

# **THE EFFECT OF APPETITIVE AND AVERSIVE OLFACTORY STIMULI ON ACOUSTIC STARTLE RESPONSE IN RATS**

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## **ABSTRACT**

The amplitude of the startle response of conditioned rats was shown to be unaffected when in the presence of an olfactory stimulus that was associated with an appetitive reward or a neutral stimulus. During conditioning, rats were trained to distinguish between the odors amyl acetate and cineole by pairing amyl acetate with a reward of sucrose solution and cineole with a lengthened blackout period. It was hypothesized that the amyl acetate would be associated with a pleasurable emotional state and therefore would attenuate the startle response. When the amplitude of the startle response was measured, the results did not show any significant differences in the presence of amyl acetate, cineole, or the control of no odor. Therefore, the experiment did not show any conclusive data for or against the attenuation of startle when an olfactory cue is linked with an assumed pleasurable state of mind.

## **INTRODUCTION**

A commonly studied reaction to unanticipated stimuli is the startle response. A startle response is defined as the reaction exhibited by an animal that has experienced a sudden change in environment, such as a loud noise or a flash of light. All mammals show some degree of startle response. For instance, humans tend to blink while their heart rates increase and adrenaline courses through their bodies. Similarly, rats will visibly startle through jumping or freezing [4]. It is believed that there is a connection among stress exhibited during startle, drug addiction, and withdrawal. Experiments that study the behavioral actions associated with startle may help scientists to understand the processes and parts of the brain involved in addiction [10]. To measure the amplitude of a rat's startle response, scientists can use many methods. One of these methods is measuring the amplitude of the force exerted by the rat's jump when startled.

It has also been confirmed that the startle response of a rat can be modulated by emotion. Therefore, scientists often relate the emotional state of a rat with its emotional context in order to examine a wide range of sensations [4]. In order to accurately test a rat's acoustic startle response in relation to an appetitive or aversive state, the rat's environment must first be associated with the specific state. It has been proven that training is more effective when the olfactory sense is used as cues rather than the visual or auditory sense [5]. The olfactory sense is used by rodents, such as rats, to recognize nourishment as well as danger in their environment, making it arguably the most important sense for their survival [6]. Therefore, researchers often use olfactory cues rather than visual or auditory stimuli to help the rats distinguish between different scents in fewer trials [3]. Consequently, olfactory learning as the focus of cognitive

scientific research can allow us to better understand processes involved with learning and memory.

Researchers rely on the established idea that when rats are subjected to a stimulus known to instill fear, their emotional states change. Fear is the most common stimulus used for these kinds of experiments because it is easy to evoke and measure. As a rat experiences startle response triggered specifically by odors, lights, or noises, there are a multitude of spontaneous psychological and physiological reactions. Due to unexpected disturbances in the environment, rats can display responses that reflect their emotional states. They may, for example, demonstrate immobility, increase the level of stress hormones secreted, stretch their attention, and avoid the stimulus [9]. It has been repeatedly shown that rats' startle responses are potentiated when a stimulus, which places the rats in a fearful emotional state, is applied to the rats' environment before a startle-inducing stimulus [6]. For comparison, it is similar to how humans seem to flinch more severely when they are in a heightened emotional state due to fear. This reaction to the stimulus, whether it is a shock, odor, or a noise, is well documented and logical.

