Video Article

# Olfactory Context Dependent Memory: Direct Presentation of Odorants

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## Abstract

Information is retrieved more effectively when the retrieval occurs in the same context as that in which the information was first encoded. This is termed context dependent memory (CDM). One category of cues that have been shown to effectively produce CDM effects are odors. However, it is unclear what the boundary conditions of these CDM effects are. In particular, given that olfaction has been called an implicit sense, it is possible that odors are only effective mnemonic cues when they are presented in the background. This assertion seems even more likely given that previous research has shown odors to be poor cues during paired associate memory tests, where odors are in the focus of attention as mnemonic cues for other information. In order to determine whether odors are only effective contextual mnemonic cues when presented outside the central focus of an observer, an olfactory CDM experiment was performed in which odorants were presented directly, rather than ambiently. Direct presentation was accomplished with the aid of an olfactometer. The olfactometer not only allows for direct presentation of odorants, but provides other methodological benefits, including the allowance of trial by trial manipulations of odorant presentations and, relatedly, time-specific releases of odorants. The presence of the same odor during both encoding and retrieval enhanced memory performance, regardless of whether the odor was presented ambiently or directly. This finding can serve as a basis for future olfactory CDM research which can utilize the benefits of direct presentation.

## Video Link

The video component of this article can be found at https://www.jove.com/video/58170/

## Introduction

Retrieval is enhanced when it occurs in the same context as encoding[1,2]; this is termed context dependent memory (CDM). Many stimuli have been shown to be effective contextual cues for enhancing retrieval[3]. In line with this general research, it has been shown that information that is learned and retrieved in the presence of the same odor is remembered better than information that is retrieved in the presence of a different odor[4] or information that is neither learned nor retrieved in the presence of an extra olfactory context[5]; this has been termed olfactory context dependent memory (OCDM).

The use of odors as contextual mnemonic cues provides researchers with new opportunities to investigate various questions related to both CDM and olfactory processing. Indeed, the relationship between olfaction and memory has been shown to be unique[6,7,8]. Memories for odors seem to be more resistant to forgetting than memory for other stimuli[6] (though this is not always found[9]). Furthermore, autobiographical memories that are cued by odors are experienced differently than autobiographical memories cued by other stimuli[7].

There are other interesting differences between olfaction and the "major" (*i.e.,* visual and auditory) senses. For example, when humans see or hear stimuli, we are frequently able to explicitly identify them and we often consciously adjust our behavior in line with the information they present. However, we are frequently unable to identify odors[10]. Furthermore, although humans are constantly surrounded by odorous substances, we do not always have the conscious experience of perceiving an odor. Accordingly, it has been proposed that olfaction is an implicit sense, and that odors mostly influence our behavior when we do not consciously perceive them[11].

If olfaction truly is an implicit sense, it poses the question as to whether odors must necessarily be outside the focus of awareness for them to act as effective contextual mnemonic cues. Some evidence would suggest this is the case, as odors have been shown to be less effective cues in paired-associates paradigms then other stimuli[12,13]. To this point all studies that have shown evidence of OCDM have utilized ambient presentation of odorants, whereby the odorants are presented in the background and subjects learn some other target information[4,14]. Often, the subjects are never explicitly told about the odors.

The focus on ambient odorant presentation up to this point has been logical, as odorants are generally present in the background of an experience, but odors are infrequently at the center of our attentional focus. That being said, ambient odorant presentation necessarily means that researchers relinquish control over some aspects that may be of interest. Chief among these aspects is time. With ambient presentation odorants must be present for a long period of time, with no control over the moment of presentation or odor perception. However, presenting odorants directly, for example with the aid of an olfactometer, allows the researcher to manipulate the time of the odorant presentation in relation to the onset time of other stimuli. Further aspects of control that are gained through direct presentation include the ability to alternate between two or more odorants during a single session, presentation to one or both nostrils, presentation of "no odor" trials, and even the ability to manipulate the intensity of odor/concentration of odorant. In short, ambient odorant presentation limits experimenters to between subjects or block-wise manipulations, while direct odorant presentation allows for odor manipulations on a trial by trial basis.

