Brief Communication

Activation of Midbrain Structures by Associative Novelty and the Formation of Explicit Memory in Humans

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Recent evidence suggests a close functional relationship between memory formation in the hippocampus and dopaminergic neuromodulation originating in the ventral tegmental area and medial substantia nigra of the midbrain. Here we report midbrain activation in two functional MRI studies of visual memory in healthy young adults. In the first study, participants distinguished between familiar and novel configurations of pairs of items which had been studied together by either learning the location or the identity of the items. In the second study, participants studied words by either rating the words' pleasantness or counting syllables. The ventral tegmental area and medial substantia nigra showed increased activation by associative novelty (first study) and subsequent free recall performance (second study). In both studies, this activation accompanied hippocampal activation, but was unaffected by the study task. Thus midbrain regions seem to participate selectively in hippocampus-dependent processes of associative novelty and explicit memory formation, but appear to be unaffected by other task-relevant aspects.

The hippocampal formation plays a critical role in episodic memory (Vargha-Khadem et al. 1997; Düzel et al. 2001; Eichenbaum 2001), and growing evidence from studies in animals and humans suggests that one major contribution of the hippocampus to episodic memory is the encoding of novel stimuli (Tulving et al. 1996; Wan et al. 1999; Lisman and Otmakhova 2001; Vinogradova 2001; Ranganath and Rainer 2003).

Encoding of novel stimuli is associated with synaptic plasticity processes in the hippocampus for which the induction of long-term potentiation (LTP) is generally believed to play an important role (Morris and Frey 1997; Frey and Morris 1998; Morris et al. 2003; Pittenger and Kandel 2003). This link is supported by parallel disruptive effects of impaired NMDA-receptor-dependent (Tsien et al. 1996) and dopaminergic (Li et al. 2003) neurotransmission on LTP and memory performance in animals. LTP induction in the hippocampus is regulated by neuromodulatory input from a wide range of brain regions (Frey and Morris 1998). Exposure to novel stimuli, for instance, activates midbrain dopaminergic neurons which also target the hippocampus (Schultz et al. 1993; Schultz 2000). Rats freely moving in a novel spatial environment have a reduced threshold for LTP induction in a narrow time window, and this facilitation of LTP in the CA1 region can be blocked by D1/D5 receptor antagonists (Li et al. 2003). In aged animals, dopamine agonists can promote hippocampus-dependent learning (Hersi et al. 1995; Bach et al. 1999).

The functional relationship between hippocampal encoding of novel stimuli and dopaminergic neuromodulation and the temporal coupling of dopaminergic activity to novel stimuli within 50–200 msec (Schultz 1998; Lisman and Otmakhova 2001; Vinogradova 2001; Li et al. 2003) implies that increased midbrain activity might be detectable in humans when the hippocampus itself is activated by encoding of novel stimuli. Here we report two event-related functional magnetic resonance imaging (fMRI) studies that show increased activity in midbrain regions including the ventral tegmental area and the medial substantia nigra under conditions that also elicit increased hippocampal activation.

In the first study—an associative recognition memory experiment involving a spatial and a nonspatial condition—novel items elicited an increased neural response in the hippocampus (Düzel et al. 2003). We now report the hemodynamic responses to novel and familiar stimuli in the midbrain area. In the second set of experiments—a memory encoding study with a level of processing (LOP) manipulation—the neural responses to novel words in two different study tasks (deep and shallow study of items) were compared as a function of subsequent remembering or forgetting in a free recall task (for similar approaches, see Fernández et al. 1999; Otten et al. 2002). We report the coactivation of the hippocampal formation and midbrain areas during successful encoding.

Both studies were conducted on the same GE 1.5 T Signa MRI system (General Electric Medical Systems) using a standard quadrature head coil. The data of the first experiment are derived from a recently reported study of associative recognition memory (Düzel et al. 2003). In summary, 10 healthy volunteers participated in the experiment on two days. On a given day, subjects studied, over five trials, pairs of one of five faces and either the type (face-identity condition) or the location (face-location condition) of one of four tools (schematic drawing of a hammer, saw, screwdriver, drill). The order of conditions over days (face-identity first vs. face-location first) was counterbalanced across subjects. Each face was presented in the center of a back projection screen, and the tools appeared in one of the four corner positions. The study task was followed by an associative recognition test, and fMRI data were acquired at this stage. Novel test

