Verbal memory improved by D-amphetamine: influence of the testing effect

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Objective The improvement of long-term retention of verbal memory after an acute administration of D-amphetamine in recall and recognition tasks has been ascribed to an influence of the drug on memory consolidation. Because recent research has demonstrated that intermediate testing is of overriding importance for retention, we investigated whether D-amphetamine modulates the repeated testing effect in verbal long-term recognition.

Method Forty men participated in two double blind placebo controlled studies. In Experiment 1, we manipulated the number of recognition tests and in Experiment 2, we compared repeated with nonrepeated testing of the same items.

Results Drug effects were observed on delayed tests only, leaving immediate recognition unaffected. Number of intermediate recognition tests and repeated testing of the same items were not affected by D-amphetamine.

Conclusions We conclude that the D-amphetamine memory enhancement is not related to the testing effect. This result supports that D-amphetamine modulates other aspects of the consolidation process, probably related to context effects. Copyright © 2010 John Wiley & Sons, Ltd.

key words — testing effect; D-amphetamine; memory consolidation

INTRODUCTION

There is now ample evidence that central-nervous stimulants, such as D-amphetamine, administered in moderate doses, can enhance long-term memory in animals and humans (e.g. Gold and Van Buskirk, 1975; McGaugh, 2000; McGaugh and Roozendaal, 2009; Wood and Anagnostaras, 2009). Experiments with post-training drug administration have provided strong evidence that the memory enhancing effect of the drug is not a consequence of influences on acquisition processes or performance, because participants were drug-free during acquisition and retention testing (Doty and Doty, 1966; Krivanek and McGaugh, 1969). Instead, research suggests that D-amphetamine acts on memory consolidation, a process of stabilization through which memories become increasingly resistant to interference in the absence of further practice (Lechner et al., 1999; McGaugh, 2000; McGaugh and Roozendaal, 2009; Soetens et al., 1993, 1995). However, the exact nature of ‘memory consolidation’ is still not understood and no consensus has been reached on what specific processes are covered by the term (e.g. Moskovitch and Nadal, 1998).

In the present study, we investigated whether the enhancing effect of D-amphetamine on consolidation may be caused by an indirect effect of the drug on intermediate retrieval. Roediger and co-workers demonstrated that repeatedly recalling an item increases the likelihood that this item will be recalled at a later time, a phenomenon known as the testing effect (Butler and Roediger, 2007; Karpicke and Roediger, 2009; Roediger and Karpicke, 2006a,b). Evidence suggests that retrieval processes during a test episode are responsible for better retention of the material (Karpicke and Roediger, 2009). Because the effect of testing is so pervasive, D-amphetamine may influence processes related to repeated retrieval in successive recall or recognition tests or to processes related to retrieval in general, resulting in a positive D-amphetamine effect on long-term memory.

On first sight, an influence of D-amphetamine on retrieval seems to be at odds with the fact that the memory enhancement was only found after delayed tests (McGaugh, 2000), at a time that participants were no longer under the influence of the drug. This shows that retrieval itself is not enhanced by the drug.
However, because intermediate testing has a strong influence on long-term retention, the drug could indirectly influence processes related to consolidation if repeated testing interacts with d-amphetamine.

There is evidence from animal studies that d-amphetamine may modulate retrieval processes. For example, Sara and Deweer (1982) showed that mice were better in maze learning when they were under the influence of d-amphetamine during acquisition and retention after 3 weeks. The effect could not be explained by state dependent learning, because the positive effect was still there when the animals were retested after a delay of 24 h.

Also in human learning, there are indications that intermediate retrieval may be influenced by d-amphetamine. This can indirectly be derived by comparing the memory-enhancing effect of d-amphetamine on recall and recognition studies. Several researchers have found that an acute d-amphetamine administration enhances long-term retention of verbal memory traces (Breitenstein et al., 2004; Rapoport et al., 1980; Soetens et al., 1993, 1995; Weingartner et al., 1982; Zeeuws and Soetens, 2007). However, the positive effect of d-amphetamine was stronger and appeared earlier for recall as compared to recognition (Soetens et al., 1995). For example, in the study of Soetens et al. (1995) participants had to learn lists of unrelated words, which had to be recalled or recognized after different delays. In recall experiments, a significant d-amphetamine facilitation effect was found 1 h after list learning and the effect remained stable for at least three days. In recognition experiments on the other hand, the facilitating effect of d-amphetamine only became significant after 1 week (see also Zeeuws et al., 2010). The testing effect can explain why intermediate recall leads to a better long-term retention than intermediate recognition (Kang et al., 2007) because of repeated testing of the same material in free recall tasks. Indeed, in most recall studies participants have to recall the same words on each free recall test, whereas in recognition different target items are presented on each test.

If d-amphetamine enhances memory consolidation by an indirect effect on retrieval, such an effect should emerge only when participants are intermittently tested under drug influence. However, the results on this issue are equivocal. In a recall experiment, Soetens et al. (1995) tested participants immediately after learning, which was approximately 1.5 h after pill intake, at the time the behavioural effects of the drug peak (Angrist et al., 1987). There was a significant influence of the drug after 1-h delay (HD), supporting the claim that the drug interacted with immediate retrieval.

