Odor cueing during sleep improves consolidation of a history lesson in a school setting

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Abstract

Sleep is a key factor in memory consolidation. During sleep, information is reactivated, transferred, and redistributed to neocortical areas, thus favoring memory consolidation and integration. While they occur spontaneously, these reactivations can also be induced using external cues linked to the acquired information, such as sound or odor cues. Hence, the target memory reactivation during sleep represents an advantageous tool to improve school content in real-life settings. In this study, our goal was to improve consolidation of complex information such as a history lesson, using one session of study at school in the presence of an odor, and one reactivation round while sleeping in their homes on the same night of the acquisition, without using additional study sessions. We found that complex information can be associated with an odor in the classroom, and one session of reactivation during the first night of sleep in the students' houses improves its consolidation. These results bring new evidence for the implementation of reactivation during sleep in real-life settings.

Introduction

Memories are not immediately fixed after acquisition. On the contrary, they are labile, followed by a stabilization process known as consolidation, where the memory trace becomes protected against interference[1]. Sleep plays a principal role in memory consolidation. During sleep, recently acquired information is spontaneously reactivated, transferred, and redistributed to neocortical stores favoring memory consolidation and integration[2]. Although, it is important to highlight that not all memories are equally benefited during sleep. The emotionally salient stimuli, the rewarded information, the intention, and the instruction to remember, that is, all the information that involves future relevance, is the one shown to be particularly benefited by sleep[3,4].

In recent years, sleep has been proposed as a tool to improve education[5,6]. Preliminary results showed that sleeping after learning enhances subsequent retrieval also in school settings[7–9]. However, we should take into account that not necessarily all the information acquired at school is taken as relevant for our brain and thus, it can easily be forgotten. Nevertheless, there are plenty of studies showing that when a cue (e.g. odor or sound) previously linked to a task (e.g. word-pairs) is presented again during sleep, particularly during the first Non-Rapid Eye Movement sleep cycles (NREM), memory strengthening and/or protection against interferences emerges as a result of the induced reactivation[3,10–13]. Thus, the presentation of specific cues during learning and subsequent sleep could guide which content would be consolidated in the sleeping brain (e.g. a school history lesson), independent of its relevance. In this way, the target memory reactivation during sleep becomes a promising tool to improve school content in real-life settings.

Recently, Neumann et al. (2020) published the first study of memory reactivation during sleep involving vocabulary learning in a regular school setting. Participants learned different sets of German-English vocabulary pairs presented to the students at school without odor[14]. Then they went through four different conditions of odor presentation: 1) during studying time at home and sleeping at night for 7 days, and in the vocabulary test on day 7; 2) during studying time at home and sleeping at night for 7 days; 3) studying time at home only; 4) no presentation of the odor. Interestingly, they found that the odor reactivation during the whole night without sleep monitoring, improved memory retention as well as in controlled experiments in the Lab only if the odor had been previously presented during the learning sessions.

Here, we aim to study the effect of cueing a more complex school curricula content of sixth-grade students, presenting the odor cue while learning a history lesson at school and reactivating the memory during the first night of sleep after acquisition, in their homes. For that, students performed a two-day experiment (Fig. 1). On day 1, a history lesson about the city of Petra and the Nabatean was given in a school class by their own teacher in the presence of coconut odor. Students took an immediate test to measure the initial level of learning (short-term testing). Half of the students received the same odor during the first hour and a half of sleep at home (auto-administered) (Reactivation group), while the other half received violets odor (control condition, No reactivation group). Finally, seven days later they were tested again in the absence of the odor (long-term testing) (Fig. 1a).

Results

To study the effect of odor cueing during sleep on memory consolidation in a school setting, we conducted a two-day experiment (Fig. 1). On day 1, the students received a history lesson provided by their teacher in the presence of an odor (coconut) and were immediately evaluated (short-term testing). Half of the participants received the same odor (coconut, Reactivation group) or a control odor (violets, No Reactivation group), during the first night of sleep. They were finally evaluated on day 8, (long-term testing) (Fig. 1a).