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stimuli in the face-identity task consisted of a studied face paired with one of the three tools it had not been paired with during learning, presented in the same location as the original tool. Novel test stimuli in the face-location task consisted of a studied face presented with its paired tool, but in a new location. Subjects were asked to dissociate novel and old stimulus configurations. Each configuration was presented for 3 sec with a 12-sec interstimulus interval. During each recognition test, five old and five novel configurations were presented randomly intermixed. Each functional run during the recognition test consisted of 54 whole-brain volumes (T2* echo planar gradient echo sequence, TR 3.2 sec, TE 40 msec, 30 × 5 gap 1-mm slices perpendicular to the hippocampal axis, field of view = 20 mm, matrix size = 64 × 64, voxel size = 3.13 × 3.13 × 6 mm; for details of data acquisition and analysis see Düzel et al. 2003). Each subject contributed seven study/test runs (except one, who contributed six) to the data set. Analysis was performed using SPM99 (Wellcome Dept. of Imaging Neuroscience, UCL, London). Statistical modeling was carried out using a two-stage mixed effects model. In the first stage, neural activity was modeled by a δ function at stimulus onset for each individual subject. The ensuing BOLD response was modeled by convolving these δ functions with a canonical hemodynamic response function (hrf). The resulting time courses were down-sampled for each scan to form covariates in a General Linear Model. Separate covariates were modeled for all conditions of interest (old vs. new spatial and nonspatial stimulus configurations) and for a constant representing the mean across scans. Parameters for each covariate were estimated by an ordinary least-squares fit to the data. In the second stage, contrasts of the parameter estimates of the individual participants were submitted to a second-level random effects analysis (images of each contrast of interest across the canonical hrf were entered into one-sample t-tests). Because of our a priori hypotheses about the role of the hippocampus and midbrain areas during associative recognition, a level of $P < .01$ (uncorrected) was chosen as the activation threshold. This threshold is slightly lower than the threshold recently used (Düzel et al. 2003).

Sixteen young (age range 18–31), healthy, right-handed native speakers of German (11 female, 5 male) participated in Experiment 2. The experiment consisted of three scanning sessions. Each session comprised three study phases with a deep study task (pleasantness judgment: indication of a pleasant or unpleasant word) and three study phases with a shallow study task (phonemic syllable counting: indication of a word consisting of exactly two syllables). Subjects responded via button press using their right and left index fingers. Response hands were counterbalanced across participants. Each study trial consisted of the presentation of a central fixation cross for 250 msec, a word for 1500 msec, and a further fixation cross for 1000 msec. Twenty trials were presented during each study phase. Study lists were followed by a distracter task consisting of four moderately difficult arithmetic operations. Here, subjects indicated via button press whether the result of two- to three-digit additions was correct. After the distracter task, subjects were prompted to freely recall all studied words they could remember and respond overtly. The duration of the free recall phase was 90 sec. EPI images were acquired during study at a TR of 2.0 sec and a TE of 35 msec. Images consisted of 23 interleaved axial slices (64 × 64, voxel size = 3.13 × 3.13 × 6 mm [slice thickness = 5 mm with 1-mm gap], 544 volumes per session; the first four volumes of each session were discarded). SPM2 (Wellcome Dept. of Imaging Neuroscience) was used for preprocessing and data analysis. EPI images were corrected for acquisition delay, realigned, normalized (voxel size: 3 × 3 × 3 mm), smoothed (Gaussian kernel of 8 × 8 × 8 mm), and high-pass-filtered (128 sec). Statistical analysis was performed using the mixed effects model as described for Experiment 1. The covariates of the general linear model for the individual subjects' contrasts were the conditions of interest (deep remembered, deep forgotten, shallow remembered, shallow forgotten), one covariate time-locked to each speech event (overt response in free recall), six for the rigid-body movement parameters derived from realignment, a 20-sec epoch for the distracter task, and a constant representing the mean over scans. The second-level analysis was computed as in Experiment 1. The significance level was set to .0001 (uncorrected), with a minimum of 24 adjacent voxels.

Ninety percent of the configurations in Experiment 1 were correctly recognized in both tasks, and there were no significant reaction time differences between correctly recognized old (1820.34 msec) and new (1864.25 msec) configurations and no interaction with task. The fMRI results of Experiment 1 are summarized in Figure 1. Compared to old configurations, novel configurations elicited increased activation of the right anterior hippocampus (x, y, z = 24, −14, −16), regardless of the relevant task dimension (face-identity/face-location). The hippocampal activation was greater in the spatial (face-location) compared to the nonspatial (face-identity) task. Midbrain structures were also activated by novel configurations, and this activation was also lateralized to the right side. However, unlike the hippocampus, the midbrain structures did not show the additional pattern of higher activation in the face-location task compared to the face-identity task, and thus did not show evidence of higher activation by spatial task requirements.