Conversely, Zeeuws and Soetens (2007) did not find a drug effect on intermediate recall when they compared participants with or without an intermediate test after 1 h. Although there was both a significant effect of the drug and a significant effect of repeated testing 1 h after list learning, the extra free recall test, under influence of the drug, did not alter the d-amphetamine facilitation effect after one day. These results seem to indicate that the drug effect was independent of repeated testing of the same items in free recall.

In sum, earlier studies suggest that the influence of d-amphetamine on long-term retention is (1) unrelated to retrieval and (2) not caused by a boosted drug effect on enhanced consolidation by repeated testing. Nevertheless, because the study of Zeeuws and Soetens is the only one that investigated the influence of d-amphetamine on intermediate tests, it is necessary to obtain more conclusive evidence regarding d-amphetamine effects on repeated testing. To determine whether the differential drug effect on recall and recognition could be attributed to intermediate testing, we investigated the influence of d-amphetamine on repeated testing in recognition memory. In Experiment 1, we manipulated the number of recognition tests to find out whether the number of tests and whether intermediate testing under drug influence modulate the d-amphetamine memory improvement. In Experiment 2, we investigated whether intermediate testing of exactly the same target items in a recognition test modulates the drug influence. Therefore, we presented the same target items repeatedly in a recognition task (similar as in free recall tests) and compared the d-amphetamine influence with a normal recognition task where different items were tested at different delays. If d-amphetamine interacts with repeated testing, it would mean that the influence of the drug on long-term retention is caused by a boosted drug effect on enhanced consolidation by repeated testing.

**EXPERIMENT 1**

In this experiment, we tested whether the improvement of recognition memory with d-amphetamine is modulated by intermittently testing participants at a time that the drug is still active. Intermediate tests have a strong influence on long-term retention both in recall (e.g. Karpicke and Roediger, 2009) and recognition (Kazen and Solis-Macias, 1999), suggesting that they enhance consolidation. Because d-amphetamine is assumed to influence consolidation, an interaction with repeated testing may be possible.

Whereas the influence of d-amphetamine on long-term recall is well documented (e.g. McGaugh and Roozen-daal, 2009), there are only two studies showing a d-
amphetamine enhancement in recognition (Soetens et al., 1995; Zeeuws et al., 2010). In these studies, a positive effect of D-amphetamine was found in recall, 1 h after learning, whereas a positive effect on recognition emerged after 1 week. An important difference between both experiments in the study of Soetens et al. was that in the recognition task participants were not tested after 1 h. Because behavioural effects of the drug are maximal 90–120 min after pill intake, D-amphetamine could have modulated consolidation indirectly through its influence on intermediate retrieval in the free recall task. Consequently, the extra test after 1 h may have been responsible for the stronger D-amphetamine effect in the recall experiments. To study this possibility, we conducted a recognition experiment with two conditions: one with a recognition test after HD and the other without the extra test. Because Zeeuws and Soetens (2007) did not find an influence of D-amphetamine on repeated testing in free recall, we also expected no influence of the additional recognition test on the magnitude of the drug facilitation effect. Furthermore, as before, we expected the enhancing effect of the drug to occur 1 week after list learning in both conditions with and without an intermediate recognition test.

Method

Participants. Twenty-four paid male volunteers, between the ages of 18 and 25 (12 without additional test after 1 h = 21.3 ± 1.54; 12 with additional test = 20.9 ± 1.83), were recruited by an advertisement in the university newspaper. Participants were excluded if they were currently using any type of medication or drug, if they had a history of drug abuse, or if they had a physical or mental illness. All participants had normal or corrected-to-normal vision. They all provided written informed consent prior to participating. None of the volunteers had previously participated in a similar study and all were unaware of the nature of the administered drug. Earlier studies have demonstrated that inexperienced participants were unable to detect the difference between drug and placebo. Moreover, those that did recognize the drug, did not produce a different pattern of memory improvement. The Ethical Commission of the Faculty of Medicine of the Vrije Universiteit Brussel approved the experimental protocol.

Materials

Word lists. There were four lists of 70 unrelated, frequent Dutch one-syllable words, each consisting of 3, 4 or 5 letters. In each session one list of 70 words was used as target list and another list as distractor list. Study and distractor lists were counterbalanced across participants and sessions as much as possible according to a Latin square design. The words in each list were matched in frequency according to the norms provided by Uit den Boogaart (1975). For each test, there was a random order for target and distractor words. The first and last five words of each list were not used for testing to avoid primacy and recency influences.

