varying $P_{i \times j}$ values. Setting $P_{i \times i} = 1$ (within all genotypes) did not significantly reduce the fit ($G_2 = 0.81$, not significant), suggesting similar mating propensity among genotypes. Asymmetries ($P_{i \times j} \neq P_{j \times i}$) can therefore be presumed to be due to mate choice rather than mating propensity. Parameters and support limits (asymptotically equivalent to 95% confidence intervals[23]) were estimated under the simpler six-parameter model.

## Live female courtship experiments

We placed two or three males (more than 5 days old) of different genotypes in an insectary and introduced a single virgin female (1–5 days old). Courtship (sustained hovering by the male over the female) was recorded over a period of 10 min. The female genotype was then substituted, with genotype order randomized. On mating, pairs were quickly and gently separated, which did not disrupt subsequent behaviour. Males were never reused, but females were drawn randomly from a pool of three to four individuals per genotype. In all, 840 min of observations were made in 19 replicates with all three male genotypes and a further nine with MP and MG males alone.

## Colour pattern models

Between five and fifteen males in a $2 \times 2 \times 2$ m insectary were presented with dissected natural wings or a colour pattern model, fixed to a length of flexible wire on a lightweight handle. Models were manipulated to simulate *Heliconius* flight in the centre of a spherical area (60 cm diameter) demarcated by a bamboo cross. Randomly ordered pairs of 5-min experiments were carried out: (1) a control flight with a model of the male's own colour pattern and (2) an experimental flight with a different colour pattern. Entry to the sphere was recorded as 'approach' and sustained fluttering directed at the model as 'courtship'. At least ten replicates were carried out per comparison. Each procedure was repeated with real female wings and paper models colour-matched using commercially available permanent marker pens. Reflectance spectra of real and paper models were similar (Supplementary Information), and male behaviour towards wings and models did not differ significantly (see below).

Numbers of approaches ($X_A$) and hovering courtship interactions ($X_H$) are given in the Supplementary Information. We estimated the probabilities $Q_{i \times j}$ that males of type $j$ approached or courted models of type $i$ relative to that of their own type $j$, using likelihood. Thus, for MP males with MP versus CP models, the actual probabilities are $Q_{A cp \times mp}/(Q_{A cp \times mp} + 1)$ that males approach CP and $1/(Q_{A cp \times mp} + 1)$ that they approach MP. The $\log_e$ likelihood for this experiment is therefore $\Sigma[X_{A cp \times mp} \log_e\{Q_{A cp \times mp}/(Q_{A cp \times mp} + 1)\} + X_{A mp \times mp}\log_e\{1/(Q_{A cp \times mp} + 1)\}]$, where $X_{A cp \times mp}$ is the number of MP males approaching CP and $X_{A mp \times mp}$ is the number approaching MP. Similarly $Q_{H i \times j}$ parameters were estimated for probability of hovering courtship of the model. Estimates were obtained for paper models as well as real wings, giving a total of 20 parameters. The summed $\log_e$ likelihood was maximized over all experiments by varying the $Q_{i \times j}$ parameters. Subsequently, all comparisons within *H. melpomene* and $Q_{A mp \times cp}$ parameters were set to 1 without loss of fit ($G_{10} = 11.02$, not significant). Parameter values for the resultant ten-parameter model are shown in Fig. 3. Real and paper model parameters do not differ significantly ($G_5 = 3.70$), giving a combined five-parameter model.

