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Reward-Related fMRI Activation of Dopaminergic Midbrain Is Associated with Enhanced Hippocampus-Dependent Long-Term Memory Formation

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Summary

Long-term potentiation in the hippocampus can be enhanced and prolonged by dopaminergic inputs from midbrain structures such as the substantia nigra. This improved synaptic plasticity is hypothesized to be associated with better memory consolidation in the hippocampus. We used a condition that reliably elicits a dopaminergic response, reward anticipation, to study the relationship between activity of dopaminergic midbrain areas and hippocampal long-term memory in healthy adults. Pictures of object drawings that predicted monetary reward were associated with stronger fMRI activity in reward-related brain areas, including the substantia nigra, compared with non-reward-predicting pictures. Three weeks later, recollection and source memory were better for reward-predicting than for non-reward-predicting pictures. FMRI activity in the hippocampus and the midbrain was higher for reward-predicting pictures that were later recognized compared with later forgotten pictures. These data are consistent with the hypothesis that activation of dopaminergic midbrain regions enhances hippocampus-dependent memory formation, possibly by enhancing consolidation.

Introduction

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; Straube et al., 2003a, 2003b).

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Recently, evidence is accumulating that LTP induction and maintenance in the area CA1 of the hippocampus are critically modulated by dopaminergic input from midbrain neurons (Frey et al., 1990, 1991; Frey and Morris, 1998; Li et al., 2003; for a review, see Jay, 2003). Exposure to novel stimuli, for instance, activates midbrain dopaminergic neurons, which also target the hippocampus (Schultz, 1998). 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).

These findings are paralleled by data showing aversive effects of dopamine depletion on memory performance. In aged humans, deficits in episodic memory are better accounted for by D2 receptor binding than by age (Bäckman et al., 2000). In aged animals, dopamine agonists can promote hippocampus-dependent learning (Hersi et al., 1995; Bach et al., 1999). Animals show impairment of object recognition memory and spatial memory after neurotoxic lesions to dopaminergic neurons (Gasbarri et al., 1996; Schröder et al., 2003) as well as reduced maze learning after D2 antagonists injected into the rat hippocampus (Umegaki et al., 2001). In humans, the reduced level of striatal dopamine in methamphetamine abusers is correlated with verbal memory impairment (Volkow et al., 2001). In patients with early Alzheimer's disease, hippocampal D2 receptor availability is correlated with verbal memory performance (Kemppainen et al., 2003). In healthy human subjects, levodopa enhances learning success and long-term retention of repetitively presented words (Knecht et al., 2004).

The link between memory formation and dopaminergic neuromodulation has recently also been supported by functional magnetic resonance imaging (fMRI) data in healthy humans (Schott et al., 2004). We have shown that activity of the ventral tegmental area and medial substantia nigra accompanied hippocampal activity related to memory formation, in that both structures were activated by novelty and in relation to subsequent free recall performance (Schott et al., 2004).

It has been hypothesized that dopaminergic input to the hippocampus enhances consolidation in the hippocampus. Activation of D1/D5 receptors in CA1 enhances consolidation of inhibitory avoidance in rats, whereas blockade of these results in amnesia (Bernabeu et al., 1997). Dopaminergic neuromodulation is required for the maintenance of LTP (i.e., "late LTP") in hippocampal CA1 (Frey et al., 1990, 1991; Huang and Kandel, 1995). The prolonged maintenance of LTP—late LTP—requires the synthesis of new macromolecules during its induction, a property which is similar to processes during the consolidation of memory at the systems level. If the hypothesis that dopaminergic input to the hippocampus enhances consolidation in the hippocampus is correct, conditions that are associated with increased activity of dopaminergic midbrain regions should increase episodic memory performance after long retention de-

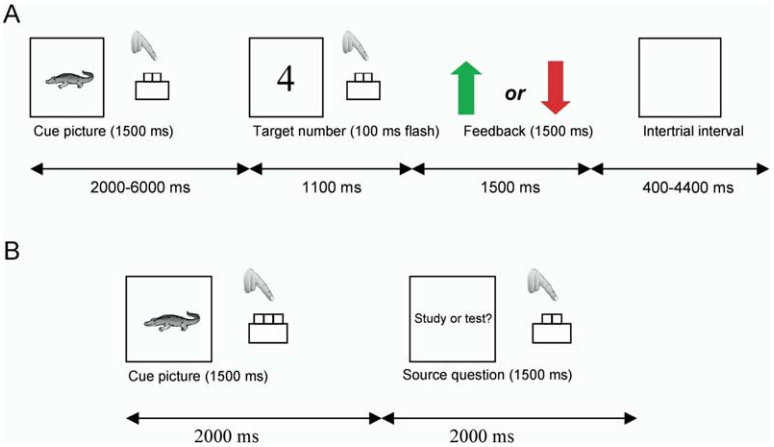

Figure 1. Experimental Design

(A) Trial sequence for study phase, exemplarily shown for a rewarded trial. On neutral trials, a question mark appeared instead of the green or red arrow.