Drug treatment. A moderate dose of 10 mg D-amphetamine was used (average 0.14 mg/kg). We opted for this constant dose in order to replicate the design of earlier studies (Soetens et al., 1995; Zeeuws et al., 2010) where we found a positive effect of D-amphetamine after one week in a recognition experiment, using the same drug dose. This made it possible to compare results across studies. Identical and unmarked capsules were provided for drug and placebo. One hour before the start of the study phase the drug was administered orally. Pharmacokinetic data indicate that plasma levels of orally administered D-amphetamine peak 120–180 min after administration, cardiovascular effects occur at 60–120 min and behavioural effects peak 90–120 min after D-amphetamine intake (Angrist et al., 1987; Brauer and de Wit, 1996; Fabian and Silverstone, 1997). Participants remained in the laboratory between the time of administration and the start of the study phase.

Subjective ratings. Subjective state was recorded using the Profile of Mood State (POMS) with the following variables: tension, depression, anger, fatigue and vigour (Wald and Mellenbergh, 1990). Participants completed the questionnaire on each session before drug administration (baseline), 2 h after administration and after 1 day.

Procedure

Participants were tested in individual cubicles at the same time in the morning and in the same laboratory. The drug was administered orally 1 h before the study phase. Participants were informed that they would see a series of words and that they should try to remember these words for a later (unspecified) memory test. A double blind, counterbalanced, placebo-controlled design was used, with sessions being separated by a 1-week washout period.

In each session participants had to study a list of 70 words. The words were presented one by one in the centre of a computer screen at a constant rate of 2 s/word with an interstimulus interval of 250 ms. Participants were randomly divided in two groups of
12: one group did the experiment without additional test (Condition 1) and the other group with additional test (Condition 2) 1 h after list learning. All participants were asked to do a recognition test immediately after the presentation of the study list (immediate recognition, Imm), after 1-day delay (DD) and after 1-week delay (WD). The group with additional test were additionally tested on their recognition memory 1 h after the study phase (HD). All tests were self-paced, with test items being presented one by one in a random order in the centre of the screen. In Condition 1 the 60 target items from the study list were divided across 3 recognition tests, so that 40 words were presented on each test, of which 20 were target items and 20 were distractors. In Condition 2 a recognition test comprised 30 words, of which 15 were target and 15 were distractor words. Each item was accompanied by a label at the bottom of the screen, instructing the participants to press ‘1’ if they thought the word had been previously presented, or press ‘3’ if they thought it had not been presented. Participants did not receive feedback about their performance.

Recognition performance was recorded as the number of hits and false alarms. Signal detection theory was applied so that recognition discriminability (d') and response bias (C) could be calculated (MacMillan and Creelman, 2005).

Results

A 2 × 3 × 2 mixed ANOVA was conducted on recognition performance with Drug Treatment (10 mg d-amphetamine and placebo) and Test Delay (Imm, DD and WD) as within-subjects factors, and Test Frequency (with or without additional test) as between-subjects factor. The analysis was run on the proportion of hits with d-amphetamine as compared to placebo. There was a main effect of Test Delay, F(2,44) = 105.92, MSE = 0.01, p < 0.001, suggesting a substantial degree of forgetting between immediate recognition and the WD test. Importantly, Drug Treatment and Test Delay interacted, indicating more forgetting with placebo relative to d-amphetamine, F(2,44) = 4.75, MSE = 0.02, p = 0.01.

There was no main effect of Test Frequency, and no interaction with Test Delay, F(1,22) = 2.50, MSE = 0.08, p = 0.13; F(2,44) = 1.82, MSE = 0.01, p = 0.17, respectively. Importantly, there was no interaction between Drug Treatment and Test Frequency, F(1,22) = 0.32, MSE = 0.02, p = 0.57, which shows that the d-amphetamine facilitation effect was not influenced by an additional test opportunity after HD. Finally, no three-way interaction emerged, F(2,44) = 0.21, MSE = 0.02, p = 0.81, demonstrating that the time course of d-amphetamine improvement was not altered by the extra test.

Although there was no three-way interaction, we nevertheless conducted planned comparisons to verify the pattern of drug effects in both test-frequency conditions. because the additional recognition test was absent in Condition 1 (Without Intermediate Test). The data were further analysed with Planned Contrasts to unravel the precise timing of the d-amphetamine facilitation effect. The between-subjects factor order of Drug Treatment was left out of the analysis, because this factor did not show a main effect and did not interact with the other factors. The proportion of hits and false alarms, and response bias (C) are listed in Table 1. Figure 1 shows recognition discriminability (d') for all conditions as a function of Test Delay.

Proportion of hits

A significant Drug Treatment effect, F(1,22) = 5.07, MSE = 0.02, p = 0.03, illustrated the overall higher proportion of hits with d-amphetamine as compared to placebo. There was a main effect of Test Delay, F(2,44) = 105.92, MSE = 0.01, p < 0.001, suggesting a substantial degree of forgetting between immediate recognition and the WD test. Importantly, Drug Treatment and Test Delay interacted, indicating more forgetting with placebo relative to d-amphetamine, F(2,44) = 4.75, MSE = 0.02, p = 0.01.