Odor cues during sleep improved memory retention

Repeated measures ANOVA analysis revealed a “group” per “session” interaction $F(1,43) = 4.76$, $p = 0.035$, $\eta^2_p = 0.1$. Thus, we performed simple effects analyses of “group” within each level of “session”. On one hand, we found that the groups reached a similar score on day 1 (Fig. 1b, R group: 58.0 ± 4.1; NR group: 57.5 ± 3.1, simple effects $F(1,43) = 0.010$, $p = 0.919$, $\eta^2_p < 0.001$). On the other hand, the Reactivation group showed a significantly higher score on day 8 than the No reactivation group (Fig. 1b, R group: 50.0 ± 3.4; NR group: 37.1 ± 3.6, simple effects $F(1,43) = 6.68$, $p = 0.013$, $\eta^2_p = 0.1$). Simple effects of “session” within each level of “group” showed that there was a significant memory decay between the short term and long term memory sessions for the No Reactivation group and a trend for the Reactivation group (Fig. 1C, simple effects, $F_{NR}(1,43) = 26.54$, $p < 0.001$, $\eta^2_p = 0.4$; $F_R(1,43) = 3.94$, $p = 0.053$, $\eta^2_p = 0.08$).

Additionally, we found that the Reactivation group had a significantly smaller normalized memory change (less memory decay on day 8 according to what the participants had learned on day 1) than the No reminder group (Fig. 1c, R group: -6.2 ± 8.8, NR group: -33.7 ± 6.6; Two-sample t-test $t(43) = -2.51$, $p < 0.016$, Cohen’s $d = 0.75$). Furthermore, memory change of the No Reactivation group significantly differed from zero (One-sample t-test(22) = -5.08, $p < 0.001$, Cohen's d = -1.06), whereas memory change of the Reactivation group did not differ from zero (One-sample t-test(21) = -0.71, p = 0.485, Cohen's d = -0.15).

Control measures

There were no significant differences between groups for the state anxiety at day 1 (R group: 33.6 ± 1.3; NR group: 36.6 ± 2.1, $F(1,40) = 1.391$, $p = 0.245$, $\eta^2_p = 0.034$), no significant differences for the state anxiety at day 8 (R group: 34.8 ± 2.0; NR group: 34.1 ± 2.0, $F(1,35) = 0.048$, $p = 0.828$, $\eta^2_p = 0.001$), no significant differences for the trait anxiety inventory (R group: 39.0 ± 1.9; NR group: 39.8 ± 2.8, $F(1,34) = 0.054$, $p = 0.818$, $\eta^2_p = 0.002$) neither for the Pittsburgh sleep quality index (R group: 5.4 ± 0.4; NR group: 5.8 ± 0.8, $F(1,34) = 0.191$, $p = 0.665$, $\eta^2_p = 0.006$).

Discussion

Here we show that targeted memory reactivation during sleep improves memory consolidation of complex information such as a history lesson in a school setting, moreover, only one session of cueing during sleep on the first night is sufficient to obtain a significant effect. In comparison to Neumann et al. (2020) in which the authors applied the cue during 7 consecutive nights, we observed a significant effect with only one night of reactivation[14]. It remains to be evaluated if the benefit of additional rounds of reactivation is greater than just one reactivation.

Another difference between Neumann et al. (2020)[14] and our study, is that we presented the odor at school during the class, whereas Neumann et al present it during individual study sessions at volunteers' houses. This result demonstrates the successful association between the odor and the lesson in the school setting allowing subsequent reactivation at home during sleep, without having to undergo additional sessions of study outside the classroom. Nevertheless, the disadvantage of presenting the odor cue in the classroom is the risk that if the classroom is not properly depleted of the odor after the lesson, the cue could potentially end up associated with other information.

Importantly, reactivation studies showed that cues presented during Slow Wave Sleep (SWS) benefit declarative memory consolidation while those presented during REM have no effect[10]. Thus, considering that the first 40 minutes of sleep (first sleep cycle) are rich in SWS, reactivation could be restricted to the first part of sleep, with no need for the subject to be exposed to the odor all night. Furthermore, odor cues do not interfere with sleep structure as much as sound cues do[15], even so, they can modify sleep architecture[16,17]. Hence restricting subjects' exposure to the odor is desirable.