(B) Trial sequence for the delayed memory test. After the remember/know/new decision, participants indicated the source of recognized items.

lays, when the processes underlying consolidation should be finished.

A condition that reliably activates dopaminergic neurons in the midbrain is reward anticipation (for a review, see Wise, 2004). In animal studies, single neurons are activated by conditioned stimuli and not by unconditioned rewarding stimuli after completion of the conditioning procedure (Schultz et al., 1992; Schultz, 1998). Human fMRI studies have demonstrated the activation of dopaminergic areas (dorsal and ventral striatum, globus pallidus, substantia nigra) by reward-predicting stimuli (Knutson et al., 2001a, 2001b; O'Doherty et al., 2002; McClure et al., 2003; Kirsch et al., 2003). Results indicate that some of these areas are no longer activated by reward receipt when the contingency between predicting stimulus and reward delivery has been learned (Knutson et al., 2001b; O'Doherty et al., 2002) and that rewarding outcomes are registered by mesial frontal cortex instead (Knutson et al., 2003).

To study the link between dopaminergic midbrain activity and hippocampus-dependent memory formation in humans, we used an event-related fMRI design in a reward anticipation paradigm with a modulated monetary incentive delay task (Knutson et al., 2000). Pictures of living and nonliving things served as cues predicting whether a following number comparison task was rewarded or not (Figure 1). Participants were asked to indicate whether they expected a reward or neutral trial but not told that a memory task for these pictures would follow. The difficulty of the number comparison task was adjusted so that approximately 80% of reward trials were followed by a correct response and a reward, and the rest were followed by a mild punishment. The fMRI data of the study phase were analyzed according to performance in an immediate and a delayed subsequent memory test (difference due to memory [DM] analyses; for a review, see Paller and Wagner, 2002). In the immediate memory test, approximately 2 min after the study phase, participants made old/new judgments on randomly mixed studied and unstudied pictures. In the delayed test (3 weeks later), participants were shown the same test pictures in a remember/know recollection paradigm (Tulving, 1985). For each recognized picture, a source memory question followed asking whether the picture had been first presented in the study or test phase. This approach made it possible to study neuromodulation by comparing the encoding activation of reward-predicting and neutral items that were recognized and forgotten after short and long retention intervals.

Given the reward anticipation property of the dopaminergic system, we expected the reward cue pictures to be associated with an increased hemodynamic response in the dopaminergic reward system including the midbrain dopaminergic areas without making the pictures themselves rewarding stimuli, or reward delivery dependent on the reaction to the cue pictures or on learning success. We expected that reward anticipation should improve hippocampus-dependent long-term memory formation. In the delayed memory test, this should be associated with improved recollection and higher source memory accuracy of reward-predicting pictures compared with the neutral pictures. This behavioral improvement of long-term memory should be accompanied by increased activity of dopaminergic midbrain areas and of the hippocampus at the time of presentation of the reward-predicting stimuli that were recognized after 3 weeks delay. The absence of such increased activity for reward-predicting stimuli recognized in the immediate test would be compatible with animal data that dopaminergic neuromodulation improves hippocampus-dependent consolidation. Its presence already for reward-predicting stimuli recognized in the immediate test, on the other hand, would make a consolidation account less likely.

Results

Reward Task

Participants correctly recognized the cues signaling rewarded or neutral trials (97% ± 1%). Reaction times for reward-predicting cues were significantly shorter than those for neutral cues (708 ± 27 ms versus 770 ± 20 ms; $p < 0.001$). In the number comparison task, reaction times and correct response rates differed significantly across conditions. Participants gained money on an average of 81% ± 2% of reward trials, approximating the targeted 80% correct response rate, whereas the