Table 1. Hits, false alarms and response bias’ for both conditions and Drug Treatment in Experiment 1 as a function of Test Delay (mean and standard error)

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Imm</th>
<th>HD</th>
<th>DD</th>
<th>WD</th>
</tr>
</thead>
<tbody>
<tr>
<td>With additional test at 1-h delay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hits</td>
<td>0.71 (0.04)</td>
<td>0.71 (0.04)</td>
<td>0.50 (0.05)</td>
<td>0.48 (0.03)</td>
</tr>
<tr>
<td>FA</td>
<td>0.12 (0.03)</td>
<td>0.17 (0.07)</td>
<td>0.14 (0.04)</td>
<td>0.28 (0.04)</td>
</tr>
<tr>
<td>C</td>
<td>0.35 (0.12)</td>
<td>0.32 (0.13)</td>
<td>0.67 (0.12)</td>
<td>0.37 (0.09)</td>
</tr>
<tr>
<td>Without additional test at 1-h delay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hits</td>
<td>0.79 (0.04)</td>
<td>/</td>
<td>0.64 (0.05)</td>
<td>0.53 (0.03)</td>
</tr>
<tr>
<td>FA</td>
<td>0.11 (0.03)</td>
<td>/</td>
<td>0.21 (0.04)</td>
<td>0.26 (0.04)</td>
</tr>
<tr>
<td>C</td>
<td>0.18 (0.12)</td>
<td>/</td>
<td>0.24 (0.12)</td>
<td>0.30 (0.09)</td>
</tr>
</tbody>
</table>

Amph, d-amphetamine; Pla, placebo; Imm, immediate recognition; HD, 1-h delay; DD, 1-day delay; WD, 1-week delay.
conditions. The results of the Planned Contrasts, with Bonferroni correction, were in accord with previous findings. For the condition with additional test there was no drug effect on the immediate recognition test, on the HD test and on the DD test, $F(1,22) = 0.96$, $p = 0.34$; $F(1,11) = 0.01$, $p = 0.92$; $F(1,22) = 0.88$, $p = 0.36$, respectively. D-Amphetamine facilitation only emerged after 1 week, $F(1,22) = 13.69$, $p < 0.001$. For the condition without additional test, the analysis revealed the same pattern. There was no effect of D-amphetamine on immediate recognition and after DD, $F(1,22) = 0.01$, $p = 0.95$; $F(1,22) = 2.19$, $p = 0.15$, respectively. Again, a D-amphetamine enhancement appeared after 1 week, $F(1,22) = 11.87$, $p = 0.002$. The present data confirm that D-amphetamine positively affects long-term retention of verbal material in human memory without affecting initial encoding processes. More importantly, an additional recognition test after HD, while participants were still under D-amphetamine influence, did not alter the drug effect.

Proportion of false alarms

A repeated measures ANOVA on the proportion of false alarms, showed a tendency for a drug effect, with a slightly higher proportion for placebo relative to D-amphetamine, $F(1,22) = 3.39$, MSE = 0.02, $p = 0.08$. No effect of Test Frequency was observed, $F(1,22) = 0.51$, MSE = 0.05, $p = 0.48$, and no interaction between Test Frequency and Drug Treatment emerged, $F(1,22) = 0.37$, MSE = 0.02, $p = 0.55$. False alarm rates increased over time, $F(2,44) = 32.49$, MSE = 0.01, $p < 0.001$, and the increase was the same for both drug treatments, $F(2,44) = 0.95$, MSE = 0.01, $p = 0.39$, and both conditions, $F(2,44) = 1.56$, MSE = 0.01, $p = 0.22$. Finally, no Drug Treatment × Test Delay × Test Frequency effect was observed, $F(2,44) = 0.11$, MSE = 0.01, $p = 0.89$. Planned Contrasts revealed no significant effect of Drug Treatment on any Test Delay in any condition (all $p > 0.10$).

Recognition discriminability ($d'$)

Participants with D-amphetamine showed a better ability to distinguish between targets and distractors than with placebo, $F(1,22) = 4.45$, MSE = 0.60, $p = 0.047$. Also a significant main effect of Test Delay on discriminability was found, $F(2,44) = 102.25$, MSE = 0.37 $p < 0.001$. More importantly, the interaction between Drug Treatment and Test Delay was significant, $F(2,44) = 5.30$, MSE = 0.25, $p = 0.009$, again illustrating the change of D-amphetamine enhancement over time (see also Figure 1).

The analysis showed no main effect of Test Frequency, $F(1,22) = 0.01$, MSE = 1.59, $p = 0.97$, and no interaction between Test Frequency and Test Delay, $F(2,44) = 0.09$, MSE = 0.37, $p = 0.92$. There was also no interaction between Drug Treatment and Test Frequency, $F(1,22) = 0.49$, MSE = 0.60, $p = 0.49$, indicating that the D-amphetamine facilitation effect on verbal memory was independent of the number of intermediate tests. Finally, no three-way interaction emerged, $F(2,44) = 0.78$, MSE = 0.25, $p = 0.46$, show-
ing that for recognition discriminability the pattern of drug enhancement over time was not modulated by the extra test.