1. Maynard Smith, J. Sympatric speciation. *Am. Nat.* **100**, 637–650 (1966).
2. Bates, H. W. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. *Trans. Linn. Soc. Lond.* **23**, 495–566 (1862).
3. Vane-Wright, R. I. in *Diversity of Insect Faunas* (eds Mound, L. A. & Waloff, N.) 56–70 (Blackwell Scientific, Oxford, 1978).
4. Turner, J. R. G. Adaptation and evolution in *Heliconius*: a defense of neo-Darwinism. *Annu. Rev. Ecol. Syst.* **12**, 99–121 (1981).
5. Mallet, J. & Joron, M. Evolution of diversity in warning colour and mimicry: Polymorphisms, shifting balance and speciation. *Annu. Rev. Ecol. Syst.* **30**, 201–233 (1999).
6. Turner, J. R. G. Adaptive radiation and convergence in subdivisions of the butterfly genus *Heliconius* (Lepidoptera: Nymphalidae). *Zool. J. Linn. Soc.* **58**, 297–308 (1976).
7. McMillan, W. O., Jiggins, C. D. & Mallet, J. What initiates speciation in passion vine butterflies? *Proc. Natl Acad. Sci. USA* **94**, 8628–8633 (1997).
8. Mallet, J., McMillan, W. O. & Jiggins, C. D. in *Endless Forms: Species and Speciation* (eds Howard, D. J. & Berlocher, S. H.) 390–403 (Oxford Univ. Press, New York, 1998).
9. Linares, M. *Adaptive Microevolution Through Hybridization and Biotic Destruction in the Neotropics* (Thesis, Univ. Texas, Austin, Texas, 1989).
10. Gilbert, L. E. in *Ecology and Evolution Taking Flight: Butterflies as Model Systems* (eds Boggs, C. L., Watt, W. B. & Ehrlich, P. R.) (Univ. Chicago Press, Chicago, Illinois, in the press).
11. Brower, A. V. Z. Parallel race formation and the evolution of mimicry in *Heliconius* butterflies: a phylogenetic hypothesis from mitochondrial DNA sequences. *Evolution* **50**, 195–221 (1996).
12. Mallet, J. Causes and consequences of a lack of coevolution in Müllerian mimicry. *Evol. Ecol.* **13**, 777–806 (1999).
13. Dobzhansky, T. Speciation as a stage in evolutionary divergence. *Am. Nat.* **74**, 312–321 (1940).
14. Butlin, R. Speciation by reinforcement. *Trends Ecol. Evol.* **2**, 8–12 (1987).
15. Noor, M. A. F. Reinforcement and other consequences of sympatry. *Heredity* **83**, 503–508 (1999).
16. Mallet, J. & Barton, N. H. Strong natural selection in a warning color hybrid zone. *Evolution* **43**, 421–431 (1989).
17. Dieckmann, U. & Doebeli, M. On the origin of species by sympatric speciation. *Nature* **400**, 354–357 (1999).
18. Kirkpatrick, M. Reinforcement and divergence under assortative mating. *Proc. Roy. R. Lond. B* **267**, 1649–1655 (2000).
19. Craig, T. P., Itami, J. K., Abrahamson, W. G. & Horner, J. D. Behavioral evidence for host-race formation in *Eurosta solidaginis*. *Evolution* **47**, 1696–1710 (1993).
20. Feder, J. L. *et al.* Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. *Proc. Natl Acad. Sci. USA* **91**, 7990–7994 (1994).
21. Rundle, H. D., Nagel, L., Wenrick Boughman, J. & Schluter, D. Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**, 306–308 (2000).
22. Mallet, J. & Gilbert, L. E. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biol. J. Linn. Soc.* **55**, 159–180 (1995).
23. Edwards, A. W. F. *Likelihood* (Cambridge Univ. Press, Cambridge, 1972).

# Lesions of the human amygdala impair enhanced perception of emotionally salient events

**Adam K. Anderson**[\*][†] **& Elizabeth A. Phelps**[‡]

\* *Department of Psychology, Yale University, New Haven, Connecticut 06520, USA*
‡ *Department of Psychology, New York University, New York, New York 10003, USA*

**Commensurate with the importance of rapidly and efficiently evaluating motivationally significant stimuli, humans are probably endowed with distinct faculties[1,2] and maintain specialized neural structures to enhance their detection. Here we consider that a critical function of the human amygdala[3,4] is to enhance the perception of stimuli that have emotional significance. Under conditions of limited attention for normal perceptual awareness—that is, the attentional blink[5,6]—we show that healthy observers demonstrate robust benefits for the perception of verbal stimuli of aversive content compared with stimuli of neutral content. In contrast, a patient with bilateral amygdala damage has no enhanced perception for such aversive stimulus events. Examination of patients with either left or right amygdala resections shows that the enhanced perception of aversive words depends specifically on the left amygdala. All patients comprehend normally the affective meaning of the stimulus events, despite the lack of evidence for enhanced perceptual encoding of these events in patients with left amygdala lesions. Our results reveal a neural substrate for affective influences on perception, indicating that similar neural mechanisms may underlie the affective modulation of both recollective[7–9] and perceptual experience.**

The amygdala supports substantial projections to primary and higher-order sensory areas and the hippocampal formation[10]. Thus, the amygdala is strategically placed to allow emotional value[4,11] both to modulate perceptual sensitivity to incoming information and to bolster its post-encoding consolidation into memory. Much evidence has shown that the amygdala is involved in the latter of these

† Present address: Department of Psychology, Stanford University, Jordan Hall, Building 420, Stanford, California 94305-2130, USA

two functions, as it is critical for enhanced memory of emotionally arousing events[7–9,12–14]. It is unknown, however, whether the human amygdala also provides a means for affective value to modulate perception itself, thus influencing the likelihood that stimulus events reach awareness.

We examined the importance of the amygdala in the affective modulation of perception by using the attentional-blink effect[5,6]. The attentional blink shows that, after identifying a single target stimulus, there is a transient impairment in awareness for a subsequently presented second target[5,6,15]. Consistent with the notion of a differential sensitivity toward significant events, this deficit in perceptual awareness is greatly attenuated for aversive verbal stimuli[16]. Considering that the comprehension of meaning from word forms is left largely intact after amygdala lesions[8,17,18], the use of linguistic events allows analysis of the influence of affective content on perceptual encoding, independent of a primary deficit in appreciation of emotional significance. Here we examined the attenuation of the attentional blink for affectively significant words in a patient with bilateral damage to the amygdala (S.P.)[9], and in 10 patients with unilateral lesions of the right or left amygdala.