Table 1. Anatomical Locations of Regions Responding to Reward Anticipation

Structure	Left/Right	Talairach Coordinates x	y	z	z Score
Insula (BA 13)	R	45	9	–3	4.41
Anterior cingulate (BA 25)	L	–3	5	–8	4.06
Medial frontal gyrus (BA 6)	L	0	2	50	4.38
Precentral gyrus (BA 4, BA 6)	L	–33	–8	64	4.16
	R	45	–9	47	4.34
	R	45	–11	61	4.23
Cingulate gyrus (BA 23)	R	6	–16	28	3.88
Postcentral gyrus (BA 5, BA 3)	L	–39	–26	57	3.47
	L	–36	–46	63	4.04
Inferior parietal lobule (BA 40)	L	–50	–30	40	3.71
	L	–53	–36	29	3.58
	R	68	–25	23	4.03
Precuneus (BA 7)	R	18	–44	55	4.03
	R	9	–61	56	3.63
Fusiform gyrus (BA 37)	L	–50	–53	–18	3.87
Putamen	L	–15	11	–8	3.86
	R	15	3	–8	3.80
Caudate	R	9	9	8	3.70
Globus pallidus	L	–18	–3	–7	3.90
Substantia nigra/midbrain	R	6	–21	–12	3.54
Thalamus	L	–15	–22	18	3.71
Cerebellum	R	3	–65	–27	3.62

Data are thresholded at $p < 0.0005$ (uncorrected), and only clusters with >10 voxels are reported.

average correct response rate for nonrewarded trials was 68% ± 4% ($p < 0.01$). Reaction times in rewarded trials were significantly shorter (519 ± 18 ms) than those in nonrewarded trials (562 ± 17 ms; $p = 0.001$).

The fMRI contrast between reward cues and neutral cues revealed significant ($p < 0.0005$) activations of the dopaminergic system: large parts of striatum (bilateral putamen, right caudate, bilateral nucleus accumbens), left globus pallidus, and right substantia nigra (Table 1; Figure 2). Other areas known to be activated by reward anticipation such as insula, anterior cingulate, and thalamus were also activated.

Unlike this anticipation contrast (i.e., reward-predicting cue versus neutral cue), reward outcome (reward versus neutral outcome) was not associated with activation of the dopaminergic system. Instead, reward outcome was associated with significant activations in right middle frontal gyrus (Brodmann area [BA] 10), secondary visual areas, fusiform gyrus, cerebellum, anterior cingulate, posterior cingulate, and thalamus ($p < 0.005$) when compared to neutral outcome. This expected dissociation between reward anticipation and reward outcome was further confirmed by masking the outcome contrast (reward versus neutral outcome) with the anticipation contrast (reward-predicting cue versus neutral cue; thresholded at 0.0005). The only common activation resulting from this masking was in the thalamus ($p < 0.005$; min. cluster size 5 voxels).

Immediate Memory Task

Hit rate (correct "old" responses to studied pictures) did not differ across conditions (average 77% ± 2%). The average rate of correct rejections (correct "new" responses to unstudied pictures) was 86% ± 2%.

At the time of test, the fMRI contrast between hits and misses (incorrect new responses to studied pictures) revealed higher activity for hits in the right temporal lobe (BA 20; $p < 0.005$). Hits belonging to previously rewarded categories elicited higher activity in left insula, right amygdala, left cuneus, superior tempo-

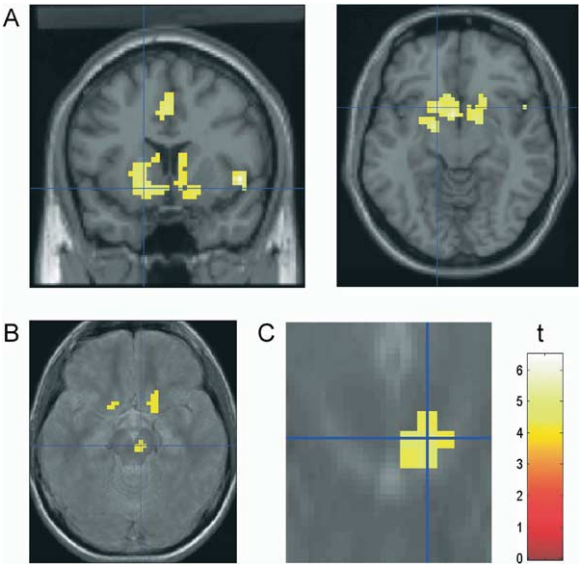

Figure 2. Reward Anticipation Response

The contrast for reward-predicting versus neutral cues revealed (A) significant striatal, cingulate, and insular activations, displayed for a coronal and the corresponding transversal slice, and (B) activation of substantia nigra, displayed for a transversal slice. (C) Magnified coronal section of the same nigral activation. In (B) and (C), activation maps are superimposed on a magnetization transfer picture. $p < 0.0005$; peak voxel (A) (x, y, z) = –15, 11, –8; peak voxel (B and C) (x, y, z) = 6, –21, –12.

