Pharmacological Dissociation of Novelty Responses in the Human Brain

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Repeated processing of the same information is associated with decreased neuronal responses, termed repetition suppression (RS). Although RS effects (i.e., the difference in activity between novel and repeated stimuli) have been demonstrated within several brain regions, such as the medial temporal lobe, their precise neural mechanisms still remain unclear. Here, we used functional magnetic resonance imaging together with psychopharmacology in 48 healthy human subjects, demonstrating that RS effects within the mesolimbic system are differentially modulated by cholinergic and dopaminergic stimulation. The dopamine precursor levodopa (100 mg) attenuated RS within the hippocampus, parahippocampal cortex, and substantia nigra/ventral tegmental area, and the degree of this reduction correlated with recognition memory performance 24 h later. The acetylcholinesterase inhibitor galantamine (8 mg), in contrast, reversed RS into repetition enhancement, showing no relationship to subsequent recognition memory. This suggests that novelty sensitive neural populations of the mesolimbic system can dynamically shift their responses depending on the balance of cholinergic and dopaminergic neurotransmission, and these shifts can influence memory retention.

Keywords: acetylcholine, dopamine, functional magnetic resonance imaging, novelty, repetition suppression

Introduction

Repetition suppression (RS) is a basic form of neural adaptation occurring in several brain regions, including the ventral visual stream, downstream brain regions of the medial temporal lobe (MTL, including parahippocampal cortex and hippocampus), and origins of neuromodulatory projections such as the dopaminergic midbrain (substantia nigra/ventral tegmental area, SN/VTA) and cholinergic basal forebrain (Wilson and Rolls 1990; Tulving et al. 1996; Strange et al. 1999; Henson et al. 2000; Ranganath and Rainer 2003; Yamaguchi et al. 2004; Bunzeck and Duzel 2006). RS signals are fundamental for goal-directed behavior because they regulate how much attention and salience is attributed to novel as opposed to familiar information (Sokolov 1963; Knight 1996; Mesulam 1998; Lisman and Grace 2005). Disruptions in RS, particularly those caused by neurotransmitter hypo- or hyperactivity, are suggested to underlie specific behavioral disorders in neurological and psychiatric conditions such as Parkinson’s disease (Dagher and Robbins 2009), Alzheimer’s disease (Mesulam, M 2004), and schizophrenia (Kapur 2003).

Despite the evidence from animal studies regarding the clinical importance of adaptation to familiar stimuli, there is only sparse physiological evidence to support the idea that both dopaminergic (Lisman and Grace 2005; Lisman et al. 2011) and cholinergic (Hasselmo and McGaughy 2004; Hasselmo and Giocomo 2006) neurotransmission play an important role here. For instance, Lisman and Grace (2005) proposed that the hippocampus can generate a neural novelty signal, which propagates to the dopaminergic midbrain (i.e., SN/VTA) via a polysynaptic pathway. This is suggested to lead to the release of dopamine to the hippocampus where it can regulate synaptic plasticity. Dopamine can stabilize and maintain hippocampal plasticity through a protein synthesis-dependent mechanism thereby improving the consolidation of hippocampus-dependent long-term memory for novel events (Bethus et al. 2010). However, as discussed in a revision of the hippocampus–SN/VTA model (Lisman et al. 2011), dopamine-release in the hippocampus could have effects that go beyond consolidation: Accordingly, dopamine could alter encoding-related activity within the mesolimbic system (i.e., how the hippocampus responds to novelty). This possibility is compatible with frameworks that link dopamine responses with stimulus saliency (Grace 1991; Kapur 2003; Lodge and Grace 2007) and mechanisms that ensure adaptive memory and behavior (Shohamy and Adcock 2010).

Likewise, although several studies have investigated the effects of cholinergic drugs on neural novelty processing, the relationship to more fine-grained parametric variations of familiarity remains unclear. For instance, environmental novelty increases hippocampal acetylcholine levels (Thiel et al. 1998), which are closely linked with successful memory encoding, but not retrieval (Hasselmo 2006). Conversely, disruption of the cholinergic system during encoding, for instance, by cholinergic antagonists can lead to disruptions of mnemonic functions (Rogers and Kesner 2003, 2004). Therefore, these models predict that cholinergic stimulation should enhance encoding and reduce retrieval. However, this contrasts with findings showing that cholinergic stimulation reduces evoked synaptic potentials in CA1 (Winson and Abzug 1978; Herreras et al. 1988; Hasselmo 1999), which could result in reduced neural activity to novel items.

