

Cross-Modal Effects of Olfactory Sense on Memory Recall

Forum Patel, Kaitlin Heller, Morgan Buchholz, Ryan Walter, Gabriel Neves

Background and Significance

Memory and learning are aspects of the human conscience that have fascinated researchers for centuries. Over the course of our lives, we make constant use of these processes and are regularly adding, removing, or editing information. Uncovering these processes could potentially give rise to treatments that enhance one's memory and learning ability and possibly even minimize side effects of neurodegenerative diseases such as Alzheimer's. Furthermore, individuals with Korsakoff's syndrome who have severe deficits in memory, lack impairment in olfactory memory, suggesting that there is a separate pathway between olfaction and memory (Woronzuk *et al.*, 2001).

The olfactory sense is connected to one's memory by the direct connection to the central nervous system's (CNS) limbic system – a system that's associated with memory and emotion – through the nerve fibers of the olfactory bulb (Buchanan *et al.*, 2003). These nerve fibers extend posteriorly towards the hypothalamus, amygdala, and hippocampus. Both the hippocampus and amygdala are structures that are well known for being associated with both short and long-term memory. The hippocampus has also been determined to have olfactory functions. Synapses between neurons from the olfactory sensory tract and neurons of the hippocampus exist and have been documented by neuroscientists utilizing the aid of functional magnetic resonance imaging (fMRI) (deOlmos *et al.*, 1978). Such synapses are an essential component of the communication between these neurons and overall retrieval of any information stored in the limbic system; which leads specialists to believe that this could be the beginning explanation for why organisms have olfactory memory.

Employing the already established physiological connections between the sense of smell and memory, we postulated that if a particular odor could be coupled to a single memory, for example a picture, then it might be easier (faster) to recall that particular piece of memory if one is later exposed to the same scent that was previously associated with the stimulus (e.g. picture). Recent research has shown a relationship between verbal memory and olfactory memory. Most people tend to think that visual and verbal cues are easier to remember, but research shows that visual, verbal, and olfactory memories are all similar and carry the same strength in memory recollection (White *et al.*, 1997). Furthermore, there is a phenomenon that states that olfactory memory triggers more emotional responses as well as increased detail in memory; this is known as the Proust Phenomenon (Toffolo *et al.*, 2012). In addition, evidence exists in vertebrates, like chipmunks and bats, which have demonstrated utilization of odor cues to localize vital nutrients and recognize their offspring (Stevenson *et al.*, 2012; Vander Wall 1991; McKracken 1993). This experiment is aimed to answer one question; do olfactory senses influence memory recall? Our hypothesis is that the if the olfactory sense can positively affect one's ability to make memories, then one's performance in a memory test that includes the connection with olfactory senses would improve.

Methodology

The physiological activity of 26 study participants (n=26), selected from University of Wisconsin-Madison's Physiology 435 class, were monitored during the entire duration of their scent-coupled memory test (Group 1=13 participants; Group 2=13 participants). All participants were randomly assigned to one of two experimental groups. The first group (Group 1) had their memory tested with a eucalyptus spearmint scent. The second group (Group 2) was tested using a garlic scent. These specific scents were chosen because of their strong nature and also to create a contrast, as they are extremely distinct from each other. Each group participant partook in three trials. The first trial was used to assess the normal memory level of the individual, whereas the later trials will be the runs where we examine a scent coupled with the memory test.

The memory test allowed the participant to study, while seated, a group of 20 black and white images for a period of 30 seconds. These images of common objects were arbitrarily assembled as to randomize the concepts. The importance of this was to ensure that the participants were not able to connect these pictures in a greater context, such as to household items, thus allowing the research team to better categorize their memory span. Figure 1 is an example of an image used on the test. After the initial study period, the participants were given an interval time of 120 seconds where they were given no stimulus and were only expected to remain calm at the test area awaiting the memory recall test. The participants were given a blank sheet of paper, where they were asked to recollect as many images as they could during a period of 120 seconds. During the study and recollection period, it was important to test each participant in an environment where there are minimal outside distractions, like noise or movement, to enable each participant to perform at his/her best. Each memory test took a total of 4.5 minutes, and each participant was tested for a total of 13.5 minutes (three trials each). Each trial had a different set of images, so, in total, there were five completely different sets of images used in the entire study. Each group used the same three sets of images for their memory test. These trials were run subsequently with no time lapse in between trials. Each correctly recalled image would be scored as one out of twenty possible points.