Observers were asked to report the identity of two green target words occurring amongst a stream of black distractor items during rapid serial visual presentation (RSVP) (Fig. 1). Confirming the affective modulation of the attentional blink, control observers identified negative words with greater accuracy than neutral words across all temporal lags (79.8% versus 61.5%; $P < 0.0001$). A significant interaction between T1–T2 temporal lag (early versus late) and T2 valence (negative versus neutral) ($P < 0.02$) indicated that the degree of affective modulation of the attentional blink was most pronounced at early lags ($P < 0.0001$), where attentional resources were most occupied with the processing of the preceding T1.

Similar to controls, S.P. showed a normal attentional blink as evidenced by her identification accuracy increasing from early relative to later temporal lags ($P < 0.0001$). S.P.'s performance also recovered to baseline levels over a normal time course; she exhibited similar levels of T2 identification performance to controls (controls 80.4% versus S.P. 81.0%) on the longest T1–T2 lag (stimulus onset asynchrony; SOA = 910 ms). Unlike control observers, S.P. showed almost no advantage for negative compared with neutral word identification during the middle of the attentional blink at early T1–T2 temporal lags (25.0% versus 23.0%; $P > 0.80$; Fig. 2b); she fell significantly below the control range in identifying negative items at early lags (controls 72.6%, versus S.P. 25.0%; $z = 2.11$, $P < 0.02$). This impairment in identifying negative events was most evident when T2 was presented immediately after T1 (controls 74.5%, versus S.P. 12.5%; $z = 3.13$, $P < 0.001$). Furthermore, an analysis of the distribution of difference scores (negative minus neutral) showed that whereas the advantage for negative words was most prominent in controls at early lags, S.P. showed almost no advantage for negative words falling outside the control range (controls 22.6%, versus S.P. 2.1%; $z = 1.76$, $P < 0.04$; Fig. 2d).

To control for any effect of lower baseline performance in S.P., we examined one half of the control subjects ($n = 10$) that performed similarly to S.P. on neutral items (controls 47.3% versus S.P. 43.8%; $z = 0.13$). In contrast to S.P., these controls had a highly robust affective modulation of the attentional blink (negative 70.6% versus neutral 47.3%; $P < 0.0001$; Fig. 2b). An examination of the advantage of negative-item identification (negative minus neutral) again showed an impairment in affective modulation at early temporal lags (controls 26.2% versus S.P. 2.1%; $z = 1.72$; $P < 0.05$), with controls showing a more substantial proportional increase in identifying negative events compared with S.P. (83.2% versus 8.7%).

The observed impaired affective modulation of the attentional blink at early temporal lags may reflect S.P.'s difficulty in maintaining items in her memory over the interval before report. Arguing

against this notion, however, her performance in identifying T1, which occurred before T2, was even more accurate than that of controls at the longest T1–T2 lag (96.8% versus 91.5%).

An additional concern was that S.P.'s deficit was not due to impaired affective modulation of perception, but might reflect a more global deficit that disrupts any type of modulation of perceptual encoding. However, when we manipulated the perceptual discriminability of neutral targets by altering the visual similarity of the targets and distractors[6], S.P.'s word identification performance was greatly enhanced (high discriminability 78.1%, versus low discriminability 52.1%; $P < 0.001$; Fig. 2c). The influence of perceptual salience was most pronounced at early T1–T2 lags (high discriminability 66.7%, versus low discriminability 31.2%; a proportional identification benefit of over 100%). A similar analysis of S.P.'s performance in the affective salience condition showed no reliable overall advantage (negative 50.8% versus neutral 44.6%; $P > 0.41$), or a lag-dependent advantage for negative words ($P > 0.49$). This finding indicates that affective and perceptual modulatory influences on visual awareness may be dissociable, with only the latter being dependent on the amygdala.

Functional neuroimaging studies of word perception find that the presentation of emotional linguistic stimuli may specifically activate the amygdala in the left hemisphere[19], suggesting that S.P.'s selective left amygdala damage in particular may be responsible for the observed deficit. This would be consistent with evidence that verbal/nonverbal hemispheric asymmetries typically ascribed to higher cortical function in humans extend to the function of subcortical structures such as the amygdala[20–22]. To replicate the
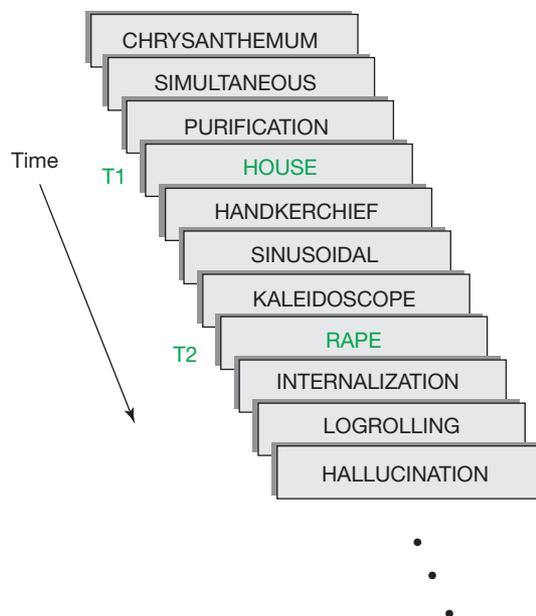