Here, we investigated 3 groups of 16 subjects who had oral administration of the dopamine precursor levodopa (100 mg, together with 25 mg Benserazid), the cholinesterase inhibitor galantamine (8 mg), or placebo in a double-blind randomized fashion. After a familiarization with indoor and outdoor images (starting ~60 min after drug intake), subjects underwent functional magnetic resonance imaging (fMRI) scanning, while novel images were randomly presented intermixed with familiar images (presented twice during familiarization) and very familiar images (presented 4 times during familiarization). We...
expected levodopa to attenuate, or even to abolish, RS effects in the SN/VTA and MTL compatible with enhanced neural salience within the MTL (Grace 1991; Kapur 2003; Grace et al. 2007). Galantamine, on the other hand, was expected to either amplify MTL RS effects (i.e., stronger responses to novelty and stronger RS; Rogers and Kesner 2003, 2004); or, alternatively, due to the known depressive effects of cholinergic stimulation on CA1, it could reduce novelty responses with opposing effects on familiar items (Winson and Abzug 1978; Herreras et al. 1988; Hasselmo 1999).

Materials and Methods

In total, 3 × 16 adult subjects participated (29 females and 19 males; mean age: 22.04 years, standard deviation = 4.42 years). All were healthy, right-handed and had normal or corrected-to-normal visual acuity. None of the participants reported a history of neurological, psychiatric, or medical disorders or any current medical problems. Subjects gave written informed consent according to the approval of the local ethics committee (University College London, UK). We monitored the heart rate and oxygen saturation throughout the fMRI session and measured the heart rate and blood pressure at several stages before and after drug administration.

Both drugs were expected to reach peak blood-plasma concentration at approximately 60–90 min after administration. One hour after drug administration, all subjects were familiarized with 100 pictures of indoor and outdoor images inside the fMRI scanner (no fMRI data were acquired). While 50 images were presented twice (these images will be called "familiar"), 50 images were shown 4 times ("very familiar"). Ninety minutes after drug administration, subjects watched the previously presented familiar and very familiar images together with 50 novel images, while fMRI data were acquired. Each image was presented for 1.5 s with an intertrial interval of 3 s. During familiarization and fMRI task, subject indicated the indoor/outdoor status of an image via button presses using their index and middle fingers.

It should be noted that our approach of first familiarizing subjects with a subset of images, which is followed by the novelty task (where novel and familiarized items are presented intermixed), is a previously established method (Bunzeck and Duzel 2006; Wittmann et al. 2007; Bunzeck et al. 2009, 2011; Guitart-Masip et al. 2010; Krebs et al. 2011). It does differ from other RS paradigms where items are repeatedly presented within one session, but has the advantage of enabling fully randomizing the order of novel and familiar items during the fMRI scanning session.

One day after encoding subjects performed an incidental recognition memory test following the "remember/know" procedure (Tulving 1985). Here, in random order all 150 previously seen pictures (50 per condition) were presented together with 50 new distracter pictures. Task: The subject first made an "old/new" decision to each individually presented picture. Following a "new" decision, subjects indicated whether they were confident ("certainly new") or unsure ("guess"). After an "old" decision, subjects indicated if they were able to remember something specific about seeing the scene at study ("remember" or "false face"), just felt familiarity with the picture without any recollective experience ("familiar" response) or were unsure that the picture was an old one (guess response). Subjects had 4 s to make each of both judgments.

All images were gray-scaled and normalized to a mean gray value of 127 and a standard deviation of 75 (RGB-space).