Figure 1: Example of a memory test image used to test the memory of each individual. There are 20 black and white figures that are meant to be disconnected. Six different sets of these images were constructed.

There were five experimental runs; however, each participant participated only in three. The first run was our control; in this run, the participants were not exposed to a scent while being shown 20 photographs, also no scent was presented when they were asked to recall the photographs two minutes later. This run provided the baseline score for the memory of the individual. This baseline test was the run that the other runs were compared to in order to assess memory gain or loss when the scent was introduced. Participants, in the second run, were exposed to scent one while viewing the photos and while being asked to recall the photos two minutes later. For the appropriate trial, scent one and scent two were placed in two separate small containers for the participant to hold to their nose during the designated time, and they were asked to breathe solely through their nose at that time. In the third run, the participants were exposed to scent one while viewing the photos but were not exposed to it while recalling the figures two minutes later. The fourth run consisted of participants being exposed to scent two while viewing the figures and while recalling the photos two minutes later (similar to the second run). In the fifth run, participants were exposed to scent two and were not exposed to scent two when being asked to recall the photos after two minutes (similar to the third run). The first group participated in runs one, two, and three. The second group participated in runs one, four, and five. At the end of the experimental trials, participants were also asked if the scent they were exposed to was generally pleasant or unpleasant. Runs where the participants were only exposed to the scent during the learning period were intended to resemble the baseline score since there would be no memory coupling in that case. However, the runs where the participants were exposed to the scent during both learning and recall should demonstrate if the participants' recall ability was increased in relation to the scent.

The physiological activity of the brain was monitored using the electroencephalogram (EEG) in order to assess brain activity in the participant to verify if there was any change between learning in the presence or absence of olfactory stimuli. The EEG monitoring software used was the BIOPAC STUDENT LAB (version BSL 4). Three small probes that measure brain activity were covered with

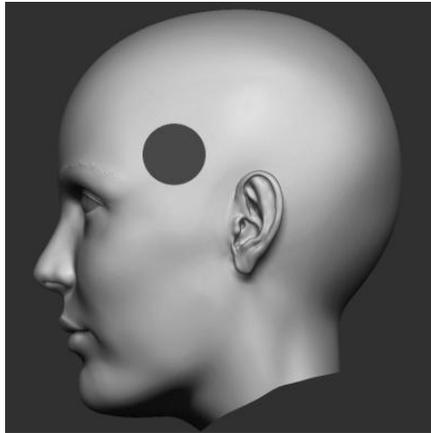


Figure 2: Left temporal region. Placement area for one of the two EEG monitoring electrodes. Electrodes were placed on temporal regions bilaterally.

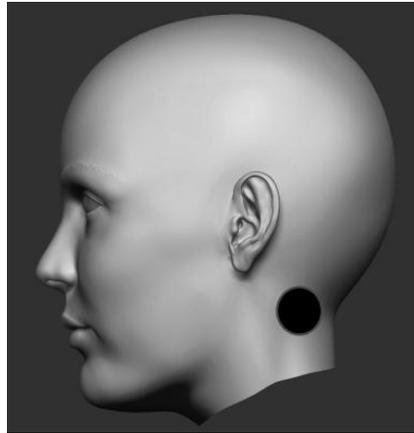


Figure 3: Left posterior cervical region. Placement area for the ground EEG electrode; right side could be used as well.