Here the fundamentals of a method for testing OCDM using direct presentation of odorants with the aid of an olfactometer are presented[15]. An olfactometer is a machine that allows for the presentation of odorants at exact points in time (for example, contemporaneously with the onset of the presentation of a visual stimulus) and for a discrete amount of time (*e.g.,* the presentation of a puff of air for 2 s). Furthermore, the olfactometer allows for the presentation to be directed below the nostrils, directly in one nostril, or directly in both nostrils. Olfactometers have long been utilized in memory research[16,17]. This protocol builds on previous research and applies it to the area of OCDM research.

## Protocol

All data collection in the original experiment[15] was performed in accordance with the ethical guidelines of the American Psychological Association as well as the World Medical Association's Declaration of Helsinki. The experiment was preapproved by the local Ethik-Kommission (equivalent to an Institutional Review Board). All subjects were made aware that their participation was voluntary and could at any time and without fear of penalty be terminated. All subjects signed informed consent.

# 1. Participant Recruitment and Pre-study Instructions

1. **Recruitment:**
    1. Recruit participants who are native speakers (to ensure a comparable understanding of the instructions and comparable processing of target information) and who have normal or corrected to normal vision. Also, either a) exclude subjects who profess below average olfactory abilities or are smokers, or b) collect information about smoking habits and olfactory ability to use in later analyses.
    2. Furthermore, given the frequency of sex differences found in olfactory studies[18], either a) recruit equal numbers of males and females, to allow for comparisons, b) perform analyses separately for each sex to ensure no differences, or c) focus on a single sex. Finally, given that olfactory performance is age dependent[19] either a) recruit all subjects from a single cohort, or b) specifically include age as a variable of interest.
    Note: The final sample used in the current study included 60 subjects (56 females, $M_{age}$ = 22.05 years, SD = 4.86), all of whom were students at the University of Hildesheim and who participated in exchange for partial course credit. Further information concerning the sample can be found in our previous article[15].
2. Provide subjects with specific pre-study instructions. 1) Subjects must not smoke for the 24 h prior to the beginning of the experiment, 2) Subjects must not eat or brush teeth for at least one hour prior to the beginning of the experiment.
3. When subjects arrive in the laboratory ensure that they have followed the pre-study instructions. This is best accomplished by having the experiment leader specifically ask the subjects whether they have complied with the instructions.
4. If they have complied, present subjects with information about the study and any ethical considerations. Have subjects sign informed consent.
5. **Situate subjects in the experimental room.**
NOTE: In the current experiment subjects were first placed in a cabin in which there was a monitor rested upon a table.
    1. Seat subjects in a comfortable chair which they can adjust as needed. Have subjects place their chins in a chin rest (in which the tubes for presenting the odors are located). Adjust the height of the chin rest to make the seating comfortable for the subject.
    2. Alternatively, use another method of presentation, including placing the tubes directly in the nostrils of the subjects or connecting the tube-ends to a mask, which ensures that the odorous material remains concentrated around the nose.

# 2. Experimental Design

NOTE: In the experiment outlined here (which corresponds to Experiment 1 from the original article[15]) the purpose was to determine whether the presence of an odor context during encoding and retrieval enhanced memory over a condition in which no extra odor context was present during both phases[5]. This is a variety of a CDM effect called context enhanced memory. The original experiment included more independent variables (odor valence and word valence) that are irrelevant for the current report. For details on the original experiment and specific information concerning the verbal material used, see the Supplementary Material from the original article[15]. The experiment is broken down into three different phases (See **Table 1** for an overview).

| Pre-study | Encoding phase | Retention interval | Retrieval phase |
|---|---|---|---|
| 1. Ensure pre-study instructions were follwed | 1. Present words one at a time | 1. 5 minute break in which subjects color in mandalas | 1. Present words one at a time |
| 2. Have subjects sign informed-concsent | 2. Subjects complete abstract/ concrete categorization task | | 2. Subjects complete old/new recognition test |
| 3. Situate subjects in experimental room | 3. Present odorants on each trial | | 3. Present odorants on each trial |
| 4. Perform practice inhalation trials | | | |

**Table 1: Overview of the experimental procedure.**

1. **For the first, encoding, phase have subjects encode the target material (words) in the presence of the contextual material (odors).**

1. Present subjects with words (one at a time) on the screen. Subjects must categorize the words as abstract or concrete. Learning is incidental, as subjects are not informed of the later memory test.
2. Have subjects categorize 90 words in total.
3. Have each trial follow the same pattern.
    1. Begin each trial with the presentation of a puff of air. The puff of air either contains odorous material or not, depending on the group.
    2. Couple the puff of air the presentation of a cross on the computer screen and have the puff of air and the cross remain for 2,000 ms.
    3. Replace the cross is replaced with a target word, and have it remain for 2,000 ms. Order word presentation randomly for each subject.
    4. Upon offset of the word, have subjects categorize the word as abstract or concrete by button press. The response triggers the immediate onset of the next trial. Present the same odorant on all 90 trials during the encoding phase.