Galantamine is licensed for the treatment of Alzheimer’s disease and develops cholinergic effects by inhibition of cholinesterase and by allosteric activation of nicotinic acetylcholine receptors, thereby enhancing the activity of hippocampal CA1 neurons through action on both nicotinic and muscarinic cholinergic receptors (Oh et al. 2006). The dopamine precursor levodopa, on the other hand, is licensed for the treatment of Parkinson’s disease and is mostly taken up and converted by dopaminergic fibers to be phasically released into the synaptic cleft. Therefore, the 2 drugs have a stimulating effect on dopaminergic (levodopa) or cholinergic (galantamine) neurotransmission.

fMRI Methods

fMRI was performed on a 3-T Siemens Allegra magnetic resonance scanner (Siemens, Erlangen, Germany) with echo-planar imaging (EPI). In the functional session, T2*-weighted images (EPI sequence; covering the whole head) with blood oxygenation level-dependent (BOLD) contrast were obtained (matrix size: 64 × 64; 48 oblique axial slices per volume angled at −30° in the antero-posterior axis; spatial resolution: 3 × 3 × 3 mm; time repetition (TR) = 2880 ms; time echo (TE) = 30 ms). The fMRI acquisition protocol was optimized to reduce susceptibility-induced BOLD sensitivity losses in both the inferior frontal and temporal lobe regions (Deichmann et al. 2003; Weiskopf et al. 2006). For each subject, fMRI data were acquired in one scanning session containing 168 volumes. Six additional volumes were acquired at the beginning of each series to allow for steady-state magnetization and were subsequently discarded from further analysis. No fMRI data were acquired during the familiarization of the presented scene pictures.

Anatomical images of each subject’s brain were collected using multichico 3-dimensional (3-D) FLASH for mapping proton density, T1 and magnetization transfer (MT) at 1-mm resolution (Helms et al. 2009) at the end of the experiment. Additionally, individual field maps were recorded using a double-echo FLASH sequence (matrix size = 64 × 64; 64 slices; spatial resolution = 3 × 3 × 3 mm; gap = 1 mm; short TE = 10 ms; long TE = 12.46 ms; TR = 1020 ms) for distortion correction of the acquired EPI images (Hutton et al. 2002). The fMRI data were preprocessed and statistically analyzed using SPM8 software package (Wellcome Trust Centre for Neuroimaging, University College London, UK) and MATLAB (The MathWorks, Inc., Natick, MA, USA). All functional images were corrected for motion artifacts by realignment to the first volume; corrected for distortions based on the field map; corrected for the interaction of motion and distortion; spatially normalized to a standard T1-weighted statistical parametric map (SMP) template (Ashburner and Friston 1999; care was taken that, in particular, midbrain regions aligned with the standard template); re-sampled to 2 × 2 × 2 mm; and smoothed with an isotropic 4-mm full-width half-maximum Gaussian kernel. The tMRI time series data were high-pass filtered (cutoff = 128 s) and whitened using an AR(1)-model.

For each subject, an event-related statistical model was computed by creating a "stick function" for each event onset (duration = 0 s), which was convolved with the canonical hemodynamic response (HR) function combined with time and dispersion derivatives (Friston et al. 1998). Modeled conditions included novel, familiar, and very familiar images as well as errors (incorrect and no responses). To capture residual movement-related artifacts, 6 covariates were included (the 3 rigid-body translation and 3 rotations resulting from realignment) as regressors of no interest. Regionally specific condition effects were tested by employing linear contrasts for each subject and each condition (first-level analysis). The resulting contrast images were entered into a second-level random-effects analysis. Here, the hemodynamic effects of each condition were assessed using a 3 × 3 analysis of variance (ANOVA) with the factors "novelty" as within-subject factor (novel, familiar, and very familiar) and "group" as between-subject factor (placebo, levodopa, and galantamine). This model allowed us to test for the main effects of novelty, main effects of group, and the interaction between both. All contrasts were thresholded at P < 0.001 (uncorrected) and corrected for multiple comparisons using small-volume correction [P < 0.05, family-wise error (FWE) correction] for a priori regions of interest where we hypothesized the effects (i.e., SN/VTA, MTL, and occipital cortex).