Table 2. Anatomical Locations of Regions Active during Successful Encoding as Tested by the Immediate Memory Test

Structure	Left/Right	Talairach Coordinates x	y	z	z Score
Inferior frontal gyrus (BA 47)	R	30	31	−14	3.50
Insula (BA 13)	L	−36	−7	25	2.74
Precentral gyrus (BA 6, BA 4)	L	−39	−13	31	3.52
	L	−15	−29	71	3.33
Parahippocampal gyrus (BA 30)	L	−12	−38	−3	4.06
Fusiform gyrus (BA 37, BA 20)	L	−45	−53	−12	3.38
	R	42	−36	−16	3.70
Cuneus (BA 18)	R	12	−72	15	3.13
Superior parietal lobule (BA 7)	L	−30	−73	45	3.13
Superior occipital gyrus (BA 19)	R	36	−83	26	3.22
Middle occipital gyrus (BA 18, BA 19)	L	−36	−93	7	3.40
	R	48	−56	−7	3.35
Cerebellum	L	−33	−59	−15	3.00

Data are thresholded at $p < 0.005$, and only clusters with >10 voxels are reported.

ral gyrus, and cerebellum when compared with hits belonging to previously unrewarded categories.

In the subsequent memory ("DM") analysis of study trials, pictures that were subsequently recognized showed a stronger activation of several brain regions, including left insula, fusiform gyrus, secondary visual areas, left prefrontal cortex, and parahippocampal gyrus when compared with pictures that were subsequently missed (Table 2; Figure 3).

Separate DM analyses of reward-predicting and neutral items revealed different patterns of brain activity. Reward-predicting pictures that were subsequently recognized were not different (at a threshold of $p < 0.005$; min. cluster size 5 voxels) from reward-predicting pictures that were subsequently missed. In contrast, neutral pictures that were subsequently recognized showed a stronger activation than neutral pictures that were subsequently missed in regions including left PFC, fusiform cortex, occipital areas, and left parahippocampal gyrus (BA 30).

Delayed Memory Task

Old items from the reward task were better recognized than new distractor items from the immediate memory task, irrespective of reward condition (63% ± 3% and 44% ± 2%, respectively; $p < 0.001$).

For the old items, there was a significantly higher rate of remember responses for previously reward-predicting than for previously non-reward-predicting pictures (29% ± 4% versus 20% ± 3% [one-tailed Student's t test]; $p < 0.05$). In the source recognition task, performance for old reward-predicting items was higher than that for old neutral items (61% ± 3% versus 55% ± 3%; $p < 0.05$). Only the source retrieval rate for old reward-predicting items, not the one for neutral or new pictures, was significantly above chance level.

The DM analysis showed an increased hemodynamic response in the dopaminergic system (right caudate, right substantia nigra), cingulate gyrus, and hippocampus/parahippocampal gyrus, for subsequently recognized versus subsequently missed items (Table 3; Figures 4A–4C). When this contrast was masked with the reward anticipation contrast (thresholded at 0.0005), significant ($p < 0.005$; min. cluster size 5 voxels), activation of left nucleus caudatus and right substantia nigra remained, as well as precentral gyrus (BA 6) and postcentral gyrus (BA 2). This showed that the same region in the substantia nigra was activated by reward anticipation and in relation to successful memory formation.

Separate DM analyses for reward-predicting and neutral items were conducted in order to assess whether activity in the substantia nigra was related to successful memory formation only for reward-predicting items. Indeed, for later recognized reward-predicting pictures, a significantly increased hemodynamic response occurred in right substantia nigra (other regions were left insula and secondary visual areas [BA 18 and BA 19]; $p < 0.005$), whereas the substantia nigra

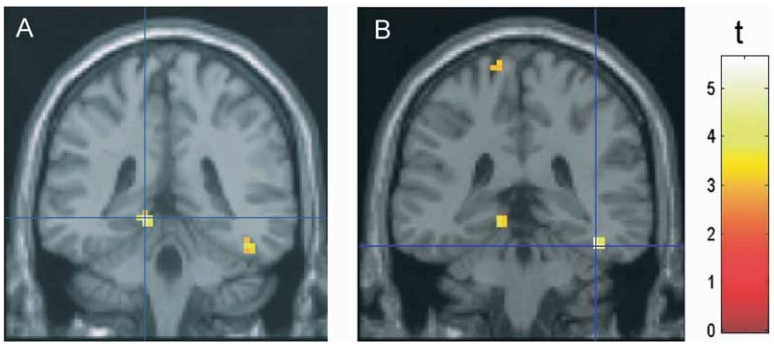

Figure 3. Subsequent Memory Effect: Immediate Test

Significant left parahippocampal (A) and right fusiform (B) activations for subsequently recognized versus subsequently forgotten pictures from the immediate memory test ($p < 0.005$). Peak voxel (A) (x, y, z) = −12, −38, −3; peak voxel (B) (x, y, z) = 42, −36, −16.