**Figure 1** Diagram of the dual-target rapid serial visual presentation (RSVP) task. Fifteen words were briefly presented sequentially in an identical central location, and observers were instructed to ignore words appearing in black (distractors) and indicate the identity of two target words occurring in green. Distractor words were longer in length to ensure sufficient masking of the target events. Responses were made by typing on a computer keyboard immediately after the stimulus sequence. The temporal lag between the first (T1) and second target (T2) was varied. T2 items occurring during early T1–T2 temporal lags (< 4 intervening distractor items, < 600 ms) are susceptible to the attentional blink (attentional blink), an attention-related impairment in perceptual encoding. As attentional resources become more available during late T1–T2 temporal lags (> 4 intervening distractor items, > 600 ms), T2 items are less susceptible to the attentional blink. In the described experiments, the affective value of T2 was manipulated and T2 identification accuracy was the critical measure.

finding of impaired affective modulation of perceptual encoding in a larger patient sample, and to assess more precisely which particular neural structures damaged in S.P. were critical for this deficit, we examined five patients with right (RTL) and five with left (LTL) amygdala lesions due to unilateral anteromedial temporal lobe resections.

When RTL and LTL observers were asked to identify neutral and negative T2 words during dual-target RSVP, we found that the effect of stimulus valence on identification was not equivalent across patient and control groups ($P < 0.02$). Similar to controls ($P > 0.27$), RTL patients showed a highly significant affective modulation of the attentional blink (negative 68.9% versus neutral 54.6%; $P < 0.009$), which was most evident at early T1–T2 lags (negative 65.7% versus neutral 44.4%; Fig. 3a). In contrast, LTL patients showed no significant advantage in negative-event identification (negative 41.1% versus neutral 36.2%; $P > 0.16$), demonstrating impaired affective modulation of the attentional blink relative to controls ($P < 0.005$).

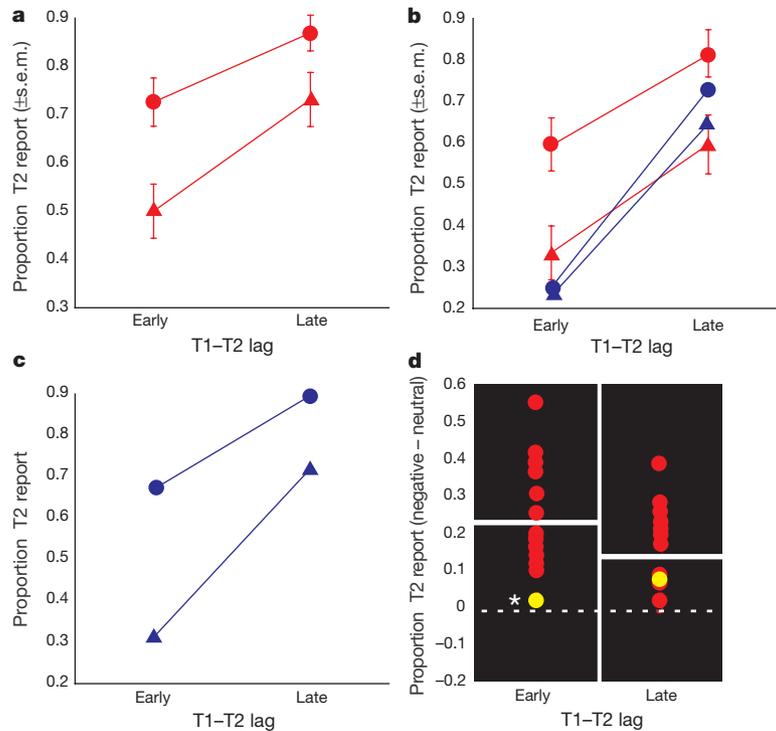When we examined the extent of the negative-event advantage