4. Odor presentation.
    1. Utilize an olfactometer to achieve direct presentation of odorants. Olfactometers come in several different varieties. Purchase or build an olfactometer that will best meet the aims of the researchers[25].
    2. Ensure that the olfactometer design is appropriate to the research question.
    3. Cut the tubes to the appropriate length and ensure that they are held in place and tightly connected.
    NOTE: The length of the tubes influences the delay between signal onset (*i.e.,* signal from program to release an odorant) and actual arrival of odorous material to the nostrils. Researchers should be aware of the delays between signals and actual presentation times, which will vary between individual olfactometers. This information can usually be found in information provided by the retailer or in instructions for building new olfactometers.
    4. Be aware of potential issues involved with odorant presentation.
        1. Ensure that the inter-stimulus interval (ISI; or the length of time between odorant presentations) is appropriate for the research question. There is no standardized or suggested ISI appropriate for all odor experiments. Conduct pilot tests to determine which ISI's are appropriate for the experiment in question.
        2. Control for tactile stimulation. Present the tubes inside a metal bar that supports the chin rest. Have the tubes end approximately 2 cm below the nostrils. Cover the top of the metal bar with a filter (*e.g.,* tissue). Replace the filter for each subject.
        3. Control for auditory stimulation. Have subjects perform the experiment inside a sound-reducing cabin with headphones on. Store the olfactometer itself in a sound-reducing container unit stored outside of the experimental cabin.

5. For presentation control, use a standard experimental control software program (see **Table of Materials**) to control odorant presentation from the olfactometer.
6. Breathing instructions.
NOTE: When using an olfactometer, it is potentially important that subjects sniff (*i.e.,* sample the olfactory material) at the appropriate moment. This is particularly important in experiments where the odorant changes between trials.
    1. To ensure that subjects sample the odorant at the appropriate time, present a visual cue (fixation cross) simultaneously with the onset of the puff of air. Furthermore, ensure that the puff of air is long enough to allow the subjects to register the visual cue and initiate inhalation (2,000 ms). It should be noted here that the inhalation instruction might act as a second task, which could potentially interact with the task(s) of interest.
    2. Alternatively, use one of several other methods for ensuring subjects sample the odorants at the appropriate time:
        1. Present subjects with a countdown, so they can prepare to inhale when the countdown is completed
        2. Present subjects with an initial cue alerting them to exhale followed by a second cue alerting them to inhale
        3. If the tubes are presented directly in the nostrils, and a discrete presentation olfactometer is used, instruct subjects to inhale anytime they feel tactile stimulation inside the nostrils.

    3. Regardless of which method is used, have subjects complete inhalation practice trials before beginning with the actual experiment.
    4. For practice trials, instruct subjects to inhale whenever they see the appearance of the fixation cross. Present a fixation cross and an air puff for 2,000 ms on each trial. Have subjects complete 10 practice trials.

7. Timing.
    1. Present the odorous material two seconds prior to the onset of the visually presented word. This is done to ensure that the olfactory context is present during visual perception.
    2. Other research questions may call for simultaneous perception. Olfactory perception takes longer than visual and auditory perception from the time of stimulus onset until perception[20], at least as measured by response times on detection tasks[21]. Therefore, depending on the manipulation, researchers must ensure that they choose appropriate inter-stimulus intervals (ISIs). Note that this will also depend on the specifics of the olfactometer used (see 4.1.)

2. **For the second phase, use a retention interval in which subjects complete a task that is unrelated to the actual memory task.**
    1. Have subjects leave the testing cabin and move to a different area, away from odorous material. This is an important step to ensure that the context is <u>reinstated</u> during the retrieval phase.
    2. Air out the testing cabin during the retention interval with ventilators or fans.
    3. Have subjects color in mandalas for 5 minutes.
    NOTE: OCDM experiments have frequently, though not always[14], utilized long retention intervals to avoid issues of adaptation and habituation. One potential benefit of direct presentation of discrete air puffs is that it may be more resistant to habituation than ambient presentation.