Table 3. Anatomical Locations of Regions Active during Successful Encoding as Tested by the Delayed Memory Test

Structure	Left/Right	Talairach Coordinates			z Score
		x	y	z	
Middle frontal gyrus (BA 46)	L	–53	30	23	3.68
Cingulate gyrus (BA 24, BA 31)	L	–6	–1	30	3.93
	L	–21	–21	45	3.79
	L	–15	–15	42	2.86
	R	15	–13	34	2.89
Middle temporal gyrus (BA 21)	L	–62	–12	–7	3.31
Parahippocampal gyrus (BA 36)/hippocampus	R	42	–27	–11	3.48
Superior parietal lobule (BA 7)	R	18	–70	59	3.36
Middle occipital gyrus (BA 19)	R	39	–84	15	4.44
Caudate	R	15	–7	28	2.89
Substantia nigra/midbrain	R	9	–21	–14	3.10

Data are thresholded at $p < 0.005$, and only clusters with >10 voxels are reported.

was not part of the DM effect for neutral items (here effects were in the right red nucleus, left cingulate gyrus [BA 24], and left parahippocampal gyrus [BA 30]; $p < 0.005$).

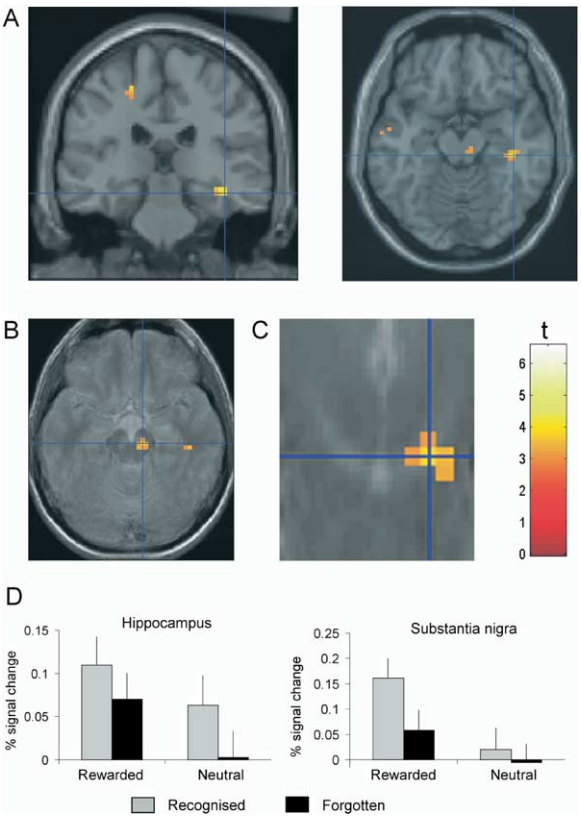

Figure 4. Subsequent Memory Effect: Delayed Test

(A–C) Significant ($p < 0.005$) activations of hippocampus (A) and substantia nigra (A–C) for subsequently recognized versus subsequently forgotten pictures from the delayed memory test, displayed (A) for a coronal and a transversal slice, (B) for a transversal slice, and (C) for the corresponding magnified coronal section. In (B) and (C), activation maps are superimposed on a magnetization transfer picture. Peak voxel (A) (x, y, z) = 42, –27, –11; peak voxel (B and C) (x, y, z) = 9, –21, –14.

(D) Comparison of the percent signal change for recognized and forgotten reward-predicting and neutral pictures, extracted from the peak voxel of hippocampus (left) and substantia nigra (right).

To further investigate the relationship between reward anticipation, memory formation, and activity of substantia nigra and hippocampus/parahippocampal gyrus, we conducted a 2 (factor reward: reward-predicting versus neutral) by 2 (factor memory: recognized versus forgotten) repeated measures analysis of variance (ANOVA) of percent signal change for the peak voxels in both regions. For the substantia nigra, this revealed significant main effects of reward [$F(1, 15) = 6.8$; $p < 0.05$] and later memory performance [$F(1, 15) = 14.0$; $p < 0.01$] as well as a reward by memory interaction [$F(1, 15) = 4.6$; $p < 0.05$; Figure 4D]. For the hippocampus, this revealed significant main effects of reward [$F(1, 15) = 4.9$; $p < 0.05$] and memory [$F(1, 15) = 19.5$; $p = 0.001$] but no interaction (Figure 4D).