Neumann et al. (2020) found a tendency to larger memory effects of the cue presentation during testing[14]. Several studies show that maintaining the enriched context during testing favors memory evocation[18]. This aspect should also be taken into account at the time of evaluation at school to obtain better performance.

It is challenging to transfer a laboratory experiment into a real-life situation, and it presents constraints that would not be present in lab conditions. For example, one limitation of our study is the low sample size. However, this allowed us to have the same teacher in both courses, lowering this way the variability between them. Moreover, data from the two courses were collected at the same time during the Covid-19 pandemic, so increasing the N size would imply the introduction of a new variable, the new stage in the pandemic, which could potentially introduce noise in our data.

All in all, here we did not only support previous data showing that target memory reactivations can be implemented in real-life settings[14] but also showed that complex information such as a history lesson can be associated with an odor in the classroom, and improving its consolidation with just one session of reactivation during the first night of sleep in the students' houses.

Methods

Participants

68 healthy high school students (27 females, 41 males), of two different 6th grades from the same school (that shared the same teacher) volunteered for the study. Their ages ranged from 17 to 18 years. None of the participants reported having any history of neuropsychiatric disorders, use of drugs, being sick during the experiment, and did not take any medication at the time of the experiment. Their parents or legal guardians signed a written informed consent approved by the Biomedical Research Ethics Committee of the Alberto C. Taquini Institute, previously to their children's participation in the study.

Data from 23 participants were excluded from the study because they did not participate in the testing session on day 8 (16), and did not match the learning criteria of 30% of correct responses on day 1 (7). Thus, the final sample was 45 participants. Unlike Neumann et al (2020) who present the odor all night long[14], our odor was present for 1.5 h to guarantee the cueing to be present during the first cycle of NREM sleep[2].

Experimental design

The study was conducted in 2 days, separated by a seven-day interval. On day 1, the classrooms were odorized using spray dispensers before the students entered the room. Both courses received a history lesson (training session) of 20 minutes provided by their own teacher (the same for the two courses) in the presence of coconut odor. To perfume the classroom we used 1 spray dispenser per 3 students. The history lesson was about the city of Petra and the Nabatean culture and commerce. When the teacher finished the lesson the spray dispensers were turned off. Afterward, the students resolved a multiple-choice exam of 13 questions about the lecture (short-term testing session). After finishing the exam, they completed the State Anxiety Inventory and the Pittsburgh Sleep Quality Index.

In each classroom, students were randomly assigned to the Reactivation or the No reactivation groups, and each one of them was provided with an automatic spray dispenser according to the group they were assigned: the Reactivation group was given the same odor that was presented in the classroom during the lesson (coconut), and the No reactivation group was provided with a different odor (violets). They were instructed to use the spray dispensers in their houses the night of the first experimental day (reactivation session). They were told to set the initiation time of the dispenser at the time they went to sleep, and the finishing time 1.30 h after. They returned the dispensers the following day. On day 8, they resolved another multiple-choice exam in the classroom (long-term testing session). After finishing the exam (without a time limit) they completed the State-Trait Anxiety Inventory and the Pittsburgh Sleep Quality Index.

Training (day 1). The training consisted of a history lesson about the ancient city of Petra and the Nabatean culture and commerce, it was presented by their teacher of Political Economics. She used a PowerPoint presentation as a tool to provide the lecture, and it was shown to the students in the classroom using a projector. Each slide was presented for 1.5 minutes as she commented on them orally.

Short-term testing session (day 1). Immediately after training, students resolved a multiple-choice exam consisting of 13 questions with 4 possible answers to each question and one correct answer. The answers to all of the exam questions were among the information written in the slides. They had no time limit to resolve the exam. Half the students of each course received the same set of 13 questions and the other half a different set of questions.