conductive adhesive gel and placed bilaterally, on the temporal region of the head, as displayed in Figure 2. The ground probe was placed on the posterior cervical region (either side), as illustrated in Figure 3. There were two monitored brain waves, and these are alpha and beta waves. Alpha waves are more prevalent in a deep relaxation state and are also believed to be involved in sensory processing/attention (Groppe *et al.*, 2013). In contrary, beta waves are associated with consciousness and reasoning,

while others have argued that functions to promote muscle tone (Groppe *et al.*, 2013). Beta wave activity differences between recall and learning periods enabled us to see a heightened or attenuated brain activity difference. When an additional olfactory stimuli is introduced, measurements of beta waves are able to be compared to their baseline activity, which are once again able to expose a heightened or attenuated wave activity. These measurements can be further correlated with memory test performance. Alpha waves, due to being prevalent in the deep relaxed state, would allow us to see whether or not individuals were not fully engaged in the experiment. Both brain waves were captured simultaneously using the previously described software while the participant remained seated during the length of the learning and recall phases. Each participant had their EEGs collected during the entire span of both study and recollection periods. The data that was collected from the EEG is the average amplitude and frequency of each brain wave using the BIOPAC's data analysis tool. This data was analyzed by looking at the amplitude and frequency of the waves. The data was taken from the EEG at 15 seconds intervals. In other words, the mean values, standard deviation and frequency values of the alpha and beta waves were recorded at the first 15 seconds of learning, 30 seconds of learning, 15 seconds of recall, and 30 seconds of recall. The changes in amplitude and frequency of the waves were compared between the learning phase and the recall phase, which indicated changes in brain activity in the recall phase in relation to the learning phase. Also, the obtained data went through one-way ANOVA tests to reveal any statistical significance between the results; statistical significance was set a p-value <0.05.

In combination to the EEG, participants had their heart rate monitored during study and recollection time; assuming a connection between heart rate and anxiety level, we looked for an addition correlation between a memory recall task and an anxiety response. The apparatus used for monitoring these vital signs is the Nonin Pulse Oximeter. In contrast with the EEG, which was continuously monitored, the heart rate was measured at specific locations during the test to assure that changes were being witnessed at relevant times. That is, after 15 seconds during the learning phase of our experiment, a heart rate measurement was taken. Another measurement of heart rate was taken during recall, however, because of the larger time frame and inconsistencies in test subject (writing speed, speed of recall, style of recall, etc.), this measurement was taken at the average "spike" of heart rate of an individual during the first 30 seconds of their memory recall. This spike is significant because this data allowed the research team to see if anxiety could have played a role, positively or negatively, in the memory scores of the participants and if there was a change in their vital signs during any of the three tests conducted. The data was analyzed by looking at the changes in heart rates when comparing the learning phase heart rate to the recall phase heart rate. This difference would indicate changes anxiety the participant experienced. We

also conducted one-way ANOVA tests to reveal any statistical significance between the gathered results; statistical significance was set a p-value <0.05.

Results

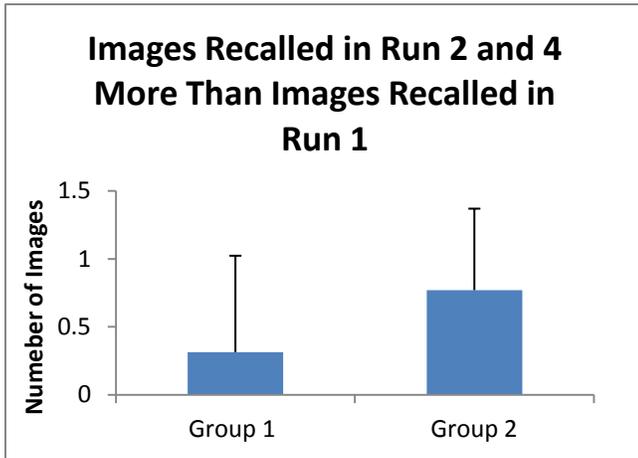
The average amount of images recalled in each run for Group 1 is presented in Table 1. The difference between the total numbers of images recalled in any run versus another was not statistically significant. Participants in Group 1 recalled on average 0.31 images more when they were exposed to the scent during recall versus when they were not exposed to the scent at such time (images recalled run 2 - images recalled in run 1) , as illustrated on Graph 1. On average, the participants of Group 1 had 0.56 less images recalled when exposed to scent solely during the learning phase versus their baseline (images recalled run 3-images recalled in run 1) , as illustrated on Graph 2. The average amount of images recalled in each run for Group 2 is presented in Table 1.