In Experiment 2, more deeply studied (32.8%; SD = 9.36) than shallowly studied items (25.0%; SD = 8.57) were recalled [two-way ANOVA for repeated measures; $F(1,15) = 64.2$, $P < .0001$]. This behavioral LOP effect was reflected by increased activity in the bilateral medial prefrontal cortex (BA 6), the left inferior frontal gyrus (BA 47), the left angular gyrus (BA 39), and the right cerebellum for deeply studied items, irrespective of subsequent recall. LOP had no influence on activation of either medial temporal or midbrain regions. Irrespective of LOP, subsequently recalled items showed an increased activity in the bilateral hippocampus (Fig. 2) compared to subsequently forgotten items. Earlier fMRI studies had shown that hippocampal activations are rather correlated with encoding success than with task-related phenomena such as intention (Reber et al. 2002). Subsequently remembered items also showed comparably higher activation in a midbrain region similar to the region that activated in response to novel stimuli in the present Experiment 1. There were no significant reaction time differences for either LOP or subsequent remembering (avg. reaction times for subsequently recalled and subsequently forgotten items: 1489 msec and 1481 msec in the deep and 1464 msec and 1444 msec in the shallow study task).

To further verify the anatomical localization of the midbrain activity observed in both experiments, we superimposed the activation maps of the contrasts of interest on a mean image of five spatially normalized magnetization transfer (MT) images (details on acquisition and image processing available upon request). On MT images the substantia nigra can be easily distinguished from surrounding structures (Eckert et al. 2004). As can be seen in Figures 1 and 2, the midbrain region that shows increased activity for novel configurations (Experiment 1) and subsequent recall (Experiment 2) overlaps partly with the substantia nigra, mostly with its medial portion, and most probably also with the ventral tegmental area, which is medial to the substantia nigra.

These results show that midbrain regions, which are likely to include mesolimbic dopaminergic structures, are activated in humans during associative novelty detection and successful encoding of novel stimuli. In both experiments the activation of mid-