Category Differences

In the immediate memory test, there was a significant difference in false alarm rate between pictures of living things (19% ± 3%) and those of man-made objects (9% ± 2%; $p < 0.01$) that did not depend on reward status. In the delayed memory test, there was a higher recognition rate for old pictures of living things (68% ± 5%) than for old pictures of man-made objects (58% ± 4%; $p < 0.05$). These results were unexpected, but the counterbalancing of the living and object categories with respect to reward status across subjects made category differences irrelevant for the analysis of reward effects.

Discussion

Reward was associated with a significant increase in correct performance and decrease in reaction time in the number comparison task when compared with the neutral trials. In the fMRI analysis, reward-predicting stimuli were associated with activation of known reward-related areas, including the substantia nigra (this activity will be referred to as "midbrain" because it might also include ventral tegmental area), nucleus accumbens, caudate nucleus, and putamen (Table 1; Figure 2). Reward outcome, on the other hand, was associated with activation in the medial prefrontal cortex (BA 10), which confirms previous findings that this region seems to register the rewarding valence of predicted outcomes (Knutson et al., 2003). This expected dissoci-

ation of the anticipation and outcome phases was supported by a lack of relevant overlap of activated areas.

In the delayed memory test, reward-predicting stimuli were given a higher rate of remember responses and were associated with a better source memory when compared with neutral items. As both a remember response and source memory require the recollection of context, reward anticipation seems to have specifically improved the hippocampus-dependent episodic memory aspect of recognition memory, but not the familiarity-based, nonepisodic aspect of recognition memory (Düzel et al., 2001; Yonelinas et al., 2002). This supports our hypothesis that the hippocampus is a major site for the neuromodulatory influence of reward on long-term memory formation.

The link between hippocampus-dependent memory formation and neuromodulation by reward was supported by the fMRI findings. We found higher activity in dopaminergic midbrain areas (most likely medial substantia nigra) and caudate nucleus for pictures that were later recognized compared with forgotten pictures in the delayed memory test (Table 3; Figure 4). This difference in activity during encoding was apparent only for the reward-predicting pictures but not for the neutral pictures. In the same late DM comparison, the higher activity in dopaminergic areas was paralleled by higher activity in the posterior hippocampus. Hippocampal activation has been associated with successful episodic memory formation in a number of previous human studies (e.g., Brewer et al., 1998; Davachi and Wagner, 2002; Schott et al., 2004). The finding of an association between hippocampal activity and activity of the dopaminergic midbrain (Table 3; Figure 4) supports the hypothesis that dopaminergic neuromodulation enhances hippocampus-dependent memory formation. This link is compatible with recent models of dopaminergic neuromodulation of hippocampus-dependent memory formation (Lisman and Otmakhova, 2001) and is consistent with evidence from rodent studies that firing of hippocampal cells increases in baited but not unbaited maze arms (Hölscher et al., 2003) and that the synchronization of hippocampal and accumbal neurons is stronger in the presence of reward (Tabuchi et al., 2000). The design of our study, in turn, might have some ecological validity for animal studies of foraging behavior.

While a subsequent memory-related activity increase in the dopaminergic midbrain was absent for the neutral stimuli, a memory-related activity increase in the hippocampus was common to both reward-predicting and neutral stimuli (Table 3; Figure 4). A stronger hippocampal activation for subsequently recognized than for forgotten neutral stimuli replicates previous reports of encoding-related hippocampal activation for stimuli that were not learned in the context of reward (Brewer et al., 1998; Davachi and Wagner, 2002; Schott et al., 2004). Such a common hippocampal activation for reward-predicting and neutral pictures also suggests that dopaminergic neuromodulation affected hippocampal memory mechanisms that were not specific to reward anticipation but were also utilized to memorize neutral stimuli.

Unlike in the late DM comparison, neither reward-predicting nor neutral stimuli showed a midbrain or hippocampal activity difference between subsequently recognized and forgotten items in the early DM comparison (Table 2; Figure 3). This absence of midbrain and hippocampal activity in the early DM comparison, in the face of their presence in the late DM comparison, indicates a stronger link between joint activation of midbrain and hippocampus with long-term memory than with immediate memory. This stronger link to long-term memory is compatible with physiological evidence that dopaminergic neuromodulation is necessary for late LTP to occur but does not affect the expression of early LTP, thereby influencing consolidation (Frey et al., 1990; Huang and Kandel, 1995). In accordance with a consolidation account for this link, we suggest that a larger proportion of reward-predicting than of neutral stimuli remained recollectable after the long delay due to improved consolidation. An alternative possibility is that the reward-related behavioral improvement in the delayed memory test merely mirrored a similar benefit already present in the immediate memory test. Despite the absence of a midbrain and hippocampal activity difference for the reward-predicting and neutral stimuli in the early DM effect, and despite equal recognition memory performance for both stimulus types in the immediate memory test, we cannot entirely rule out this latter possibility, because an accurate behavioral estimate of recollection was available only in the delayed memory test.

