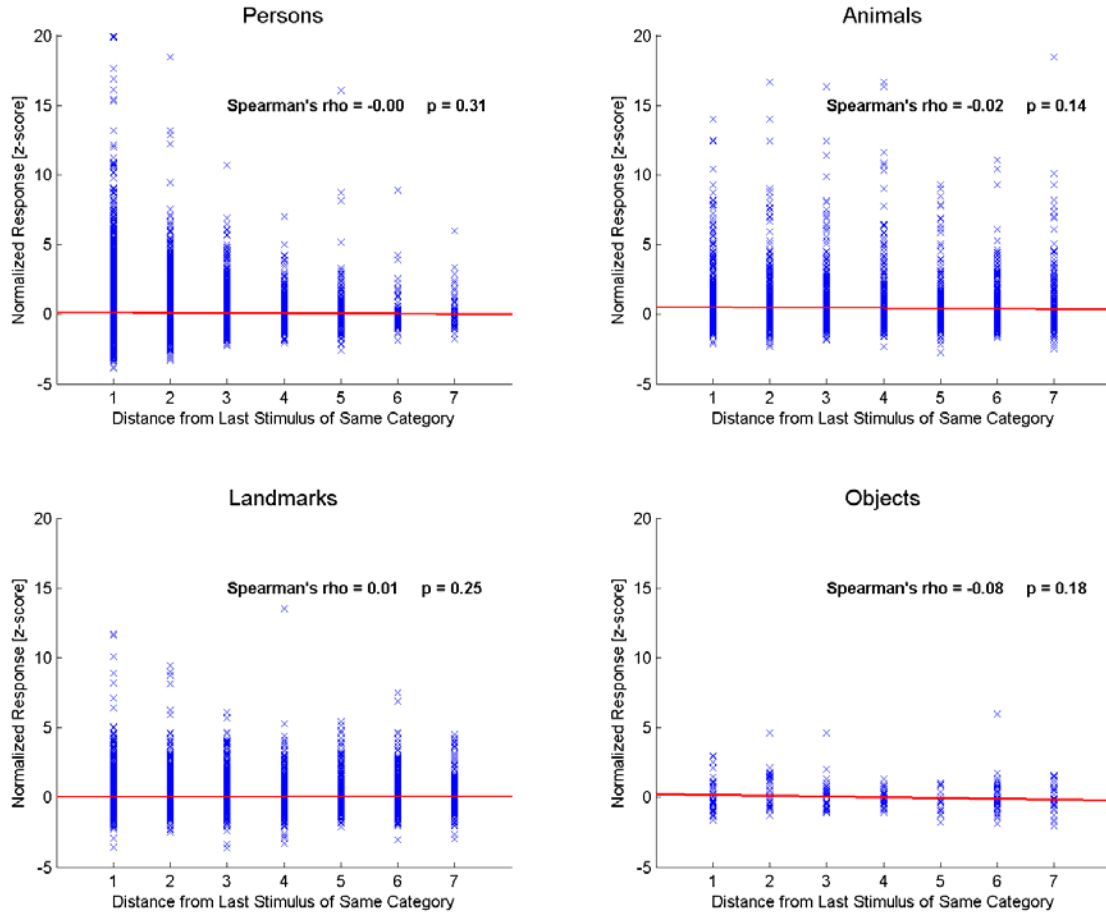
Supplementary Figure 12 Absence of within-category habituation in the neuronal responses.
Normalized response magnitudes of 131 responsive amygdala units are plotted against the time passed since the previous presentation of a stimulus from a given category. Red lines denote linear regression slopes. No significant correlation is observed for any of the four stimulus categories.