The anatomical localization of significant activations was assessed with reference to the standard stereotaxic atlas by superimposition of the SPM maps on 1 of the 2 group templates. A T1-weighted and an MT-weighted group template were derived from averaging all subjects’ normalized T1 or MT images (spatial resolution of 1 × 1 × 1 mm). While the T1 template allows anatomical localization outside the midbrain, on MT-images the SN/VTA region can be distinguished from surrounding structures, such as the red nucleus or cerebral peduncle, which appear dark (Eckert et al. 2004; Bunzeck et al. 2007).
Note that we prefer to use the term SN/VTA and consider BOLD activity from the entire SN/VTA complex for several reasons (Duzel et al. 2009). Unlike early formulations of the VTA as an anatomical entity, different dopaminergic projection pathways are dispersed and overlapping within the SN/VTA complex. In particular, dopamine neurons that project to limbic regions are not confined to the VTA, but they are distributed also across the SN (pars compacta).

All subjects were required to fill in a subjective rating scale (Pessiglione et al. 2006) and perform the “d2 test of attention” (Bates and Lemay 2004) before drug administration (T1), 30 min after drug intake (T2), and after the fMRI scan (T3) (~2 h after drug intake). Responses to the subjective ratings (Supplementary Table S1) were analyzed by a series of ANOVAs using time-point as within-subject factor (T1, T2, and T3) and drug (placebo, levodopa, and galantamine) as between-subject factor. There was no interaction between drug and time-point (P > 0.05; Bonferroni-corrected for multiple comparisons). This suggests that all 3 drug groups had similar responses in the subjective rating scale (including dimensions, such as mood, and alertness) at all 3 time-points.

Results

Accuracy and Reaction Times

During encoding, subjects distinguished between indoor and outdoor images with high accuracy (average hit rate = 0.93) in the absence of any drug effects (P > 0.05; t-tests). Reaction times (RTs; Fig. 1A) were analyzed using ANOVA with novelty (novel, familiar, and very familiar) as within-subject factor and with drug (placebo, levodopa, and galantamine) as between-subject factor. It revealed a main effect of novelty (F = 12.5, P < 0.001), which was driven by faster responses to very familiar when compared with novel images (in all groups: P < 0.05, post hoc t-test); there was no significant interaction between drug and novelty (F = 0.5, P > 0.05).

D2 test of Attention

Subjects completed the d2 test of attention (Bates and Lemay 2004) 3 times during the experiment [i.e., before drug administration, 30 min after drug intake, and after the fMRI scan (~2 h after drug intake)]. ANOVA revealed a main effect of time-point [i.e., training effects; (F = 64.55, P < 0.001)], but no significant interaction between time-points and drug (F = 1.9, P > 0.05), indicating that drugs did not have a global effect on attentional states (Fig. 1B).

Recognition Memory

Recognition memory analysis was based on both hits (remember responses, know responses, and guesses following pictures seen on day 1) and false alarms [FA: Remember, know, and guesses to distractors]. In a first step, we calculated the proportion of remember, know, and guess responses for studied and distractor images (i.e., hit rates and FA rates) by dividing the number of hits (and FA, respectively) by the number of items per condition. Secondly, corrected hit rates were obtained for remember responses (remember hit rate minus remember FA rate), know responses (know hit rate minus know FA rate), and guess responses (guess hit rate minus guess FA rate; Supplementary Table S2).

An initial between subject ANOVA with the factors novelty and drug on overall recognition memory (corrected hit rate pooled across remember, know and guess responses) revealed a main effect of novelty (F = 157.02, P < 0.001; i.e., improved recognition memory by repetition), but no interaction between novelty and drug (F = 0.26, P > 0.05; Fig. 1C). More complex ANOVAs, including corrected remember, know, and guess rates and planned direct comparisons of the corrected remember, know, and guess rates, respectively, also did not reveal any effect of drug (P > 0.05, corrected for multiple comparisons).

Relationship to Body Weight

The cognitive effects of a psychopharmacological drug can be dose-dependent, often showing linear or quadratic effects (Goldman-Rakic et al. 2000; Knecht et al. 2004; Chowdhury et al. 2012). Therefore, we tested for linear and inverted u-shape relationships between body weight-adjusted relative doses (for the levodopa group: 100 mg/body weight, mg/kg; for the galantamine group: 8 mg/body weight, mg/kg) and (1) accuracy, (2) RT, (3) performance in the d2 test of attention, and (4) recognition memory performance (corrected remember, know, and guess responses). There were no statistically significant effects (P > 0.05; corrected for multiple comparisons), further indicating no drug effects on behavior.

fMRI

fMRI data were analyzed using a 3 × 3 ANOVA with the factors novelty (within subjects) and drug (between subjects). All SPMs were thresholded at P < 0.001 (uncorrected; extent threshold k = 10 voxels), and small-volume correction was applied using a priori-defined regions of interest.