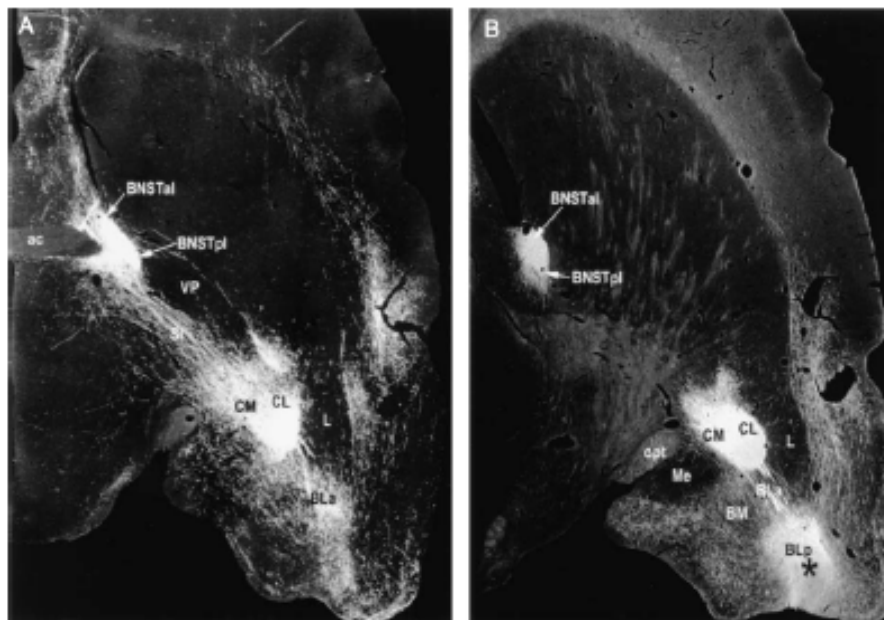
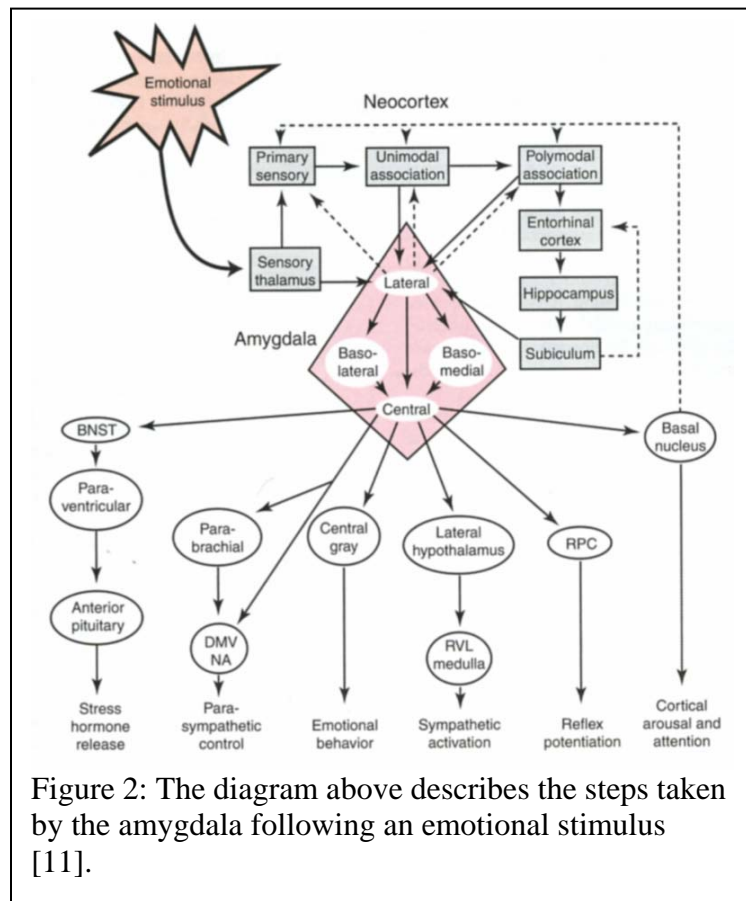


Figure 1: Photomicrographs of a rat's brain. This figure displays certain regions responsible for certain odor-induced fear responses such as freezing. Some of the regions labeled include the basolateral amygdala (Bla), the medial amygdala (Me), the medial and lateral regions of the central nucleus of the amygdala (CM and CL respectively), and the anterior and posterior areas of the bed nucleus of the stria terminalis (BNSTal and BNSTpl respectively) [10].

Odor initiated fear has activated certain regions of the brain that were once unacknowledged for their role in olfactory learning associated with the emotion of fear. Figure 1 shows an image of a rat's brain and exhibits some of the key regions. There are certain amygdalar nuclei that are stimulated from odor-induced fear. The basolateral amygdala (Bla) is thought to be the area in which stimuli relationships are created and is activated when fear-initiated responses come in the form of immobility and avoidance. In a study conducted by

Cousens and Otto, rats with lesions in the Bla exhibited a disturbance in freezing behavior; thus the Bla may be required for the accurate correlation of conditioned and unconditioned stimuli, which then allow rodents to display the fear [2]. In one study, when subjects were exposed to cat fur, there was a considerable decrease in freezing and avoidance in Bla lesioned rats [9]. In a previous study, when the medial amygdala (Me) was removed in rats, there was a significant decrease in the length of freezing time and an escalation in the number of instances of contact with a cloth that contained cat odor [9]. In addition to the medial amygdala, the central amygdala is also an essential area that allows for fear behavior, especially when the experiments involved electric shock [9]. In a study conducted by Hitchcock and Davis, the removal of both the central nucleus of the amygdala and the trans-section of the fiber bundle attaching the central nucleus to the brainstem (that arbitrates the startle response) completely impeded fear-potentiated startle [10].

The bed nucleus of the stria terminalis (BNST) is the region that collects projections released from the central amygdala. Rats, whose BNST's were inactivated, showcased reduced levels of stillness when exposed to a certain scent [9]. All of these regions of the brain seemed vital in allowing rats to use their unique sense of smell to respond to certain emotions associated with odors. Figure 2 presents the role each part of the brain plays and the steps taken to interpret an emotional stimulus.



A possible corollary to the idea of fear-potentiated startle is the possibility of pleasure-attenuated startle, although it has not been researched thoroughly. Schmid, Koch, and Schnitzler have shown that this attenuation of startle is certainly probable [7]. Their data indicates that when a light is associated with a positive stimulus (sucrose solution), experimental rats' startle response is attenuated when

exposed to a loud noise—especially when compared to naïve rats. Olfactory cues have been paired with fear to potentiate startle, but research has not been nearly as complete in pairing olfactory cues with pleasure to attenuate startle. This is interesting because of how crucial the olfactory sense is to the life of the rat and how commonly it is used in other studies. Our aim is to help further the information available in the particular milieu of rat and human behavior.