Planned Contrasts with Bonferroni correction on the two conditions separately confirmed the previous analyses. For the condition with additional test, no drug effect was found on immediate recognition, on the HD test and on the DD test, $F(1,22) = 0.93, p = 0.34; F(1,11) = 1.31, p = 0.28; F(1,22) = 2.49, p = 0.13$, respectively. However, the $\alpha$-amphetamine facilitation effect emerged after 1 week, $F(1,22) = 14.21, p < 0.001$. The pattern of results was the same in the condition without additional test, that is, no effect of $\alpha$-amphetamine on immediate recognition and the DD test, $F(1,22) = 0.18, p = 0.67; F(1,22) = 2.91, p = 0.11$, respectively, and a $\alpha$-amphetamine facilitation effect on the WD test, $F(1,22) = 16.47, p < 0.001$.

**Response bias (C)**

The analysis on $C$ revealed no main effect of Drug Treatment, $F(1,22) = 0.04, MSE = 0.18, p = 0.84$. A main effect of Test Delay showed that participants responded more conservative over time, $F(2,44) = 10.64, MSE = 0.11, p < 0.001$, but there was no interaction between Drug Treatment and Test Delay, $F(2,44) = 1.86, MSE = 0.11, p = 0.17$. There was a tendency towards responding more conservative in the condition with additional test relative to the condition without additional test, $F(1,22) = 3.97, MSE = 0.53, p = 0.06$, and the difference had the tendency to increase over time, $F(2,44) = 2.78, MSE = 0.11, p = 0.07$. Finally, no interaction between Drug Treatment $\times$ Test Delay $\times$ Test Frequency was observed, $F(2,44) = 0.07, MSE = 0.11, p = 0.93$. Planned Contrasts revealed no significant differences (all $p > 0.10$).

**Subjective ratings**

The changes in memory performance occurred in the absence of any subjective alterations in mood, as measured by the variables of the POMSs (all comparisons $p > 0.05$).

**Conclusion**

The insertion of an extra recognition test 1 h after learning, at a time that participants were still under influence of the drug, did not modulate the pattern of memory improvement with $\alpha$-amphetamine. In both conditions, a significant $\alpha$-amphetamine improvement was only observed after WD. We conclude that the $\alpha$-amphetamine enhancement is not caused by an indirect influence of the drug on intermediate retrieval.

**EXPERIMENT 2**

In this experiment, we studied the testing effect by investigating the influence of repeatedly retrieving exactly the same target items on intermediate tests. Because the strongest influence of repeated testing has been found in experiments where the same items had to be retrieved on consecutive tests (e.g. Karpicke and Roediger, 2009), item-specific retrieval processes could be responsible for a modulation of the $\alpha$-amphetamine memory enhancement. In one condition, the target items were the same on each successive recognition test and in the other condition different target items were tested on each recognition test. Repetition of target items over recognition tests was manipulated within-subjects, so that all participants did both conditions. Again, based on the study of Zeeuws and Soetens (2007), we predicted that the $\alpha$-amphetamine improvement would not be influenced by item-specific repeated testing, and that, as before, facilitation in recognition would emerge after WD.

**Method**

**Participants.** Sixteen paid male volunteers, between the ages of 18 and 25 ($21.4 \pm 1.59$), were recruited by an advertisement in the university newspaper. Exclusion criteria were the same as in Experiment 1. All participants provided written informed consent prior to the experiment. None of the volunteers had previously participated in similar studies and all were unaware of the nature of the administered drug. The Ethical Commission of the Faculty of Medicine of the Vrije Universiteit Brussel approved the experimental protocol.

**Materials**

**Word lists.** There were 8 lists of 70 unrelated, frequent Dutch words. Each word consisted of 3, 4 or 5 letters and had only one syllable. In each of 4 sessions 1 list of 70 words was used as the source for target stimuli. Half of the participants were presented with four target lists, one in each condition and Drug Treatment. The words of the other four lists were used as distractors. Targets and distractors were switched for the other participants. The words in each list were matched in frequency according to the norms provided by Uit den Boogaart (1975). For each test, there was a random order of target and distractor words. To avoid primacy and
recency effects, the first and last five words of the lists were not used for testing.

Drug Treatment and subjective ratings were the same as in Experiment 1.

Procedure
As in Experiment 1, a double blind, counterbalanced, placebo-controlled design was used. Sessions were separated by a 1-week washout period. Drug Treatment, Repeated Testing (with or without repeated testing of the same target items) and lists were counterbalanced according to a Latin square design across participants and sessions as much as possible. The drug was administered orally 1 h before the study phase. Participants were informed that they would see a series of words and that they should try to remember these words for a later (unspecified) memory test.

The 16 male volunteers participated in 4 sessions: Drug Treatment (D-amphetamine and placebo) and Repeated Testing (with or without repeated testing) were manipulated within-subjects. In each session participants had to study a list of 70 words and performed on 4 yes/no recognition tests: immediately after list learning (Immediate Recognition, Imm), after HD, after DD and after WD. Each recognition test comprised 30 words, of which 15 were target words and 15 distractors. In the condition with repeated testing, the participants were given the same 15 target words with different distractors on all successive recognition tests. In the other condition, different target and distractor words were used on different recognition tests. All recognition tests followed the same procedure as in Experiment 1. Recognition performance is reported as the number of hits and false alarms. Recognition discriminability (d') and response bias (C) were calculated using signal detection theory (MacMillan and Creelman, 2005). Also in this study, participants did not receive feedback about their performance.