An ANOVA revealed a main effect of novelty (i.e., RS or decreased HRs as a function of stimulus repetition)—but no significant interaction with the drug group—in the posterior brain regions including bilateral occipital gyrus (P < 0.05; FWE-corrected using the occipital cortex as volume) extending into the bilateral fusiform gyrus and right posterior parahippocampal gyrus (P < 0.05; FWE-corrected using bilateral fusiform and parahippocampal gyrus as volume; Fig. 2). These brain regions (occipital cortex, fusiform gyrus, and parahippocampal cortex) were a priori hypothesized to show RS effects (Henson et al. 2002; Bunzeck and Duzel 2006) and therefore used as independent masks to correct for multiple comparisons. They are also strongly innervated by acetylcholine (Mesulam, MM 2004; Zilles et al. 2004), and dopamine receptors can be found in much of the primate cortex, although there is a rostro-caudal gradient with lower densities in the visual cortex than in the frontal cortex (Lidow et al. 1991).

Furthermore, we observed a main effect of drug within the right posterior hippocampus and parahippocampal cortex (Fig. 3). Post hoc analysis of the peak voxel of these clusters revealed that these effects were driven by enhanced HRs under levodopa. Since there was no statistically significant interaction between novelty and drug (P > 0.05), this suggests a general increase of stimulus processing by levodopa independent of novelty status within these brain regions. Both effects survived at a relatively liberal threshold of P < 0.001 (uncorrected), but not when FWE correction was applied (P > 0.05, using a bilateral MTL mask including the hippocampus and parahippocampal cortex).

Critically, the neural effects of drugs on RS were assessed by combining the contrast for novelty in the placebo group (novel vs. very familiar, T-contrast) together with a significant interaction (F-contrast) between novelty and drug (using
implicit masking). This conjunctive contrast was preferred over the interaction term alone which could be driven by a wide range of activation patterns of no interest. In other words, we were specifically testing for those brain regions that show RS in the placebo group (i.e., novelty effects) and a significant modulation by drug (i.e., an interaction). Such a

Figure 1. Behavioral results. RTs during the novelty task (A), performance in the d2 test of attention (B), and recognition memory performance. Corrected hit rates (sum of remember, know, and guesses) were above chance level ($P < 0.05$) for all conditions (novel, familiar, and very familiar items) and drug groups. Error bars indicate one standard error of the mean.

Figure 2. Main effect of novelty. RS was observed in posterior brain regions including middle occipital gyrus (A), fusiform gyrus (B), and parahippocampal gyrus (C). Error bars denote one standard error of the mean, and asterisks indicate statistically significant differences (*$P < 0.05$, 1-tailed; **$P < 0.05$; ***$P < 0.01$). Activation maps were superimposed onto a $T_1$-weighted group template, and coordinates are given in the MNI space. A.u.: arbitrary unit. Activation patterns were extracted from the peak voxel in a given cluster.
response pattern was observed within the right SN/VTA (Fig. 4A), left parahippocampal cortex (Fig. 4B), and left posterior hippocampus (Fig. 4C). Post hoc analyses of the peak voxels (Fig. 4) revealed that within all 3 brain regions repeated processing of scene images led to decreased HRs under placebo (i.e., novelty effect), and—importantly—this novelty effect was diminished by levodopa (no difference between HR to novel and very familiar items) and reversed by galantamine (stronger HR to very familiar items vs. novel items). The SN/VTA effect survived correction for multiple comparisons ($P < 0.05$, using the SN/VTA as volume), whereas the activity in the parahippocampal cortex did not ($P > 0.05$, again using the MTL as mask). Note that the interaction term (group × novelty) alone revealed a similar, but slightly less-confined activation pattern as the reported conjunction.