Recently, we observed a subsequent memory-related activation of midbrain regions in an immediate memory test (Schott et al., 2004). However, unlike in the present study, this immediate memory test required free recall of studied items and thus was more difficult and tapped more directly into episodic memory than the recognition memory test used here. It is likely that a greater proportion of subsequently recallable items will undergo hippocampus-dependent consolidation than of subsequently recognizable items. Thus, if our current immediate memory task had been more difficult and tapped more directly into episodic memory, allowing the sampling of a greater proportion of items that would undergo hippocampus-dependent consolidation, we would probably have observed a subsequent memory-related activation of midbrain regions in the immediate test as well. Note, however, that in the absence of a behavioral difference in memory performance in the immediate memory test, such a finding would still be compatible with a consolidation account of reward-related memory improvement in the delayed memory test.

Our fMRI data allowed us to establish a direct relationship between the delayed memory test and neural events during the initial presentation of the reward-predicting and the neutral stimuli. This link, together with the behavioral data showing that subjects recollected that they had first seen the reward-predicting pictures during study rather than during immediate test, showed that the reward-related behavioral improvement was attributable to neural events (and the later processes of consolidation associated with these events) at the study phase rather than being an effect of the immediate memory test. Moreover, neutral stimuli have undergone the same immediate memory testing as the reward-predicting stimuli, thereby forming an effective

control for any putative influences of the immediate memory test on performance in the delayed memory test.

In summary, these results provide evidence for a relationship between activation of dopaminergic areas and hippocampus-dependent long-term memory formation. This relationship can now be used to study how dopaminergic dysfunction in aging and in diseases such as schizophrenia, Parkinson's disease, and attention deficit hyperactivity disorder affects memory formation for novel stimuli. In our study, we found this relationship under conditions in which reward was a relevant dimension, bringing up interesting parallels with imaging studies of the influence of the amygdala on long-term memory when emotion is a relevant dimension (Canli et al., 2000). The presented evidence is based on functional anatomy and will benefit from pharmacological manipulations (e.g., Honey and Bullmore, 2004; Tracey, 2001) as well as genetic imaging of the dopaminergic neurotransmitter system in future studies of memory formation.

Experimental Procedures

Subjects

Twenty-two healthy adults participated in the study. Six of the participants had to be excluded from analysis due to technical or procedural problems. Sixteen participants remained in the analysis (mean [±SD] age 22.9 ± 3 years; 8 women; 16 right-handed). All participants gave written informed consent to participate, and the study was in accordance with the guidelines of the ethics committee of the University of Magdeburg, Faculty of Medicine.

Experimental Paradigm

Before entering the scanner, participants were given a short demonstration of the task and completed a practice version lasting 3.5 min, which was already rewarded. This practice session minimized learning effects during functional data acquisition and was intended to lead to a switching of reward responses from the moment of reward receipt to the time of reward anticipation (Knutson et al., 2001b). Participants were also shown the money that they could earn by performing the task successfully. Once in the scanner, anatomical and functional scans were collected. Participants engaged in two 8 min sessions of the reward task followed by two 4 min sessions of the immediate memory task.

Each of the two rewarded sessions consisted of 60 trials lasting 4.6–8.6 s (Figure 1A). During each reward trial, participants saw a grayscale cue picture (Rossion and Pourtois, 2004) for 1500 ms, responded to it with a button press (right index or middle finger) indicating whether they expected a reward or not, waited a variable interval (delay, 500–4500 ms), and then responded to a target number (target, 100 ms) with a button press. A visual feedback (1500 ms) was given 1000 ms after the presentation of the target. The feedback was followed by a variable fixation phase (500–4500 ms).

On rewarded trials, €0.50 reward was represented by a green arrow pointing upward, and loss of €0.20 was represented by a red arrow pointing downward. On nonrewarded trials, a question mark was shown regardless of outcome. Nine participants were told that they could gain money in the reaction time task following a picture depicting a living thing (animal, fruit, vegetable, or human body part), and seven were rewarded in trials following the presentation of a man-made object. Each category constituted half of the cues.

The number comparison task (Pappata et al., 2002) required participants to decide whether the target number (1, 4, 6, or 9) was larger or smaller than 5. They responded as quickly as possible by a button press with index or middle finger. Whenever participants responded incorrectly or too slowly for the response deadline in the rewarded trials, negative feedback was given; otherwise they received positive feedback. The response deadline was adjusted individually based on reaction times in the immediately preceding session to attain a correct response rate of ~80%. The times of occurrence of target buttons as well as numbers were counterbalanced for each session. Participants were asked to pay attention to the cues in order to be aware of the reward status but not told that a memory test would follow.