**Figure 2** Proportion of T2 items correctly identified at early and late T1–T2 temporal lags. **a**, Controls (*n* = 20; red) showed enhanced identification of negative (circles) compared with neutral (triangles) items during the attentional blink. **b**, S.P. (blue) showed no evidence of enhanced identification of negative (circles) compared with neutral (triangles) items during the attentional blink, and remained significantly impaired when compared with the half of the controls (*n* = 10, in red) that showed similar baseline performance on neutral items. Bars in **a** and **b** show s.e.m. **c**, Impaired affective modulation of perception in S.P. did not generalize to the influence of non-affective manipulations of stimulus salience on perceptual awareness. S.P. exhibits a large benefit for high (circles) compared with low (triangles) salience T2 events. **d**, Distribution of T2 difference scores (negative minus neutral) for controls (*n* = 20) and S.P. at early and late T1–T2 temporal lags. Yellow, S.P.; red, controls; white bar, control mean. In contrast to controls, S.P. did not show a reliable advantage for identifying negative compared with neutral items at early T1–T2 temporal lags, falling significantly outside the control range (controls 22.6%, S.P. 2.1%). Asterisk denotes significant impairment.
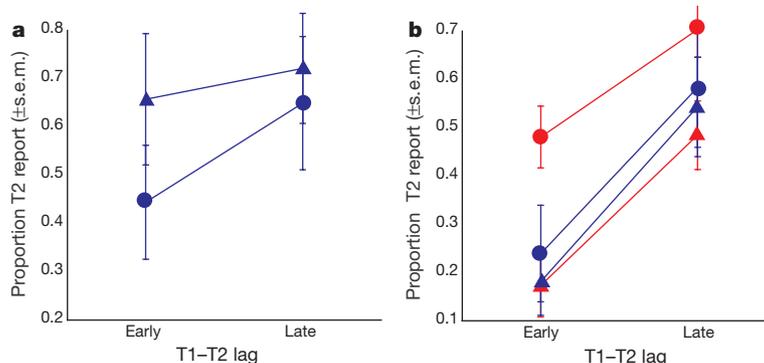


**Figure 3** Proportion of T2 correctly identified for negative (circles) and neutral (triangles) items at early and late T1–T2 temporal lags for RTL and LTL patients. **a**, RTL patients showed enhanced identification, to a similar extent as controls, of negative compared with neutral words (see Fig. 2a). **b**, LTL patients (blue) showed no evidence of enhanced identification of negative compared with neutral items, and remained significantly impaired when compared with controls (red) that showed similar baseline performance on neutral items. Bars in **a** and **b** show s.e.m.

(negative minus neutral), we found that it depended on subject group ($P < 0.03$). Both controls ($P < 0.007$) and the RTL group ($P < 0.05$) showed a significant valence-dependent advantage in stimulus identification compared with the LTL group (controls 22.6%, RTL 21.3%, LTL 5.8%). There was no significant difference between the RTL and control groups ($P > 0.80$). When we precisely matched LTL patients ($n = 5$) with control observers showing equivalent baseline responding on neutral items ($n = 5$) (LTL 36.2%, controls 32.7%), LTL patients remained differentially impaired on negative-event identification ($P < 0.003$; Fig. 3c). In contrast, an analysis of performance on the preceding T1 showed that there were no significant differences between the three groups, and near numerically identical performance between RTL and LTL patients (controls 89.6%, RTL 79.6%, LTL 80.9%; $P > 0.27$).

The impaired perceptual encoding advantage for stimuli of affective significance may be only secondary to impaired comprehension of the affective importance of these events. To investigate this hypothesis, we considered the evaluation of each T2 item used in the affective salience RSVP task according to two stimulus dimensions: valence and arousal. Control participants rated negative words as both more negative ($4.61 \pm 0.86$ versus $3.28 \pm 0.73$; $P < 0.0001$) and more arousing ($4.16 \pm 1.5$ versus $2.06 \pm 1.03$; $P < 0.0001$) than the neutral words. S.P.'s ratings similarly discriminated between the valence (negative 4.79 versus neutral 4.04) and arousal value (negative 4.82 versus neutral 1.75) of negative and neutral words, falling in the normal control range for each ($P > 0.15$ in all cases). Likewise, RTL and LTL patients exhibited intact discrimination between the valence of the target words ($RTL_{negative}$ 5.04 versus $RTL_{neutral}$ 2.78; $LTL_{negative}$ 5.17 versus $LTL_{neutral}$ 2.38), with both patient groups showing an even greater polarization between the appreciation of items of negative and neutral valence ($P < 0.04$). In addition, RTL and LTL patients provided normal ratings of arousal value ($RTL_{negative}$ 2.96 versus $RTL_{neutral}$ 1.34; $LTL_{negative}$ 3.75 versus $LTL_{neutral}$ 2.44; $P > 0.40$). Thus, the impaired influence of affective content on perceptual awareness shown in S.P. and LTL patients was not a reflection of an impaired comprehension of the affective attributes of these same stimuli. This result is similar to evidence that patients with amygdala lesions can exhibit normal subjective impressions of emotional stimuli, but show little enhanced memory for these events[7–9].

Related to our study, patients with amygdala lesions exhibit normal subjective and psychophysiological responses to aversive word types, but do not show the normal retention advantage associated with these words[8,9]. Consistent with animal models of the amygdala's role in supporting perceptual vigilance[23,24], our findings indicate that neuromodulatory influences of the human amygdala may not be restricted to episodic memory consolidation, but may extend to initial perceptual encoding[25,26], influencing the likelihood that events of affective importance reach awareness.