**Relationship to Recognition Memory**

We tested the relationship between the above-described drug-related changes in the SN/VTA, hippocampus, and parahippocampal cortex and recognition memory performance on the day after encoding (here, we simplified our analyses to the sum of corrected remember rates and corrected know rates, since there was no effect of drug on the type of memory). We found that levodopa-induced reductions in RS in the SN/VTA (i.e., the difference between HR to novel and very familiar items) correlated negatively with the subsequent recognition memory performance (Fig. 5) for novel ($r = -0.56$, $P = 0.024$) and familiar items ($r = -0.67$, $P = 0.005$); there was only a trend for very familiar items ($r = -0.43$, $P = 0.09$). In other words, the more reduced the RS effect by levodopa in the SN/VTA, the better subsequent memory performance on the following day. There was no such effect within the hippocampus or parahippocampal cortex (all $P$'s > 0.05). There were no significant effects for the galantamine or placebo group in any of these 3 brain regions ($P > 0.05$).

**DM effect**

In a final analysis, we computed the so-called “DM effect” (difference due to later memory) for all 3 drug groups. Here, hemodynamic activity during encoding was contrasted for those images that were later correctly recognized as old images versus for those images that were later forgotten (i.e., incorrectly classified as new images). To increase the power of the analysis, the effects were computed across all degrees of novelty/familiarity. There was a significant effect of drug within the medial prefrontal cortex (Fig. 6), which was driven by enhanced DM activity in the levodopa and galantamine group in contrast to the placebo group. The effect was statistically significant at a relatively liberal threshold of $P < 0.001$ (uncorrected), but not when FWE correction was applied ($P = 0.12$, FWE-corrected). Here, we used the entire brain to correct for multiple comparisons since we did not have specific a priori hypotheses regarding the PFC.
Discussion

We used fMRI in combination with psychopharmacology to test the relationship between neural novelty processing and dopaminergic and cholinergic neuromodulation. As a major finding, we can demonstrate that RS effects within the hippocampus, parahippocampal cortex, and SN/VTA are differentially affected by cholinergic and dopaminergic stimulation. This suggests that novelty sensitive neural populations of the mesolimbic system can shift their responsiveness depending on the balance of cholinergic and dopaminergic neurotransmission.

Figure 4. Activation pattern in the SN/VTA, parahippocampal cortex, and hippocampus. In all 3 brain regions, repeated processing of scene images led to RS under placebo. RS was diminished by levodopa and reversed by galantamine. Error bars denote one standard error of the mean, and asterisks indicate statistically significant differences (*P < 0.05, 1-tailed; **P < 0.05; ***P < 0.01; n.s.: not significant). A.u.: arbitrary unit. Activation patterns were extracted from the peak voxel in a given cluster.

Figure 5. Relationship between SN/VTA activity and recognition memory performance. In the levodopa group, hemodynamic activity in the SN/VTA (difference between novel vs. very familiar items) correlated negatively with memory performance on day 2 (sum of corrected remember and know rates, see text). A.u.: arbitrary unit.
Levodopa attenuated RS signals in the SN/VTA, parahippocampal cortex, and hippocampus (Fig. 4), indicating that brain regions discriminating between novel and familiar items under normal levels of dopamine were shifted toward being less responsive (i.e., “neutral”). One possible mechanism behind this observation is that the relatively slow (enteral) uptake of levodopa has led to dopamine autoreceptor-driven adaptation of dopamine neuronal firing (Grace 1991; Seeman and Madras 1998). Interestingly, 2 different areas within the hippocampus and parahippocampal cortex showed overall increased HRs irrespective of item repetition (Fig. 3). Importantly, these brain regions were neutral with respect to novel and repeated stimuli under placebo (i.e., they did not show RS or repetition enhancement under placebo), suggesting that they are typically not responsive to these types of stimuli or their repetition. Hence, increasing dopamine levels impairs the functional discrimination of memory cues in the SN/VTA, hippocampus, and parahippocampal cortex, while at the same time causing increased responsiveness in otherwise unresponsive regions of the hippocampus and parahippocampal cortex. These findings are compatible with a causal relationship between altered dopaminergic neuromodulation, so-called “aberrant” salience within the MTL, and the inability of the mesolimbic system to respond differentially to novelty and familiarity, which closely mimics some of the pathophysiological features of schizophrenia (Heckers 2001; Lisman and Otmakhova 2001; Lodge and Grace 2007).