During the immediate memory trials, participants were shown a picture for 1500 ms and asked to indicate by button press with right index or middle finger whether they recognized that picture from the reward session or not. One-third of the pictures were new, with one-half from each of the previous cue categories. Pictures were counterbalanced for studied/nonstudied status across participants. The immediate and delayed memory test sessions consisted of 90 trials each, with picture presentation lasting 2 s per trial, followed by a variable fixation phase (400–4400 ms).

Three weeks after the first study and test day, participants were given a delayed memory test on a computer screen (Figure 1B) using the same pictures as in the first memory test, including the pictures that had served as distractors. No new distractor items were added. Each picture was presented for 1500 ms, and participants were required to describe their memory for the item by a button press (right index, middle, or ring finger) according to the "remember/know" procedure (Tulving, 1985; Düzel et al., 1997). They gave a "remember" response if they recollected any aspect about the context from the study phase of the item, a "know" response if the item was familiar in the absence of any recollection, and a new response if they had no memory for the previous presentation history of an item. A delay of 500 ms followed, and then a question was shown on the screen ("study or test phase?") for 1500 ms. Participants now had to decide whether they had first seen the previous picture in the reward task or as a new item in the immediate memory task. They were told to make that judgment only if they remembered or knew that an item had been presented before. Data from one participant were excluded from the behavioral and DM analysis of the delayed test because she made too few responses to the source memory question. As the DM analysis allowed us to separately assess the importance of the encoding phase for later memory performance in each of the subsequent memory tests, the repetition of pictures from the immediate tests in the delayed test did not interfere with the analysis of reward effects during encoding.

MRI Acquisition

Echoplanar images were acquired on a GE Medical Systems Signa 1.5 T MRI scanner at a repetition time of 2 s and an echo time of 35 ms. Images consisted of 23 axial slices (64 × 64; voxel size = 3.13 × 3.13 × 6 mm [slice thickness = 5 mm with 1 mm gap]) and were acquired in an interleaved manner (1 to 23 in steps of 2, 2 to 22 in steps of 2, from bottom to top). For the reward sessions, number of volumes was 240; for the memory sessions, 120 volumes were collected. The first six volumes of each session were discarded.

Data Processing and Analysis

Data analysis was performed using Statistical Parametric Mapping (SPM2; Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK). Echoplanar images were corrected for acquisition delay, realigned, normalized (voxel size: 3 × 3 × 3 mm) into a common reference frame (Montreal Neurological Institute [MNI]), and smoothed using a Gaussian kernel of 8 × 8 × 8 mm. A high-pass filter with a cutoff of 128 s was applied to the data.

Statistical analysis was carried out using a two-stage mixed effects model. In the first stage, the hemodynamic response was modeled by convolving a delta function at stimulus onset with a canonical hemodynamic response function (HRF) (Friston et al., 1998). The resulting time courses were downsampled for each scan to form covariates that could be applied to a general linear model. The model included separate covariates for each of the conditions of interest (rewarded, not rewarded, recognized, forgotten, and combinations of these). In the second stage of the model, contrasts of the parameter estimates for each covariate were submitted to a random effects analysis, with each participant being treated as a random effect. Specifically, images of each contrast of interest (rewarded versus nonrewarded, recognized versus forgotten, and a

combination of both) for the canonical HRF were entered into one-sample Student's t tests. Beta values of peak voxels in substantia nigra and hippocampus were extracted and corrected with the maximum value of the HRF for general level of activation in the trial to yield percentage of signal change.

To better localize midbrain activity, the activation maps were superimposed on a mean image of five spatially normalized magnetization transfer (MT) images acquired previously (Schott et al., 2004). On MT images, the substantia nigra can be easily distinguished from surrounding structures (Eckert et al., 2004).

Our hypothesis of activation of the reward system was tested at a threshold of $p < 0.0005$ in a whole-brain analysis for the reward anticipation contrast (reward versus neutral cues) and reward outcome contrast (rewarded versus unrewarded). For the memory contrasts (recognized versus forgotten items), we expected midbrain and hippocampal activity differences. As there were fewer trials for each condition in the memory contrasts than in the reward contrasts, we chose a threshold of $p < 0.005$. All p values are uncorrected for multiple comparisons, with a minimum number of ten adjacent voxels, unless otherwise stated. All stereotaxic coordinates are given in Talairach space (Talairach and Tournoux, 1988). All behavioral averages are mean values ± SE.

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