Supporting the notion that the human amygdala is involved in the perceptual encoding of emotionally significant linguistic events, visually presented negative-word stimuli evoke greater activity in the amygdala compared with words of neutral value[19,27]. Although such results may suggest that the amygdala itself directly encodes linguistic events, we propose that lesions of the amygdala disrupt its ability to modulate the efficiency of word processing that takes place in other brain regions, such as the posterior fusiform gyrus[19,27]. Consistent with its larger perceptual neuromodulatory role, anatomical studies of primate amygdala projections show widespread influences throughout all levels of the ventral visual processing pathway[10], allowing the amygdala to influence cortical modules that are specialized for the processing of different informational domains.

The exact neural mechanisms by which the amygdala influences the salience of stimulus events processed in target perceptual systems remain unclear. The amygdala may modulate the fluency of perceptual encoding either by influencing processing dynamics in perceptual systems by transiently modulating cortical firing thresholds[23,25], or by influencing neuronal plasticity by altering the receptive field properties of subcortical and primary and secondary cortical regions[26,28]. Our results suggest that the result of these amygdala-mediated neural modifications may be to enhance perceptual sensitivity to events of importance to the organism, making them less dependent on attentional resources to achieve awareness. Thus, one of the critical functions of the human amygdala is to segregate the neural representations of the significant from the mundane[29], either through later recollective processes[7–9,12–14] or during stimulus encoding, shaping perceptual experience directly. □

## Methods

### Participants

S.P. is a 54-year-old right-handed female who, at the age of 48, had her right amygdala removed as a result of anteromedial temporal lobe resection for medically intractable epilepsy. Her right temporal lobe resection included partial removal of the anterior, middle and inferior temporal gyri, and complete removal of the hippocampus, para-hippocampus and projection fibres to their posterior extent. Before surgery, an additional lesion was observed in the left amygdala region. Two biopsies in this region revealed reactive gliosis consistent with mesial temporal sclerosis. Post-surgery T1- and T2-weighted magnetic resonance scans (see Supplementary Information) show abnormal signal intensity extending throughout her left amygdala. Neuropsychological and radio-logical indices suggest that the damage does not extend to adjacent temporal lobe structures in the left hemisphere[9]. S.P. received a high-school education, has taken college courses, and presents a normal neuropsychological profile[9].

In addition to S.P.'s performance, ten patients with unilateral anteromedial temporal lobectomy (TLB) including removal of the amygdala were examined: five right (RTL) and five left (LTL). Each of these patients had undergone a highly uniform surgical procedure[30] (described above) to control for medically intractable seizures. The performance of S.P. and TLB patients (RTL: age, $42.6 \pm 7.4$ yr; education, $14.4 \pm 2.6$ yr; LTL: age, 33.4 yr; education, $14.6 \pm 2.5$ yr) was compared with that of 20 (15 female and 5 male) control participants of similar age ($43 \pm 7.7$ yr) and education (14.1 yr).

### Design and procedure

The first target stimuli (T1) were 56 neutral words. In the affective salience RSVP task, the second target stimuli (T2) consisted of 28 negative (for example, rape, bastard) and 28 neutral words (for example, broom, distance). The negative word list comprised aversive words intended to be more negative and physiologically arousing in nature than their neutral counterparts[8]. The negative and neutral lists were matched for average word length, written word frequency and interletter frequency. Distractor words were neutral in content and longer (mean 11.66 letters) than the target stimuli to ensure sufficient masking.

Each trial consisted of 15 word items, 2 targets and 13 distractors. In the perceptual salience control condition, an alphanumeric character mask replaced distractor words and both T1 and T2 were neutral words. T1 and T2 were designated as targets by appearing in bright green, whereas the distractor words appeared in black. Each item in the stream was presented for 130 ms and immediately followed the subsequent item, resulting in seven lags between T1 and T2 ranging from lag 1 (no intervening items, SOA = 130 ms) to lag 7 (SOA = 910 ms). S.P. was tested at the same SOA as controls; TLB patients were tested at a slightly longer SOA of 150 ms. There were 16 trials for each factor combination of lag (1–7) and condition (negative versus neutral), for a total of 224 trials. The subjects' task was to monitor the RSVP stream and then report the two green coloured targets by typing them after the stimulus sequence (Fig. 1). After the dual-target RSVP task, we presented subjects with the T2 words and asked them to evaluate each word on two seven-point scales: valence (1, pleasant; 7, unpleasant) and arousal (1, low; 7, high).

### Apparatus

The experiment was conducted on a Macintosh computer and stimuli were presented in Geneva font, point-size 24, viewed from an average distance of 40 cm. The background was a uniform grey field.

### Analysis

For the control participant sample, data for the first target (T1) and second target (T2) contingent on correct identification of T1 were considered separately and submitted to two independent $2 \times 2$ factorial analyses of variance (ANOVAs), with condition (negative versus neutral) and T1–T2 lag (early (collapsing across lags 1–3) versus late (collapsing across lags 5–7)) entered as independent within-subject factors. Collapsing across independent lags was undertaken to highlight the essential contrast between when attentional resources for perceptual encoding were more available (late lags) versus less available (early lags).