Galantamine did not attenuate the ability of the mesolimbic system to differentiate between novel and familiar items, but it reversed mesolimbic novelty signals from RS to repetition enhancement (Fig. 4). Indeed, previous functional imaging studies (Dolan and Fletcher 1997; Donaldson et al. 2001) and intracranial recordings in both humans (Fried et al. 1997; Rutishauser et al. 2006) and nonhuman primates (Brown and Xiang 1998; Xiang and Brown 1998; Brown and Aggleton 2001) have demonstrated that the MTL can adapt to stimulus repetition in several ways. While the majority of studies found RS, some showed the opposite pattern of increased activation (i.e., they show repetition enhancement). Our data extend these observations by suggesting that whether mesolimbic brain regions show RS, repetition enhancement, or are neutral with respect to item repetition is determined by acetylcholine and dopamine levels.

Furthermore, the finding of repetition enhancement by galantamine is compatible with a view of acetylcholine that focuses on its role in directing attentional resources toward target events (Hasselmo and Sarter 2011). Accordingly, high levels of cholinergic stimulation may cause a designation of very familiar events as being targets. An interpretation of repetition enhancement in terms of target processing is compatible with data from invasive recordings of MTL activity, which have shown that the task relevance of repeated items determines the responsiveness of MTL neurons in monkeys (Miller and Desimone 1994). In this study (Miller and Desimone 1994), repeated items that were designated as targets elicited repetition enhancement in the MTL, whereas the repetition of irrelevant items did not. Our results suggest that this effect might relate to cholinergic neuromodulation (Hasselmo and Sarter 2011).

The effects of galantamine were also evident within the SN/VTA, which may have 2 reasons. First, SN/VTA dopamine neurons receive cholinergic projections from the laterodorsal and pedunculopontine (PPT) tegmental nuclei, which modulate their activity via muscarinic and nicotinic receptors (Futami et al. 1995; Oakman et al. 1995). For instance, systemic injections of the nonselective muscarinic agonist scopolamine increased dopamine release into the striatum in rats (Chapman et al. 1997; Miller and Blaha 2004), and this effect is possibly due to enhanced numbers of active dopamine neurons by inhibiting muscarinic PPT autoreceptors (Di Giovanni and Shi 2009). On the other hand, cholinergic agonists, such as the mixed muscarinic and nicotinic agonist carbachol, attenuated the striatal dopamine efflux (Miller and Blaha 2004). Therefore, these and other studies (e.g. Mark et al. 2011) point toward a close interaction between the cholinergic and dopaminergic system via cholinergic receptors at the level of midbrain dopamine neurons [see also Threlfell et al. (2012) for a mechanism of striatal dopamine release via cholinergic interneurons]. The second possibility is that galantamine influenced novelty processing in afferent pathways of the SN/VTA, thereby affecting inputs into dopaminergic neurons.

There was no significant modulation of striatal or prefrontal activity by galantamine or levodopa in response to novelty (i.e., no significant interaction between novelty × drug group). Such effects could have been expected since the striatum and
prefrontal cortex are rich in cholinergic/dopaminergic innervations and both are interconnected with the SN/VTA and the MTL (Mansvelder et al. 2006; Fields et al. 2007). Indeed, previous studies have shown that cholinergic (Furey et al. 2000; Bozazli et al. 2006) and dopaminergic (Pessiglione et al. 2006; van Schouwenburg et al. 2010; Guitart-Masip et al. 2012) drugs can change striatal and prefrontal activity albeit in different tasks. Together with the observation that there were no striatal or prefrontal novelty effects in the placebo group, this suggests that the functional effects of galantamine and levodopa, as administered in our paradigm, are most prominent in task-relevant mesolimbic structures (i.e., SN/VTA and MTL) and not more global effects in brain regions known to be innervated by dopamine and acetylcholine. In other words, both drugs had regional and functionally specific effects in a manner that argues against global changes in neurovascular coupling (Thiel 2003). Note that the absence of novelty effects in the prefrontal cortex contrast with other experiments (reviewed in Ranganath and Rainer 2003), which is likely to relate to differences in task requirements. While most previous studies have included an active discrimination between old and new items, our subjects were asked to distinguish the indoor and outdoor status of repeatedly presented scene images and hence novelty processing was implicit.