Average Images Recalled in Each Run per Group (Table 1)

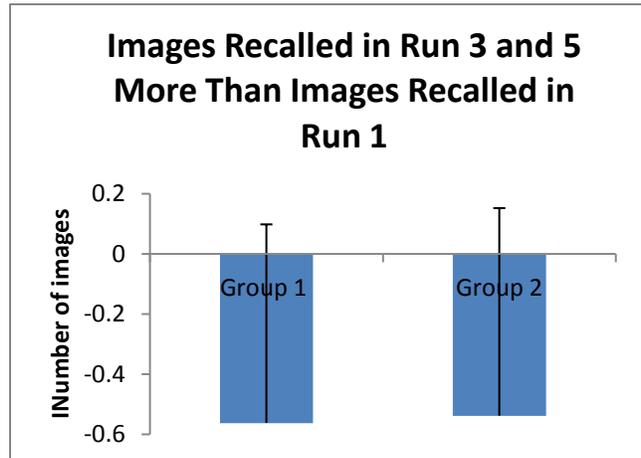
	Group 1	Group 2
1 st run	8.18	7.85
2 nd run	8.50	8.60
3 rd run	7.63	7.30

Table 1: Table summarizing the average number of images recalled in each run. These differences were not significant.

Graph 1: Graph illustrating the mean number of images recalled on runs 2 and 4 more than the number of images recalled in the baseline run (run 1). These changes were not statistically significant.



Graph 2: Graph illustrating the mean number of images recalled on runs 3 and 5 less than the number of images recalled in the baseline run (run 1). These changes were not statistically significant.



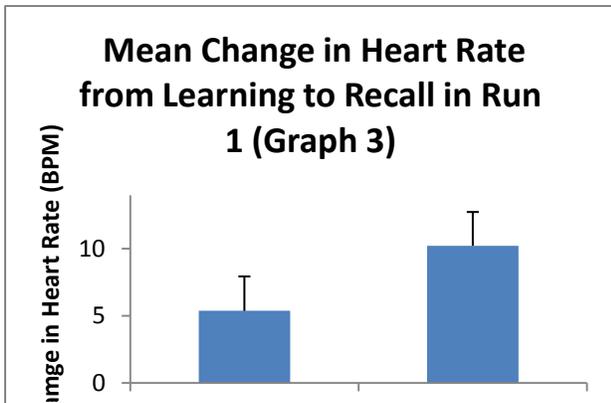
There was no statistical significance when considering differences between images recalled at the end of any run in this group. The mean number of images recalled on run 4 versus run 1 for the participants of Group 2 was of 0.77 images more. Individuals in Group 2 recalled on average 0.77 images more when exposed to the scent during recall versus when they were not exposed to the scent during recall (images recalled run 4-images recalled in run 1) , as illustrated on Graph 1. On average, Group 2 had 0.54 fewer images recalled when exposed to scent solely during the learning phase versus their baseline (images recalled run 5 - images recalled in run 1), as illustrated in Graph 2.

Both groups had significant changes in their heart rates in the recall phase when compared to the learning phase. Increases in heart rate in the recall phase in comparison to the learning phase for each run are presented in Table 2. The evidenced changes in heart rate between the recall phase and learning phase were more salient in Group 2 by 2.25 BPM in comparison to all participants. The trends of heart rate change can be seen in the appendix where Graphs 3, 4, and 5 represent the changes in heart rate during run 1, runs 2 and 4, and runs 3 and 5, respectively.