Results
A 2 × 4 × 2 repeated measures ANOVA was conducted on recognition performance with Drug Treatment (10 mg D-amphetamine and placebo), Test Delay (Imm, HD, DD and WD) and Repeated Testing (with and without repeated testing of the same targets) as within-subjects factors. For the same reason as in Experiment 1, the factor Order of Drug Treatment was removed from all analyses. The data of the proportion of hits and false alarms, and response bias (C) measures can be found in Table 2. Figure 2 shows recognition discriminability (d') for all conditions as a function of Test Delay.

Proportion of hits
The analysis on the proportion hits showed no significant Drug Treatment effect, F(1,15) = 0.75, MSE = 0.03, p = 0.40. A main effect of Test Delay indicated that there was a substantial degree of forgetting between the immediate recognition test and WD, F(3,45) = 14.91, MSE = 0.03, p < 0.001. The interaction between Drug Treatment and Test Delay showed a tendency towards more forgetting with placebo relative to D-amphetamine, F(3,45) = 2.30, MSE = 0.01, p = 0.09.

Not surprisingly, the proportion of hits in the condition with repeated testing of the same target items was higher than in the condition with different target items on each recognition test, F(1,15) = 22.99, MSE = 0.07, p < 0.001. There was an interaction between Repeated Testing and Test Delay, with a

<table>
<thead>
<tr>
<th></th>
<th>d-Amphetamine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imm</td>
<td>HD</td>
</tr>
<tr>
<td>Repeated testing of the same target items</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hits</td>
<td>0.73 (0.04)</td>
<td>0.78 (0.05)</td>
</tr>
<tr>
<td>FA</td>
<td>0.13 (0.03)</td>
<td>0.22 (0.04)</td>
</tr>
<tr>
<td>C</td>
<td>0.31 (0.12)</td>
<td>0.03 (0.14)</td>
</tr>
<tr>
<td>Different targets on each recognition test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hits</td>
<td>0.73 (0.04)</td>
<td>0.68 (0.04)</td>
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</tr>
<tr>
<td>C</td>
<td>0.40 (0.11)</td>
<td>0.30 (0.11)</td>
</tr>
</tbody>
</table>

Imm, immediate recognition; HD, 1-h delay; DD, 1-day delay; WD, 1-week delay.
steeper decline in performance when different targets were tested (see Figure 2), $F(3,45) = 35.98$, MSE = 0.01, $p < 0.001$. Importantly, there was no interaction between Drug Treatment and Repeated Testing, $F(1,15) = 0.54$, MSE = 0.03, $p = 0.47$, showing that the facilitation effect was independent of repeated testing of the same items. Finally, a three-way interaction emerged, $F(3,45) = 3.55$, MSE = 0.01, $p = 0.02$, showing that the course of d-amphetamine enhancement was influenced by repeated testing.

The results of the Planned Contrasts, with Bonferroni correction, for the condition with different targets were in agreement with the results of Experiment 1. There was no effect of the drug on immediate recognition, HD test and DD test; $F(1,15) = 1.19$, $p = 0.29$; $F(1,15) = 0.04$, $p = 0.85$, $F(1,15) = 3.88$, $p = 0.07$, respectively, and the d-amphetamine facilitation effect became significant after 1 week, $F(1,15) = 9.24$, $p = 0.008$. However, there was no drug effect in any recognition test for the condition with repeated testing: immediate recognition test, $F(1,15) = 0.11$, $p = 0.75$; HD test, $F(1,15) = 0.58$, $p = 0.46$; DD test, $F(1,15) = 0.06$, $p = 0.81$; and WD test, $F(1,15) = 0.52$, $p = 0.48$. Although this last result seems to indicate that repeated testing eliminated the d-amphetamine enhancement on long-term consolidation, we will present an alternative hypothesis for this discrepant result in the General Discussion Section.

Proportion of false alarms

There was no difference in proportion of false alarms between drug and placebo $F(1,15) = 0.13$, MSE = 0.03, $p = 0.73$. A main effect of Test Delay indicated that false alarm rate increased with delay, $F(3,45) = 11.72$, MSE = 0.02, $p < 0.001$. There was a tendency towards an interaction between Drug Treatment and Test Delay, illustrating a slightly larger increase in the proportion of false alarms for placebo relative to d-amphetamine over time, $F(3,45) = 2.49$, MSE = 0.00, $p = 0.07$.

There was no effect of Repeated Testing, $F(1,15) = 2.75$, MSE = 0.03, $p = 0.12$, and no interaction between Repeated Testing and Drug Treatment, $F(1,15) = 1.25$, MSE = 0.03, $p = 0.28$. More false alarms were reported over time in the condition with different targets, $F(3,45) = 4.77$, MSE = 0.01, $p = 0.006$. Finally, no Drug Treatment × Test Delay × Repeated Testing effect was observed, $F(3,45) = 0.51$, MSE = 0.01, $p = 0.68$. Planned Contrasts revealed no significant differences between d-amphetamine and placebo on any recognition test for both types of testing (all $p > 0.10$).