The MTL effects (the main effect of drug and interaction between novelty and drug) and the prefrontal DM effect were statistically significant only at a rather liberal threshold of $P<0.001$ (uncorrected), but not when FWE correction was applied. The anatomical location of these effects, however, conforms to our a priori hypotheses (see Introduction) and/or physiologically plausible models of dopaminergic and cholinergic neuromodulation (Mesulam et al. 1983; Fields et al. 2007), which argues against a mere alpha error. Furthermore, the main effects of group within the parahippocampal cortex and hippocampus (Fig. 3) are qualitatively very similar, and—likewise—the interaction within the hippocampus and parahippocampal cortex resembles that of the SN/VTA (Fig. 4), which also argues against random effects. Nevertheless, these results should be treated with caution and need further confirmation.

Behaviorally, there were no effects of levodopa or galantamine on overall recognition memory. At the first glance, this is at odds with theoretical models, suggesting that dopaminergic and cholinergic stimulation during encoding should enhance mnemonic functions (Lisman and Grace 2005; Hasselmo 2006; Lisman et al. 2011). However, in the case of dopaminergic stimulation, an earlier study in humans also did not show any significant effects of acute levodopa administration on explicit memory performance (Knecht et al. 2004). Instead, only after repeated intake of 100 mg levodopa over several days at which learning took place free recall improved. This suggests that, in a dopaminergic system of healthy young subjects, acute administration of 100 mg levodopa can modulate mesolimbic novelty signals, but this does not necessarily translate into better memory consolidation over a 24-h retention interval. However, the correlation between the reduction of RS in the SN/VTA by levodopa and subsequent memory performance (Fig. 5) provides further empirical evidence for a link between dopaminergic neuromodulation and long-term memory. Although the precise physiological mechanism behind this observation remains unclear, it should be noted that, in older adults and using higher doses, we have recently observed a dose-dependent benefit of levodopa for memory consolidation (Chowdhury et al. 2012). This suggests that higher doses than used here can be effective under condition when the dopaminergic system is already compromised, as is the case even in healthy older adults.

In the case of galantamine, a different explanation for the absence of learning effects is based on the notion that the high levels of acetylcholine drive encoding, but are detrimental for consolidation (Gais and Born 2004; Hasselmo 2006; Winters et al. 2006). More precisely, increased levels of acetylcholine after learning reduce consolidation possibly through interference as suggested by human and animal studies (e.g. Gais and Born 2004; Winters et al. 2006). Galantamine, as administered in our study, has a relatively long half-life of approximately 7–8 h. Since encoding of the experimentally relevant items ended approximately 2.5 h after drug intake, it seems possible that the still elevated acetylcholine levels reduced consolidation possibly by interfering with information that was perceived after learning (e.g., on the way home from the laboratory).

Finally, we would like to point out that, in contrast to our expectation, there was no relationship between drugs, memory performance, and MTL activity (Lisman and Grace 2005; Hasselmo 2006), nor were there differential drug effects on prefrontal DM activity. While the absence of effects is often difficult to interpret, the most parsimonious explanations here might lie in the relatively low single dosage and presumably higher integrity of the mesolimbic system of our healthy young subjects relative to a recent study that did report a benefit of levodopa in healthy older adults (Chowdhury et al. 2012).

Taken together, although stronger improvements of recognition memory by both drugs and an effect of galantamine on attention could have been expected (Lisman and Grace 2005; Thiel et al. 2005; Hasselmo 2006; Bentley et al. 2011), it is also clear that levodopa and acetylcholinesterase inhibitors are known to have only relatively small acute effects on behavior (Knecht et al. 2004; Bartus and Dean 2009). Therefore, future studies could include higher dosages and/or longer periods of administration (i.e., several days before encoding) of both drugs in larger sample sizes to further investigate possible behavioral benefits.

To conclude, our data show that dopaminergic and cholinergic stimulation differentially modulate mesolimbic novelty processing. While the dopamine precursor levodopa attenuated RS within the hippocampus, parahippocampal cortex, and SN/VTA, the acetylcholinesterase inhibitor galantamine reversed the effect into repetition enhancement. These findings suggest that the balance of acetylcholine and dopamine levels determines how novelty responsive brain regions within the mesolimbic system adapt to item repetition.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes
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