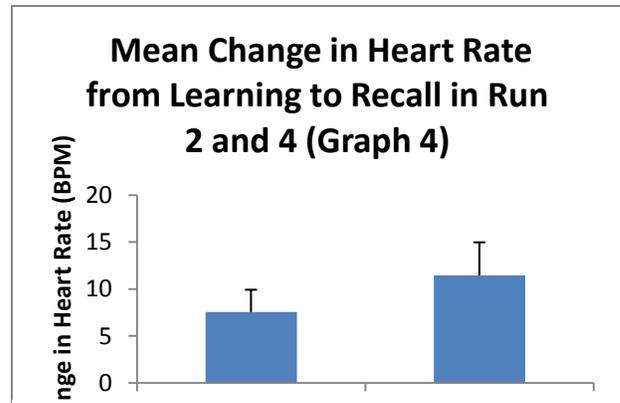
Average Increase in Heart Rate During Each Run per Group (Table 2)

	Group 1 (BPM)	Group 2 (BPM)
1st run	5.34	10.23
2nd run	7.59	11.46
3rd run	8.94	6.92

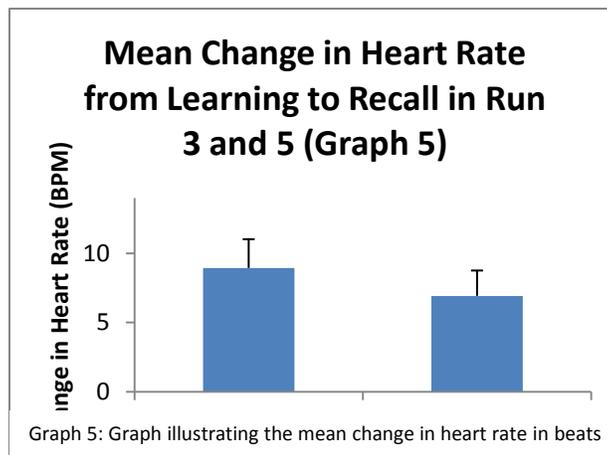
Table 2: Table summarizing the average increase in heart rate in beats per minute (BPM) between the learning and recall phases for all groups. These differences were significant.



Graph 3: Graph illustrating the mean change in heart rate in beats per minute (BPM) between the learning phase and the recall phase of run 1. These changes were significantly different.



Graph 4: Graph illustrating the mean change in heart rate in beats per minute (BPM) between the learning phase and the recall phase of runs 2 and 4. These changes were significantly different.



Graph 5: Graph illustrating the mean change in heart rate in beats per minute (BPM) between the learning phase and the recall phase of runs 3 and 5. These changes were significantly different.

Changes in alpha and beta amplitude for all participants between the learning and recall phase are available in Table 3. There was an overall increase in alpha and beta amplitude in the first two runs during the recall phase. The third run, where participants did not receive olfactory stimuli during the recall phase, there was a decrease in wave amplitude when compared to the learning phase. Similar trends were present in the changes of wave frequency with frequency increases in the recall phase of the first two runs and a decrease in the final run. The overall changes in wave frequency between the learning and recall phase are summarized in Table 4. Calculated changes in alpha and beta wave amplitude and frequency in all groups were not statistically significant.

Overall Brainwave Amplitude Change During Each Run (Table 3)

	Increase 1 st run (mV)	Increase 2 nd run (mV)	Decrease 3 rd run (mV)
α (alpha)	$3.35 \cdot 10^{-4}$	$2.59 \cdot 10^{-3}$	$8.76 \cdot 10^{-4}$
β (beta)	$8.23 \cdot 10^{-4}$	$1.77 \cdot 10^{-3}$	$2.07 \cdot 10^{-4}$
Overall Δ		α (alpha) = $6.83 \cdot 10^{-4}$ mV ↑	β (beta) = $7.95 \cdot 10^{-4}$ mV ↑

Table 3: Table summarizing the mean change in amplitude (mV) of alpha and beta waves between the learning and recall phases for all groups. These differences were not significant. Overall change in alpha and beta amplitudes are also given.

Overall Brainwave Frequency Change During Each Run (Table 4)

	Increase 1 st run (mHz)	Increase 2 nd run (mHz)	Decrease 3 rd run (mHz)
α (alpha)	0.15	0.27	0.40
β (beta)	0.15	0.31	0.42
Overall Δ		α (alpha) = 0.005 mHz ↑	β (beta) = 0.02 mHz ↑

Table 4: Table summarizing the mean change in frequency (mHz) of alpha and beta waves between the learning and recall phases for all groups. These differences were not significant. Overall change in alpha and beta frequency are also given.