4. Alternative distractor tasks can be implemented in the retention interval to accomplish different goals.

3. **For the third, retrieval phase, have subjects remember and retrieve the target information.**
    1. Have subjects return to the experimental cabin. Once again, seat subjects with their chins in the chin rest.
    2. Have subjects complete an old/new recognition test with a total of 180 trials (90 old/90 new).
    3. Have each trial follow the same pattern.
        1. Begin each trial with the presentation of a puff of air. The puff of air either contains odorous material or not, depending on the group. As this is a context enhancement experiment, the odorant presented is always the same as that presented in the encoding phase.
        2. Couple the puff of air with the presentation of a cross on the computer screen; have both the puff of air and the cross remain for 2,000 ms.
        3. Replace the cross with a word and have the subject respond (by button press) as to whether the word is old or new. The word remains until the subject responds. The order of word presentation is determined randomly for each subject.
        4. Have the subject's response trigger the immediate onset of the next trial. There are no time limits during the retrieval phase.

## 3. Stimulus Selection

1. **Target stimuli**
    1. Select target stimuli that best fit the exact research question or the type of memory test that the experimenter is planning. For the most basic design, choose stimuli that come from a non-olfactory modality (*e.g.,* words). For more information about the target stimuli used in the experiment reported here see the original article[15].

2. **Contextual stimuli**
    1. Select odorants that best fit the exact research question. Important attributes to take into account include 1) valence, 2) arousal, 3) intensity, 4) familiarity, and 5) identifiability.
    2. There are several methods that can be used when choosing the appropriate odorants.
        1. In one method, choose the odorants based on a pilot study. In this case, have external subjects (those not participating in the main experiment) rate a variety of potential odorants on various attributes. Based on the results, choose the odorants that best suit the needs of the experimental question. This was the method used in the original experiment using direct presentation and OCDM (see original article for details on chosen odorants)[15].
        2. Alternatively, the choose the odorants based on a pre-existing database containing ratings[22]. Exercise caution with this method due to large potential cultural differences in odor perception[23].
        3. Lastly, in certain cases, choose stimuli based on the subject's idiosyncratic odor ratings[24].

    3. Choose the odorous material that is best suited for the olfactometer being used.
        1. Decide on liquid or solid material—some olfactometers only allow for the use of liquid material, while others allow for the use of either liquid or solid material.
        2. Obtain odorous material from a commercial supplier, either from companies that specifically produce odorants and chemicals for research purposes, or from companies that produce products (*e.g.,* essential oils, perfumes, scented candles, *etc.*) for use by the general public.
        3. Alternatively, olfactometer permitting, collect odorous material from surrounding sources (*e.g.,* pine needles, sweaty t-shirts, fruits, *etc.*). This method may be appropriate for some research questions; exercise caution as it makes standardization (and therefore comparisons with other research projects) difficult.

## Representative Results

One of the main purposes of the current experiment was to determine whether OCDM effects could be found when the olfactory context was presented directly, rather than ambiently. There were three main dependent variables (in line with common signal detection theory analyses[26]): the proportion of hits, the proportion of false alarms, and a corrected accuracy score termed *d'*. Given the focus of interest here, the data from the two odor groups from the original experiment[15] (positive and negative odor) are combined and compared to the no odor group. Collapsing the data across these two groups provides the purest test of olfactory context enhancement.

As can be seen in **Table 2**, the olfactory context groups outperformed the no odor group in accuracy, by displaying more hits and significantly fewer false alarms as well as significantly higher *d'*. This is clear evidence that direct presentation of odorants can also lead to OCDM effects, as has been previously found for ambient presentation[4,5].

This finding is somewhat surprising, given that previous experiments have shown odors to be poorer cues of memory in paired-associates tests (where odors are presented in the foreground)[12,13] and notions that olfaction is an implicit sense[11]. Future research will be needed to determine what distinguishes using odors as paired-associates from using odors as contextual mnemonic cues.

| | | *n* | *d'* | Hits | False Alarms |
|---|---|---|---|---|---|
| Control (no odor) | | 20 | 1.54 (1.62) | .67 (.25) | .25 (.25) |
| Olfactory context | | 40 | 2.18 (.50) | .75 (.13) | .13 (.05) |
| | *t* | | 2.29 | 1.57 | 2.90 |
| | *p* | | .026 | .123 | .005 |
| | Cohen's *d* | | .53 | .40 | .67 |

**Table 2: Means, significance test values, and effect sizes for comparisons between the control and olfactory context groups.** Values in parentheses represent standard deviations.