To assess deviation from normal performance, S.P.'s data were converted to $z$ scores on the basis of the control means and standard deviations. To avoid the loss of data points, S.P.'s performance on T2 was calculated independently of T1 performance. S.P.'s data from the affective salience and perceptual salience conditions were also submitted to separate single-subject ANOVAs, in which each trial was entered as the random factor. TLB data for

T1 and T2 contingent on correct identification of T1 were considered separately and submitted to two independent mixed-design ANOVAs. In addition, control, LTL and RTL difference scores (negative minus neutral) were submitted to an additional ANOVA. The valence and arousal evaluation ratings for the control, RTL and LTL groups were submitted to separate between-group *t*-tests.

1. Bruner, J. S. & Postman, L. Emotional selectivity in perception and reaction. *J. Pers.* **16,** 69–77 (1947).
2. Niedenthal, P. M. & Kitayama, S. (eds) *The Heart's Eye: Emotional Influences in Perception and Attention* (Academic, San Diego, 1994).
3. Aggleton, J. P. *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction* (Wiley-Liss, New York, 1992).
4. LeDoux, J. E. *The Emotional Brain* (Simon & Schuster, New York, 1992).
5. Raymond, J. E., Shapiro, K. L. & Arnell, K. M. Temporary suppression of visual processing in an RSVP task: An attentional blink? *J. Exp. Psychol. Hum. Percept. Perform.* **18,** 849–860 (1992).
6. Chun, M. M. & Potter, M. C. A two-stage model for multiple target detection in rapid serial visual presentation. *J. Exp. Psychol. Hum. Percept. Perform.* **21,** 109–127 (1995).
7. Cahill, L., Babinsky, R., Markowitsch, H. J. & McGaugh, J. L. The amygdala and emotional memory. *Nature* **377,** 295–296 (1995).
8. LaBar, K. S. & Phelps, E. A. Arousal mediated memory consolidation: Role of the medial temporal lobe in humans. *Psychol. Sci.* **9,** 490–493 (1998).
9. Phelps, E. A. *et al.* Specifying the contributions of the human amygdala to emotional memory: a case study. *Neurocase* **4,** 527–540 (1998).
10. Amaral, D. G., Price, J. L., Pitkanen, A. & Charmichael, S. T. in *The Amygdala* (ed. Aggleton, J. P.) 1–66 (Wiley-Liss, New York, 1992).
11. Nishijo, H., Ono, T. & Nishino, H. Single neuron responses in amygdala of alert monkeys during complex sensory stimulation with affective significance. *J. Neurosci.* **8,** 3570–3583 (1988).
12. Cahill, L. *et al.* Amygdala activity at encoding correlated with long-term, free recall of emotional information. *Proc. Natl Acad. Sci. USA* **93,** 8016–8021 (1996).
13. Hamann, S. B., Ely, T. D., Grafton, S. T. & Kilts, C. D. Amygdala activity related to enhanced memory for pleasant and aversive stimuli. *Nature Neurosci.* **2,** 289–294 (1999).
14. Canli, T., Zuo, Z., Brewer, J., Gabrieli, J. D. & Cahill, L. Event-related activation in the human amygdala associates with later memory for individual emotional experience. *J. Neurosci.* **20,** RC99, 1–5 (2000).
15. Vogel, E. K., Luck, S. J. & Shapiro, K. L. Electrophysiological evidence for a postperceptual locus of suppression during the attentional blink. *J. Exp. Psychol. Hum. Percept. Perform.* **24,** 1656–1674 (1998).
16. Anderson, A. K. Affective influences on attentional dynamics during perceptual encoding: Investigations with the attentional blink. *J. Exp. Psychol. Gen.* (submitted).
17. Anderson, A. K. & Phelps, E. A. Expression without recognition: Contributions of the human amygdala to emotional communication. *Psychol. Sci.* **11,** 106–111 (2000).
18. Adolphs, R., Tranel, D., Damasio, H. & Damasio, A. R. Fear and the human amygdala. *J. Neurosci.* **15,** 5879–5891 (1995).
19. Strange, B. A., Henson, R. N. A., Friston, K. J. & Dolan, R. J. Brain mechanisms for detecting perceptual, semantic, and emotional deviance. *NeuroImage* **12,** 425–433 (2000).
20. Anderson, A. K., Spencer, D. D., Fulbright, R. K. & Phelps, E. A. Contribution of the anteromedial temporal lobes to the evaluation of facial emotion. *Neuropsychology* **14,** 526–536 (2000).
21. Adolphs, R., Damasio, H., Tranel, D., Cooper, G. & Damasio, A. R. A role for somatosensory cortices in the visual recognition of emotion as revealed by three-dimensional lesion mapping. *J. Neurosci.* **20,** 2683–2690 (2000).
22. Funayama, E. S., Grillon, C. G., Davis, M. & Phelps, E. A. A double dissociation in the affective modulation of startle in humans: Effects of unilateral temporal lobectomy. *J. Cogn. Neurosci.* (in press).
23. Kapp, B. S., Wilson, A., Pascoe, J. P., Supple, W. & Whalen, P. J. in *Neurocomputation and Learning: Foundations of Adaptive Networks* (eds Gabriel, M. & Moore, J.) 53–90 (MIT Press, Cambridge, Massachusetts, 1990).
24. Whalen, P. J. Fear, vigilance, and ambiguity: Initial neuroimaging studies of the human amygdala. *Curr. Dir. Psychol. Sci.* **7,** 177–188 (1998).
25. Morris, J. S. *et al.* A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain* **121,** 47–57 (1998).
26. Morris, J. S., Friston, K. J. & Dolan, R. J. Experience-dependent modulation of tonotopic neural responses in human auditory cortex. *Proc. R. Soc. Lond. B* **265,** 649–657 (1998).
27. Isenberg, N. *et al.* Linguistic threat activates the human amygdala. *Proc. Natl Acad. Sci. USA* **96,** 10456–10459 (1999).
28. Weinberger, N. M. in *The Cognitive Neurosciences* (ed. Gazzaniga, M. S.) 1071–1089 (MIT Press, Cambridge, Massachusetts, 1995).
29. Adolphs, R. & Tranel, D. in *The Amygdala: A Functional Analysis* (ed. Aggleton, J. P.) 587–630 (Oxford Univ. Press, New York, 2000).
30. Spencer, D. D., Spencer, S. S., Mattson, R. H., Williamson, P. D. & Novelly, R. A. Access to the posterior medial temporal lobe structure in the surgical treatment of temporal lobe epilepsy. *Neurosurgery* **15,** 667–671 (1984).