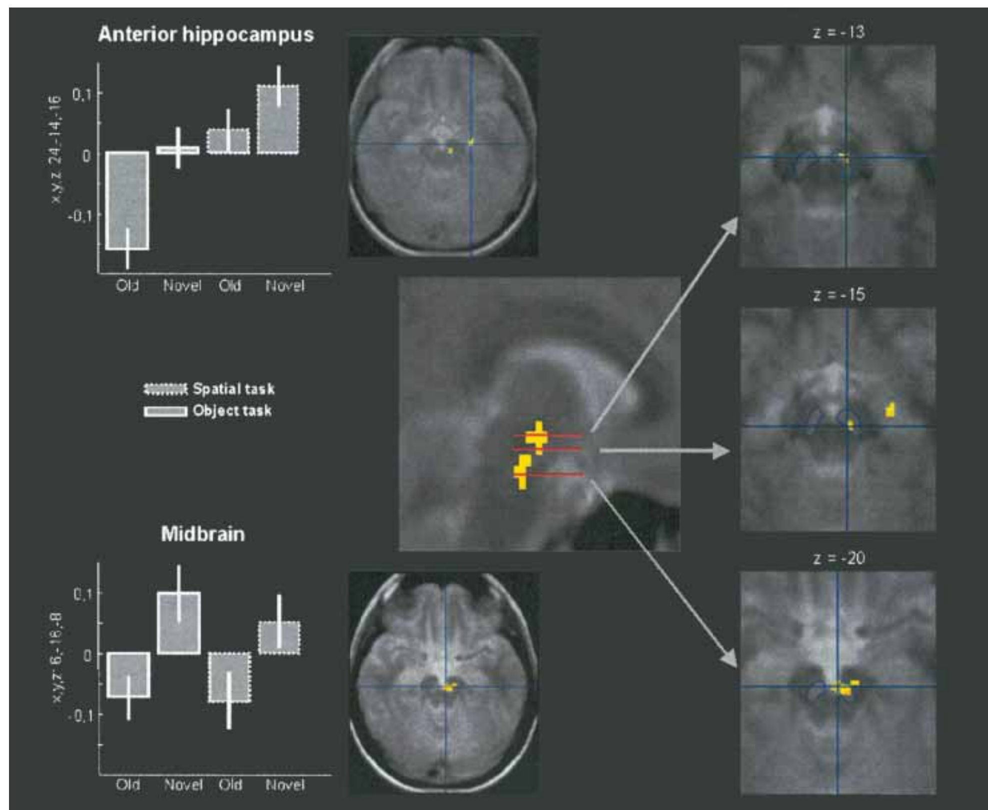

Figure 1 Experiment 1 results. Regions whose hemodynamic responses distinguish novel from old configurations, superimposed on magnetization transfer (MT) images. The substantia nigra has been segmented (blue). Bar plots show contrasts of parameter estimates (from the peak voxel) for old and novel configurations in the spatial/nonspatial conditions. Activations in the anterior hippocampus are greater for correctly rejected novel configurations compared to recognized old configurations, particularly in the spatial task. Activation in the midbrain dopaminergic area also shows a preference for associative novelty but not for spatial relationship. x,y,z: voxel coordinates in MNI (Montreal Neurological Inst.) reference space.

brain regions occurred under conditions that were also associated with hippocampal activation. This coactivation of the hippocampus and midbrain structures was detected after presenting different stimulus material (face-tool or face-location associations vs. novel words) and during different memory tasks (old/new decision vs. incidental episodic memory encoding). Importantly, the common aspect of both experiments was their 'episodic' quality. In the first experiment, the 'novelty' of stimuli was not contingent upon single items, but rather due to the rearrangement of learned configurations of item pairs with single-item information being highly familiar (Wan et al. 1999; Düzel et al. 2003). Such associative novelty is likely to be more episodic in nature and more dependent on the hippocampus than single-item novelty, which is common to other MTL structures that show decreased activity to familiar (repeated) stimuli such as the perirhinal cortex (Brown and Aggleton 2001; Henson et al. 2003). In Experiment 2, all stimuli were novel (previously unstudied words) and the retrieval task required free recall, which is typically dependent upon intact episodic memory (Vargha-Khadem et al. 1997). Thus, we observed midbrain activity under conditions where hippocampal processing of novel stimuli was very likely to reflect episodic memory rather than accompanying familiarity-related memory phenomena that can also be sustained by parahippocampal regions alone (Brown and Aggleton 2001; Düzel et al. 2001).

One possible explanation of our results might be that increased hippocampal activity during encoding of novel stimuli exerts an excitatory influence on midbrain dopaminergic neurons. The hippocampus, however, lacks direct projections to the midbrain dopaminergic areas. A potential hippocampally mediated modulation of these areas would therefore have to be conveyed indirectly through output targets of the hippocampus such as medial prefrontal cortex, the nucleus accumbens, or the lateral septal nucleus (areas which also showed increased activation for successful encoding in Experiment 2 [data not shown]; see Lisman and Otmakhova 2001), which in turn could send excitatory input to the midbrain dopaminergic areas. This excitatory activity might stimulate midbrain dopaminergic areas, which could then exert a regulatory effect on hippocampal synaptic plasticity, for example by facilitating LTP. In the so-called SOCRATIC model of Lisman and Otmakhova (2001), increased dopaminergic drive to the hippocampus improves synaptic plasticity for the encoding of novel stimuli at the CA3–CA1 synapses and prevents interference due to other incoming information via direct connections between entorhinal cortex and CA1. Alternative accounts for the functional interaction of midbrain and hippocampal areas might be that both are activated simultaneously, perhaps independently. Such a coactivation might lead to a modulatory influence of midbrain dopaminergic activity on the simultaneously active hippocampus. Finally, another possibility is that dopaminergic stimulation drives the hippocampal response to novel stimuli. In fact, aside from novelty, midbrain dopaminergic neurons respond also as a function of failure to predict reward (Schultz 1998), and it is possible that such a response does not need hippocampal processing.