Reactivation (day 1). Memory cueing during sleep was carried out the first night of the experiment. The students received a dispenser that was programmed to spray the congruent (coconut) or incongruent (violet) odor every 15 minutes depending on the group that they were assigned. Volunteers were instructed to activate the dispenser at the time they went to sleep at night of day 1 and to set the finishing time 1.5 h after that. It is important to highlight that the participants did not know which odor they received.

Long-term testing (day 8). One week later in the classroom and without any odor, each subject resolved a different multiple-choice exam from the one that they answered on day 1. Again, they had no time limit to resolve the exam. In each classroom, half the students received the questions that their partners had resolved the week before and vice-versa.

Control measures

As this study was carried out during COVID-19 pandemic, when the levels of anxiety in the population were increased and a worse quality of sleep was reported[19–21], we controlled that both groups had a similar level in both variables. For that, we evaluated the State-Trait Anxiety Inventory (STAI)[22] and the Pittsburgh sleep quality index (PSQ)[23].

Experimental groups

Subjects were randomly assigned to one of 2 conditions: “Reactivation” and, “No reactivation” groups.

“Reactivation” group (R, n = 22). Participants were trained on day 1 on the presence of coconut odor. They received the reactivation using the same odor cue during the first night of sleep and they were finally tested on day 8.

“No reactivation” group (NR n = 23). Participants were trained on day 1. They received the reactivation session using a different odor (violets), as a control condition, during the first night of sleep and they were finally tested on day 8.

Statistical analysis

The statistical analysis was made with SPSS version 25 (IBM Corporation). The scores reached at short and long-term testing sessions were calculated as the percentage of correct answers for the short and long-term tests, respectively. We also calculated the normalized memory change for each subject as [(# of correct responses at long-term testing session - # of correct responses at short-term testing session)*100/ # of correct responses at short-term testing session].

The scores at short and long-term were analyzed with repeated measures ANOVA with “group” as between-subjects factor with two levels (Reactivation and No reactivation) and “session” as within-subjects factor with two levels (short and long term testing). Furthermore, we compared the memory change between groups with a two-tailed t-test and performed two separately one-sample t-tests to compare both groups’ memory change to the value zero. Alpha was set at 0.05.

We also analyzed the State Anxiety Inventory, Trait Anxiety Inventory and PSQI scores with One-way ANOVAs with “group” as between-subjects factor with two levels (Reactivation and No reactivation). It is important to highlight that 3 students of the No reactivation group did not complete STAI at day 1, 6 the STAI at day 8, 6 the PSQI and 6 did not complete the Trait Anxiety Inventory. 2 students of the Reactivation group did not complete the STAI at day 8, 3 the PSQI and 3 the Trait Anxiety Inventory. We reported partial eta square (η^2_p) and Cohen’s d as effect size estimates.

Declarations

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Author contributions

VV, FMB and CF made substantial contributions to the conception and design of the work. CF were responsible for funding acquisition. VV and ARB ran the experiments. VV, LMT and CF performed the data analyses. VV and CF made the graph art. CF administered and supervised the project. LIB, FMB and CF were responsible for resources. VV and CF contributed by drafting the work. VV, ARB, LMT, LIB, FMB and CF contributed to revising it critically.

The authors declare no competing financial interests.

Data availability statement

The raw data supporting the conclusions of this article will be online available upon acceptance of the manuscript.