Correspondence and requests for materials should be addressed to A.K.A. (e-mail: adam.k.anderson@stanford.edu).

---

# α-CaMKII-dependent plasticity in the cortex is required for permanent memory

**Paul W. Frankland**\*, **Cara O'Brien**†, **Masuo Ohno**\*, **Alfredo Kirkwood**† & **Alcino J. Silva**\*

\* *Departments of Neurobiology, Psychiatry and Psychology, Brain Research Institute, University of California, Los Angeles, California 90095-1761, USA*
† *Department of Neuroscience and Mid/Brain Institute, Johns Hopkins University, Baltimore, Maryland 21218, USA*

**Cortical plasticity seems to be critical for the establishment of permanent memory traces[1–3]. Little is known, however, about the molecular and cellular processes that support consolidation of memories in cortical networks[4,5]. Here we show that mice heterozygous for a null mutation of α-calcium-calmodulin kinase II (α-CaMKII$^{+/-}$) show normal learning and memory 1–3 days after training in two hippocampus-dependent tasks. However, their memory is severely impaired at longer retention delays (10–50 days). Consistent with this, we found that α-CaMKII$^{+/-}$ mice have impaired cortical, but not hippocampal, long-term potentiation. Our results represent a first step in unveiling the molecular and cellular mechanisms underlying the establishment of permanent memories, and they indicate that α-CaMKII may modulate the synaptic events required for the consolidation of memory traces in cortical networks.**

A homozygous null mutation of α-CaMKII blocks hippocampal long-term potentiation (LTP) and learning[6,7], and disrupts experience-dependent plasticity in the sensory cortex without affecting cortical topography[8,9]. In contrast, α-CaMKII heterozygous mice learn normally in hippocampus-dependent tasks under moderate-to-intensive training conditions[7]. We were, therefore, able to study the impact of reduced levels of α-CaMKII on the establishment of permanent memories. We trained α-CaMKII$^{+/-}$ mutants and their wild-type littermate controls in contextual fear conditioning. In contextual fear conditioning, an association is formed between a distinctive context and an aversive event that takes place in that context. When placed back in the context, mice exhibit a range of conditioned fear responses, including freezing (the absence of all but respiratory movement). This is a particularly robust and long-lasting form of learning that is dependent on the hippocampus[10,11]. Importantly, post-training lesions of the hippocampus disrupt recent, but not remote, contextual fear memories[10].

After training with three shocks, α-CaMKII$^{+/-}$ mutants and wild-type mice were placed back in the context at different times (1, 3, 10, 17 or 50 days), and freezing behaviour was recorded using automated procedures[12]. To avoid the confounding effects of extinction, separate groups of mice were tested at each retention delay. Whereas wild-type mice showed stable contextual freezing at all test delays, contextual freezing declined sharply at longer test delays in α-CaMKII$^{+/-}$ mutants (genotype × delay interaction, $F(4, 102) = 17.6$, $P < 0.01$) (Fig. 1a).

During training, α-CaMKII$^{+/-}$ mutants were around 20% more active than the wild-type mice before shock delivery ($F(1, 102) = 14.8$, $P < 0.05$) (data not shown). We therefore assessed contextual memory with a different measure that is not affected by differences in basal activity. In the same mice, we calculated activity suppression by comparing activity levels before a shock delivery during training with activity levels during the test, thus normalizing for initial differences in activity[12]. Although wild-type and α-CaMKII$^{+/-}$ mice showed indistinguishable levels of activity suppression one day after training, at longer delays, activity