Recognition discriminability ($d'$)

The ability to distinguish between targets and distractors was the same for both drug treatments, $F(1,15) = 0.04$, MSE = 1.55, $p = 0.85$, and discriminability decreased over time, $F(3,45) = 25.95$, MSE = 0.65 $p < 0.001$. More importantly, Drug Treatment and Test Delay interacted significantly, $F(3,45) = 3.22$, MSE = 0.26, $p = 0.03$, suggesting a change in the d-amphetamine facilitation effect over time (Figure 2).

Furthermore, repeated testing improved memory, $F(1,15) = 23.21$, MSE = 1.08, $p < 0.001$, and the factor
Repeated Testing interacted with Test Delay, $F(3,45) = 21.16$, $MSE = 0.41$, $p < 0.001$, showing the increasing advantage of repeated testing with delay. Importantly, there was no interaction between Drug Treatment and Repeated Testing, $F(1,15) = 1.05$, $MSE = 0.82$, $p = 0.33$, showing that repeated testing of the same target items did not affect the d-amphetamine facilitation effect on verbal memory. Finally, there was no three-way interaction, $F(3,45) = 1.53$, $MSE = 0.38$, $p = 0.22$.

Planned Contrasts, with Bonferroni correction, revealed no significant drug effect for the condition with different targets on the immediate recognition test and on the HD test, $F(1,15) = 0.48$, $p = 0.50$ and $F(1,15) = 0.26$, $p = 0.62$, respectively. Unexpectedly, there was a significant drug effect after DD, $F(1,15) = 5.34$, $p = 0.04$. As before, the d-amphetamine facilitation effect appeared at the one week delay test, $F(1,15) = 11.99$, $p = 0.003$. For the condition with repeated testing of the same targets no Drug Treatment effect could be found on any recognition test, not on the immediate recognition test, $F(1,15) = 0.05$, $p = 0.83$, after HD, $F(1,15) = 0.52$, $p = 0.48$, after DD, $F(1,15) = 0.75$, $p = 0.40$, nor after WD, $F(1,15) = 0.45$, $p = 0.51$.

Response bias (C)

The analysis on C revealed no main effect of Drug Treatment, $F(1,15) = 0.03$, $MSE = 0.23$, $p = 0.86$, and of Test Delay, $F(3,45) = 1.55$, $MSE = 0.23$, $p = 0.21$. Neither was there an interaction between Drug Treatment and Test Delay, $F(3,45) = 0.86$, $MSE = 0.08$, $p = 0.47$.

There was more conservative responding in the condition with repeated testing relative to the condition with different targets, $F(1,15) = 9.74$, $MSE = 0.35$, $p = 0.007$. Furthermore, an interaction was found between Repeated Testing and Test Delay, $F(3,45) = 5.23$, $MSE = 0.07$, $p = 0.004$, showing that repeatedly testing the same items counteracted forgetting. The interaction between Repeated Testing and Drug Treatment was not significant, $F(3,45) = 0.01$, $MSE = 0.033$, $p = 0.95$. Finally, no three-way interaction was observed, $F(3,45) = 0.97$, $MSE = 0.08$, $p = 0.42$. Planned Contrasts revealed no significant effect of Drug Treatment on any Test Delay or Type of Testing (all $p > 0.10$).

Subjective ratings

The changes in memory performance occurred in the absence of any subjective alterations in mood, as measured by the variables of the POMSs (all comparisons $p > 0.05$).

Conclusion

Repeatedly testing the same items on intermediate recognition tests improves long-term retrieval compared to the testing of different items on each test. However, this improvement is not altered by d-amphetamine, demonstrating that the influence of the drug on consolidation is independent of repeated retrieval of exactly the same items.

GENERAL DISCUSSION

The aim of the present study was to find out whether the improvement of long-term retention with d-amphetamine could be ascribed to an influence on intermediate testing. While the majority of studies investigating the influence of stimulants on long-term retention assume that d-amphetamine enhances the consolidation process, it has never been made explicit what such a modulation of consolidation processes entails. Because recent investigations have drawn attention to the crucial influence of the testing effect on long-term learning (e.g. Karpicke and Roediger, 2009), we investigated the hypothesis that d-amphetamine improvement could be related to influences of the drug on processes related to intermediate testing. Because in most research there is more repeated testing in recall compared to recognition, we determined whether this could explain why the improvement with d-amphetamine is faster and more pronounced in free recall than in recognition (Soetens et al., 1995).

In Experiment 1, we investigated the influence of repeated testing on the d-amphetamine memory enhancement by adding an extra recognition test 1 h after list learning, at the time that the participants were still under the influence of the drug. In Experiment 2 we directly measured the influence of repeated testing of the same items in consecutive recognition tests on the d-amphetamine improvement.