## Discussion

The method described here, in which an olfactometer is used to directly present odorants as contextual stimuli, represents an expansion of the utility of olfactometers in olfactory memory research[16,17]. Specifically, this method allows for an expansion of the area of OCDM research. Previous research had shown that odors are indeed effective contextual mnemonic cues[4,14], but all research to this point has utilized ambient presentation of odors. By using the method described here, it was shown that OCDM effects can also be found when odorants are presented directly, by means of an olfactometer[15].

This methodology, with appropriate modifications, provides several benefits over ambient presentation. For one, direct presentation allows for presentation of odorants at specific times. This can help researchers answer several questions, such as determining whether an odor must be present contemporaneously with target material in order to be bound together, and eventually act as an effective contextual mnemonic cue. Another benefit is that direct presentation allows for trial by trial manipulations of odorant presentation. This can help researchers learn whether odors function similarly to other stimuli, which can be effective cues when altered rapidly between trials[27]. Other benefits include 1) allowing for presentation to a single nostril on one trial, 2) allowing for the presentation of multiple odorants on a single trial, and 3) allowing for the presentation of different intensities across trials. This list of the benefits of this methodology is not exhaustive but highlights its potential utility to future researchers.

Another potential benefit of using an olfactometer in OCDM research is that direct presentation may be less susceptible to adaptation and habituation than ambient presentation of odorants. Past OCDM research, which has exclusively utilized ambient presentation, has frequently made use of short encoding phases and long retention intervals, potentially to avoid problems of adaptation and habituation[14]. Any procedure that would allow for longer encoding phases and shorter retention intervals could have large practical benefits for future research in terms of allowing for more encoding phase manipulations and making organization of subjects easier. Future research is needed to investigate whether direct presentation is, indeed, less susceptible to adaptation and habituation than ambient presentation.

The use of an olfactometer to directly present odorants also has several limitations. For one, directly presenting odorants to subjects increases the likelihood that olfaction is in the center of attention. Direct presentation is, therefore, not suited to OCDM research that attempts to investigate whether a subject must be consciously aware of the olfactory context for odors to serve as effective contextual mnemonic cues. Furthermore, given that odors are generally outside the focus of our awareness on a day to day basis, the direct presentation method probably provides less external validity than the ambient presentation method.

There are several issues that researchers should consider when determining which type of olfactometer to use or build and other issues that researchers should consider about their experiment, depending on the properties of the olfactometer they have. For example, the original experiment made use of a discrete presentation olfactometer with three separate channels[15]. This particular olfactometer had an estimated flow rate of 97.4 mL/s and the tubes that led from the odorant jar to the end just below the subject's nose was 4.3 m long. A discrete presentation olfactometer presents puffs of air at discrete times. This could potentially lead to issues related to tactile information, which need to be considered by the researcher when planning the experiment (see 2.1.4.4.2). The number of channels determines the number of different odorants that can be used in a single experiment. Some research questions may require a large number of odorants to be presented to a subject in a single experimental sitting, and researchers must therefore ensure that their olfactometer has the appropriate number of channels. One more issue to consider is the length of the tubes used in the olfactometer, which determine the delay between presentation trigger and actual delivery to the subject. Researchers should test to ensure that the timing is appropriate for their research question.

When a researcher investigating OCDM is deciding whether to utilize direct or ambient presentation of odors, we would suggest the following, based on the goals of the particular research project. Ambient odor presentation should generally be preferred when matters of ecological validity are of primary concern to the researcher. Direct presentation may be preferable when matters of experimental control are of primary importance. Specifically, direct presentation should be preferred when investigating topics such as a) the role of attention towards the olfactory context, b) inter-nasal differences, c) trial by trial manipulations of presented context, and d) differences in presentation time of olfactory context. Of course, this list is not exhaustive, and there may be other situations where direct presentation of olfactory context is preferable to ambient presentation.

## Disclosures

The authors have nothing to disclose.

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