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Figures

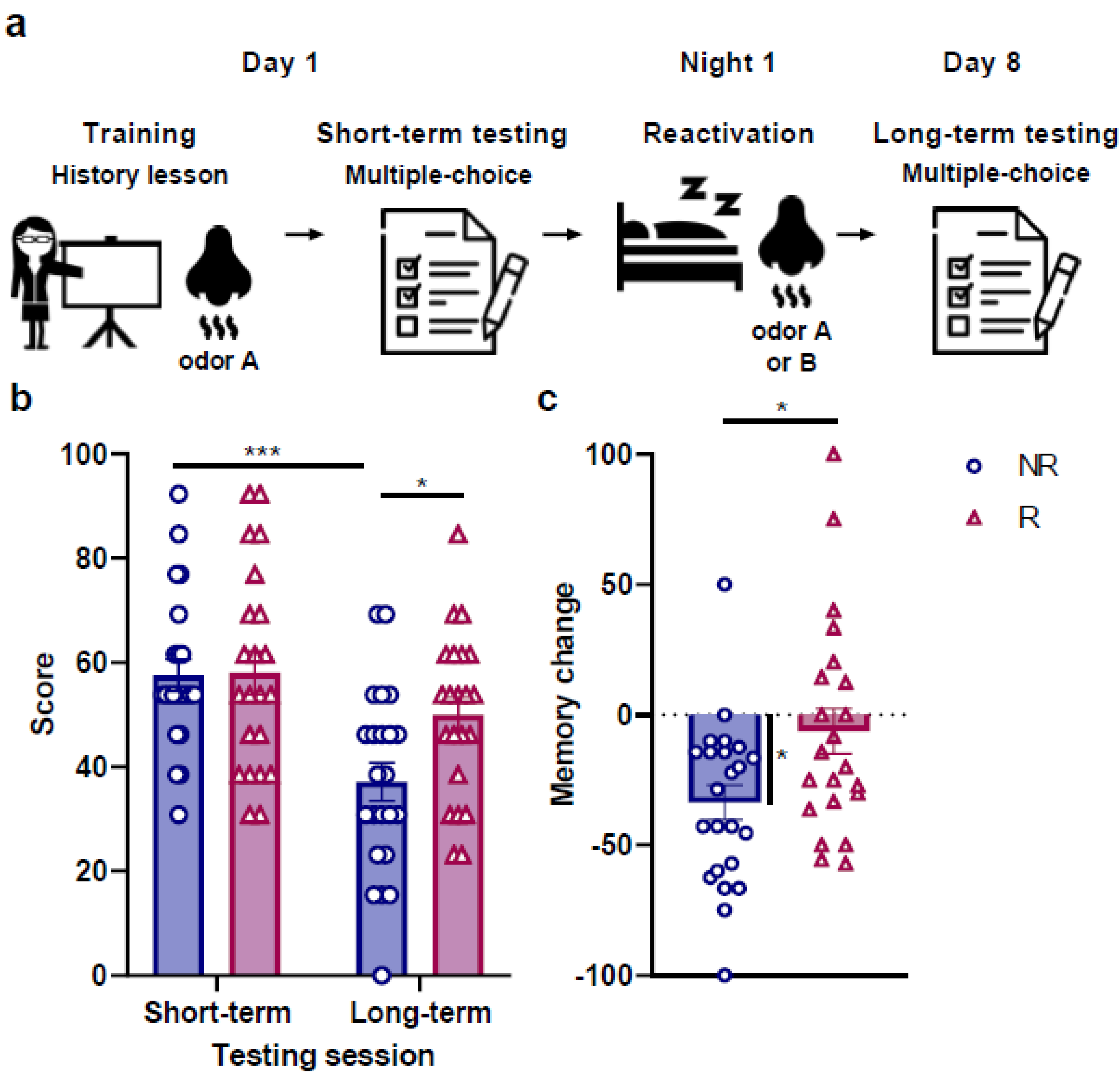

Figure 1

a, The history lesson was presented by their teacher using a PowerPoint presentation on a projector screen, each slide was shown for 1.5 minutes as she commented on them orally (day 1). Short-term testing: They resolved an evaluation consisting of 13 multiple-choice questions with 4 possible answers and one correct answer; they had no time limit to solve it and all the right answers were previously presented on the slides during the training. Night 1 Reactivation: Volunteers used in their houses the dispensers containing the congruent odor A (coconut) or the incongruent odor B (violets) (depending on which group they were assigned) at the time they went to sleep for 1.5 h, it sprayed every 15 minutes. Day 8 Long-term testing: They resolved another evaluation consisting of 13 multiple-choice questions with 4 possible answers and one correct answer; they had no time limit to solve it and all the right answers were previously presented on the slides during the training on day 1. **b,** Mean score at testing sessions on days 1 and 8 ± SEM for Reactivation and No reactivation groups. **c,** Mean memory change ± SEM for Reactivation and No reactivation groups. *, $p < 0.05$; ***, $p < 0.001$.