In general, the current experiments clearly support the facilitating effect of an acute administration of d-amphetamine on the long-term retention of verbal memory in healthy men (see also Breitenstein et al., 2004; Rapoport et al., 1980; Soetens et al., 1995; Weingartner et al., 1982; Zeeuws and Soetens, 2007; Zeeuws et al., 2010). In agreement with these earlier studies, there was no improvement with the drug on immediate tests, but the effect only emerged after a long delay.

More importantly, Experiment 1 showed that adding an extra recognition test did not alter the magnitude of the d-amphetamine improvement. With or without an additional test, the enhancing effect of the drug
only emerged after WD. This result shows that d-amphetamine does not act indirectly upon consolidation by influencing memory retrieval processes. Although participants were still under drug influence 1 h after learning, inclusion of an HD recognition test did not modulate the drug enhancement. These data are in agreement with a previous study, where the number of retrieval attempts was manipulated in a free recall task (Zeeuws and Soetens, 2007). Also in that study, the number of intermediate free recall tests did not modulate the memory enhancement with d-amphetamine. Although in general the number of intermediate tests is of utmost importance for long-term retention (Butler and Roediger, 2007; Karpicke and Roediger, 2009; Kazen and Solis-Macias, 1999; Roediger and Karpicke, 2006a,b), the enhancement caused by amphetamine does not seem to be related to this testing effect. It could be argued that in a recognition task, the items are different on each test, whereas in most of the studies showing the testing effect, the same items are tested repeatedly on different delays. However, Chan et al. (2006) demonstrated that the memory amelioration of intermediate testing is not limited to item-specific effects, and that also testing semantically or episodically related items, such as belonging to the same list of words, improve long-term memory for target items. Nevertheless, we did not find an effect of an extra recognition test and moreover, there was no interaction with d-amphetamine, demonstrating that such a mechanism cannot explain the d-amphetamine improvement in this experiment. Based on these observations it is unlikely that d-amphetamine acts upon the testing effect.

Experiment 2 revealed a positive effect of repeated testing of the same items in recognition. The amount of recognized items did not decline across successive tests in contrast to the condition with nonrepeated testing. This finding is not new and adds up to the well-documented findings of memory enhancement with repeated testing in free recall (e.g. Erdelyi and Kleinbard, 1978; McDaniel et al., 1998; Mulligan, 2001), and associative recall (Karpicke and Roediger, 2009; Roediger and Karpicke, 2006a,b), but also in repeated recognition (Erdelyi and Stein, 1981; Kazen and Solis-Macias, 1999). Importantly, repeated testing of the same target items was not affected by an acute administration of d-amphetamine. On the contrary, the drug effect even disappeared completely in this condition. So, even though a positive testing effect was observed on recognition performance, it was independent of any d-amphetamine improvement. This finding confirms the results of Experiment 1 and those of Zeeuws and Soetens (2007) with recall experiments. In contrast to our predictions and to the results of recognition studies with nonrepeated testing (Soetens et al., 1995; Experiment 1 of this study), we did not find a d-amphetamine facilitation effect after WD in the condition with repeated testing. A plausible reason for the absence of drug facilitation could be a ceiling effect (Kazen and Solis-Macias, 1999). Indeed, performance was relatively high in the immediate recognition test, even in the placebo condition, so that a further improvement with d-amphetamine was difficult to obtain. Anyhow, the data showed that repeated testing of the same items certainly did not enhance the d-amphetamine improvement on verbal memory.

The present findings are highly relevant with respect to the differential time course of d-amphetamine improvement observed in recognition and recall memory. It has been suggested that repeated testing of the same items in free-recall experiments could explain this difference. However, this hypothesis has to be refuted on the basis of both, the present study with repeated testing in recognition, and the previous study manipulating repeated testing in recall (Zeeuws and Soetens, 2007). An alternative explanation for the differential time course between recall and recognition may be a difference in context-dependency. Storage of contextual information allows an event to be encoded uniquely in memory, so that it can be distinguished from other similar events in memory. In a recent study, using a source memory paradigm, Zeeuws et al. (2010) showed that d-amphetamine did not only enhance long-term memory for target items, but that also the knowledge about the temporal context of acquisition was better retained.

In summary, we replicated the d-amphetamine facilitation effect in long-term recognition of verbal material in humans. In two experiments, we demonstrated that the d-amphetamine enhancement cannot be ascribed to processes related to the testing effect. Neither the number of recognition tests, nor the repeated testing of the same items on consecutive tests influenced the d-amphetamine memory enhancement. It shows that the positive effect of d-amphetamine on long-term verbal retention is independent of possible enhancements of consolidation caused by repeated testing. Accordingly, these data suggest that the differential time course of the drug effect between recall and recognition cannot be explained by influences on intermediate retrieval. Together with previous studies, the present results suggest that d-amphetamine affects processes operating after initial encoding, making recently formed memory traces more stable and less vulnerable to forgetting, which are independent of processes related to intermediate
testing sessions. At this time, most of the evidence seems to point to a d-amphetamine improvement in the retention of the context in which the target items have been presented.

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