Our findings of increased activation of the hippocampus and the midbrain areas during explicit memory processes suggest that—irrespective of task and stimulus types—transmitter net-

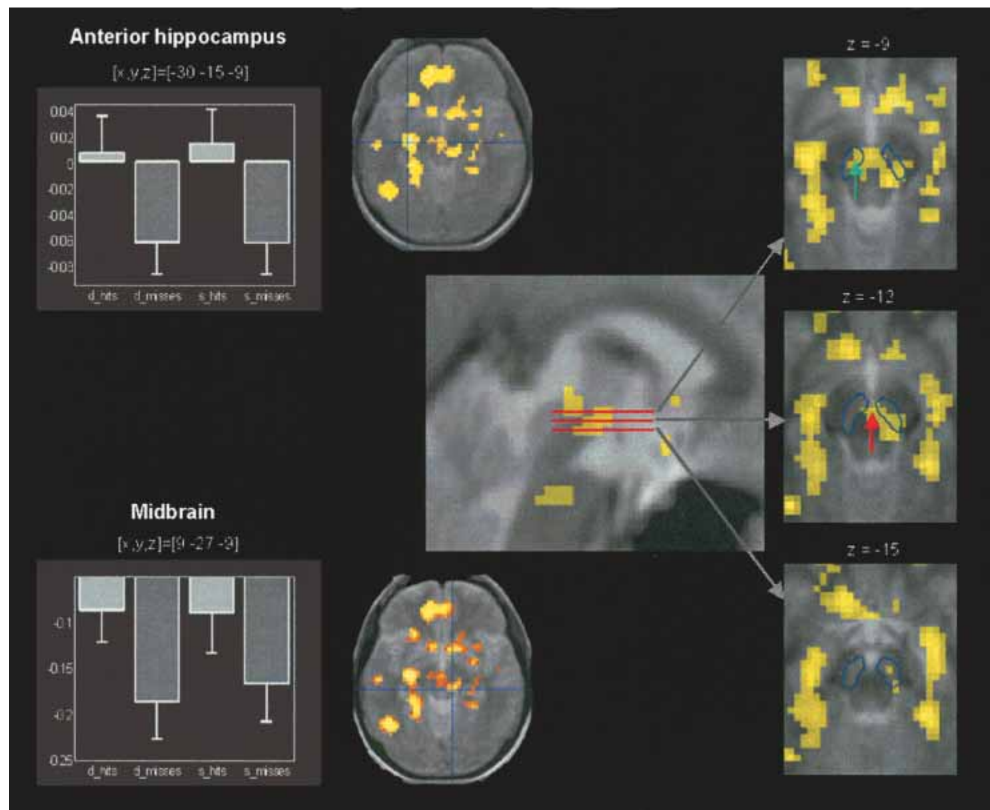

Figure 2 Experiment 2 results. Superimposition of the midbrain activations onto the average MT image. The substantia nigra has been segmented (blue). Bar plots depict the contrasts of parameter estimates for deep remembered (d_hits), deep forgotten (d_misses), shallow remembered (s_hits), and shallow forgotten (s misses). Green arrow: medial substantia nigra. Red arrow: approximate location of the ventral tegmental area.

works identified in animal studies can be studied in humans indirectly via their large-scale neural signature. However, our observation is, thus far, a purely anatomical one. Recent developments in the integration of pharmacological and molecular genetic approaches into neuroimaging (Egan et al. 2003; Mattay et al. 2003; Thiel 2003) might help to further elucidate the role of neuromodulatory transmitter systems in human memory processes.

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REFERENCES

Bach, M.E., Barad, M., Son, H., Zhuo, M., Lu, Y.F., Shih, R., Mansuy, I., Hawkins, R.D., and Kandel, E.R. 1999. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc. Natl. Acad. Sci.* **96:** 5280–5285.

Brown, M. and Aggleton, J.P. 2001. Recognition memory: What are the roles of the perirhinal cortex and the hippocampus. *Nat. Rev. Neurosci.* **2:** 51–61.

Düzel, E., Vargha-Khadem, F., Heinze, H.J., and Mishkin, M. 2001. Brain activity evidence for recognition without recollection after early hippocampal damage. *Proc. Natl. Acad. Sci.* **98:** 8101–8106.

Düzel, E., Habib, R., Rotte, M., Guderian, S., Tulving, E., and Heinze, H.J. 2003. Human hippocampal and parahippocampal activity during visual associative recognition memory for spatial and non-spatial stimulus configurations. *J. Neurosci.* **23:** 9439–9444.

Eckert, T., Sailer, M., Kaufmann, J., Schrader, C., Peschel, T., Bodammer, N., Heinze, H.J., and Schoenfeld, M.A. 2004. Differentiation of idiopathic Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, and healthy controls using magnetization transfer imaging. *Neuroimage* **21:** 229–235.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., et al. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112:** 257–269.

Eichenbaum, H. 2001. The hippocampus and declarative memory: Cognitive mechanisms and neural codes. *Behav. Brain Res.* **127:** 199–207.