Discussion

Our initial hypothesis did not have any statistical significance. The only significance that was seen is between recall and heart rate. The rest of the statistical analyses showed trends toward our hypothesis but did not significantly support it. This is shown by the average improvement of participant's ability to remember images when exposed to the same scent while learning and recalling and could mean that the olfactory sense helped trigger the image recollection. Performance decreased when participants were exposed to the scent during learning but not during recall. Two possible explanations for our insignificant results are that either the smell was distracting to the participant or memory recall was more difficult in the absence of the original scent. This conclusion is supported by the fact that the EEG showed decreases in amplitude and frequency of brain waves. In addition, heart rate increased during recollection, and it is possible that the participants were anxious about the recollection process, or that the act of recalling caused higher levels of stressed and increased heart rate. Higher levels of stress, due to a time limit, may make it more difficult to recall images. Another trend seen in the results was that Group 2 had higher heart rates than Group 1. The only difference between the groups was the presence of the garlic scent for Group 2 opposed to the aroma therapy soap in Group 1. This could indicate that the smell of garlic was unpleasant or more aggravating when present for longer periods of time. The EEG also showed an overall increase in the amplitude and frequency of the alpha and beta waves that were recorded, which

may be due to the increased effort during recall. In other words, when actively recalling a memory the brain will produce waves with higher amplitude and frequency. However, in experiment runs with scent during learning but no scent during recall, there was a decrease in amplitude and frequency most likely caused by the absence of the olfaction sensory input. The EEG also showed that beta waves changed more than alpha waves, which is congruent with the idea that participants were actively engaged in the experiment.

Although we found significant results in the heart rate data between learning and recall runs, the rest of our data trended towards our initial hypothesis but did not have any statistical significance. The trends suggest that with a larger, more diverse sample, our results could significantly support our hypothesis that one can recall more in the presence of a scent (pleasant or unpleasant). In addition, not all participants used the complete two-minute recollection time, which could account for missing images they might have recalled had they waited the full time. This difference in wait time could also affect the EEG data in that some participants have more EEG data than others had they used a longer time to recall the images. Furthermore, the two-minute wait period between learning and recall posed a lot of variance between participants. In other words, each participant's waiting period was different: some had more distractions from the proctors or other students, while some had a quiet setting with no other external disturbances. This difference in distractions could skew the number of images recalled and in the future more care should be taken in controlling these variables. Additionally, it was noticed that participants could potentially improve their performance from the first run to the last run by discovering a strategy once finding out how the test was administered. In other words, participants can perform better if a better strategy to memorize the pictures was acquired between runs. This could be assessed with a post-study questionnaire in future experiments.

In future studies, it may be worthwhile to run a longer study. Running a longer study would allow participants to be assessed over two or more days and data could be collected for both scents from each participant to provide a within group comparison. In addition, it would be beneficial to increase the time span between runs to longer than two minutes to allow for participants to recover from smelling the scent so the runs do not bias each other. Finally, a longer set time between runs would eliminate the problem of images from previous runs being recollected in subsequent runs, as many participants found this to be an issue. The short time frame does not allow for the brain to reset and concentrate on the newly added images of the current run.

It would be ideal to use a more accurate EEG analysis by increasing the number of electrodes around the head to gain access to memory association areas of the brain, such as the hippocampus. Since the hippocampus is in the medial part of the brain, using only three electrodes temporally, as in our study, may not access these working regions, therefore, skewing the data and underestimating the changes in amplitude between the alpha and beta waves. Similarly, it would be beneficial to find consistent equipment that could be utilized. However, trends were found between EEGs and images recalled as was hypothesized and significance was found with heart rate between groups. This evidence allows us to conclude that even with minor errors, this study can provide a valid base for olfactory senses and memory recollection. Our results have shown that with further research, a connection between the olfactory sense and memory may or may not be possible. Furthermore, since there was a significant difference in heart rate between groups and between learning and recall, further studies could focus on heart rate and memory recall alone. The results of this study can be used to narrow the focus of olfactory memory and provide new ground for future studies.

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