Fernández, G., Effern, A., Grunwald, T., Pezer, N., Lehnertz, K., Dumpelmann, M., Van Roost, D., and Elger, C.E. 1999. Real-time tracking of memory formation in the human rhinal cortex and hippocampus. *Science* **285:** 1582–1585.

Frey, U. and Morris, R.G. 1998. Synaptic tagging: Implications for late maintenance of hippocampal long-term potentiation. *Trends Neurosci.* **21:** 181–188.

Henson, R.N., Cansino, S., Herron, J.E., Robb, W.G., and Rugg, M.D. 2003. A familiarity signal in human anterior medial temporal cortex? *Hippocampus* **13:** 301–304.

Hersi, A.I., Rowe, W., Gaudreau, P., and Quirion, R. 1995. Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acetylcholine release in memory-impaired aged rats. *Neuroscience* **69:** 1067–1074.

Li, S., Cullen, W.K., Anwyl, R., and Rowan, M.J. 2003. Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat. Neurosci.* **6:** 526–531.

Lisman, J.E. and Otmakhova, N.A. 2001. Storage, recall, and novelty detection of sequences by the hippocampus: Elaborating on the SOCRATIC model to account for normal and aberrant effects of dopamine. *Hippocampus* **11:** 551–568.

Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., Kolachana, B., Callicott, J.H., and Weinberger, D.R. 2003. Catechol O-methyltransferase val158-met genotype and individual

variation in the brain response to amphetamine. *Proc. Natl. Acad. Sci.* **100:** 6186–6191.
Morris, R.G. and Frey, U. 1997. Hippocampal synaptic plasticity: Role in spatial learning or the automatic recording of attended experience? *Philos. Trans. R Soc. Lond. B Biol. Sci.* **352:** 1489–1503.
Morris, R.G., Moser, E.I., Riedel, G., Martin, S.J., Sandin, J., Day, M., and O'Carroll, C. 2003. Elements of a neurobiological theory of the hippocampus: The role of activity-dependent synaptic plasticity in memory. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **358:** 773–786.
Otten, L.J., Henson, R.N., and Rugg, M.D. 2002. State-related and item-related neural correlates of successful memory encoding. *Nat. Neurosci.* **5:** 1339–1344.
Pittenger, C. and Kandel, E.R. 2003. In search of general mechanisms for long-lasting plasticity: Aplysia and the hippocampus. *Philos. Trans. R Soc. Lond. B Biol. Sci.* **358:** 757–763.
Ranganath, C. and Rainer, G. 2003. Neural mechanisms for detecting and remembering novel events. *Nat. Rev. Neurosci.* **4:** 193–202.
Reber, P.J., Siwiec, R.M., Gitelman, D.R., Parrish, T.B., Mesulam, M.M., and Paller, K.A. 2002. Neural correlates of successful encoding identified using functional magnetic resonance imaging. *J. Neurosci.* **22:** 9541–9548.
Schultz, W. 1998. Predictive reward signal of dopamine neurons. *J. Neurophysiol.* **80:** 1–27.
———. 2000. Multiple reward signals in the brain. *Nat. Rev. Neurosci.* **1:** 199–207.
Schultz, W., Apicella, P., and Ljungberg, T. 1993. Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J. Neurosci.* **13:** 900–913.
Thiel, C.M. 2003. Cholinergic modulation of learning and memory in the human brain as detected with functional neuroimaging. *Neurobiol. Learn. Mem.* **80:** 234–244.
Tsien, J.Z., Huerta, P.T., and Tonegawa, S. 1996. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* **87:** 1327–1338.
Tulving, E., Markowitsch, H.J., Craik, F.E., Habib, R., and Houle, S. 1996. Novelty and familiarity activations in PET studies of memory encoding and retrieval. *Cereb. Cortex* **6:** 71–79.
Vargha-Khadem, F., Gadian, D.G., Watkins, K.E., Connelly, A., Van Paesschen, W., and Mishkin, M. 1997. Differential effects of early hippocampal pathology on episodic and semantic memory. *Science* **277:** 376–380.
Vinogradova, O.S. 2001. Hippocampus as comparator: Role of the two input and two output systems of the hippocampus in selection and registration of information. *Hippocampus* **11:** 578–598.
Wan, H., Aggleton, J.P., and Brown, M.W. 1999. Different contributions of the hippocampus and perirhinal cortex to recognition memory. *J. Neurosci.* **19:** 1142–1148.

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