Our experiment is most comparable to Schneider and Spanagel's recent experiment, in which an odor paired with a pleasurable emotional context effectively attenuated the amplitude of a rat's startle response. The pleasurable state was created through appetitive reward conditioning in the presence of an orange odor. The rat was only exposed to one odor, and the odor was only paired with one consequence, a sweet condensed milk reward. This conditioning emotionally charged the odor with a pleasurable state of mind, and such a mentality caused the startle response to be attenuated [8]. The ultimate goal of our experiment was to find if a relationship existed between the emotional state of a rat and the rat's response to startle, when trained under conditions different from those of Schneider and Spanagel.

With previous tests providing solid evidence that a response to olfactory learning is attainable in rats, we believe that an experiment to explore a pleasurable state in rats is feasible. This knowledge of past research led us to predict that once conditioned to identify scents with a certain emotional state, rats given a startle response test will show attenuation in the amplitude of its startle response when exposed to the scent connected with a reward. Conversely, rats will either display a potentiation in startle response or a baseline response when exposed to the scent connected with a punishment.

## **METHODS**

### Subjects

Subjects in this experiment included twelve male Sprague-Dawley rats, each approximately 6 months old and initially weighing between 387 g and 481 g. After the onset of the experiment, the rats were first restricted to 85-90% of their normal free-feeding weight, although water was freely available. Six rats were experimentally naïve, while six had previously experienced startle testing. All were bred at Drew University and singly housed in suspended wire-mesh cages. All were kept on a 12:12 hour light and dark cycle, with lights on at 0700 hrs. All except two rats were trained solely during the light cycle.

### Procedure

*Nose-poke shaping.* During this step of the experiment, the rats were conditioned to poke their noses into a port. All shaping occurred in six identical sound-attenuating standard conditioning boxes (32.25 cm × 25.5 cm × 25.0 cm) made from Plexiglas, stainless steel, and plywood. Inside each of the conditioning boxes, an exhaust fan contributed to background noise while a standard Med Associates Inc. (St. Albans, VT) house light bulb provided light. The inner boxes consisted of floors made from stainless steel rods. During shaping, subjects were trained to nose-poke by incentive of sucrose water (20%). When appropriate, the sucrose water was transported to stainless steel plates (located on one wall of each box) via transparent pliable plastic tubing. In each conditioning box, the tubing was connected to a computer-controlled syringe (each containing 25 mL of sucrose solution), which released 0.0625 mL of sucrose solution upon detection of nose-poke responses. At the nose-poke port, responses were detected by photoelectric beams and recorded by the Med-PC (Med Associates Inc.) computer program.

One day prior to the nose-poke shaping, subjects were handled for 15 minutes and weighed. On day one rats were trained in 30 minute intervals, then placed back into their cages. They continued to be maintained at 85-90% of their normal free-feeding weight. On day two, the rats were trained for 30 minutes using the same procedure and apparatus. Overnight training for two of the rats occurred from day four to day five. Subjects were discontinued from shaping upon achievement of at least 75 successful nose-pokes in a 30 minute period.

*Odor preference test.* This phase of the experiment served to find out which odor the rats initially preferred. A radial arm maze (Lafayette Instrument Co., Lafayette, IN) was set up with two open arms. Bedding placed at the end of one arm was infused with the amyl acetate odor. At the end of another arm, bedding was infused with the cineole odor. One rat at a time was placed at the center platform of the radial arm maze. The rats were allowed to freely move into and between the arms. If the rat made an entrance into an arm with all four of its paws inside, it was counted as having crossed over to that side. The amount of time spent in each arm and in the center platform was recorded. The procedure lasted for 10 minutes per rat.

*Odor discrimination training.* The odor discrimination phase of the experiment trained the rats to distinguish between amyl acetate (associated with a positive stimulus) and cineole (associated with a negative stimulus). The go/no-go model of discrimination learning used in previous studies was implemented in the discrimination phase [2]. In this model, the subjects were trained to perform a certain task under one set of stimuli (go) and not perform the task under another set of stimuli (no-go). Training occurred in six 32.25 cm x 25.5 cm x 25 cm sound-attenuating Plexiglas chambers. Each chamber was equipped with an exhaust fan which provided white noise in the background.

Subjects were trained to insert their nose into the odor port, which stimulated the release of either amyl acetate or cineole. When the amyl acetate odor was released, the subjects were trained to make a response at the water cup. When subjects moved to retrieve water from the cup, the photoelectric beam was broken, resulting in the release of 0.0625 mL of 20% sucrose solution during a 5-second period. Subsequently, lights turned off for 5 seconds. This cycle of sucrose solution and 5-second lights-out was meant to be a reward for the rats.

When the cineole odor was released, the subjects were trained not to go for the sucrose solution in the water cup. If the subjects attempted to retrieve sucrose solution from the cup, the lights were turned off for a 10-second interval during which no successive nose-pokes could be attempted (punishment).

Odorized air moved in from a flow-dilution olfactometer (custom-built) connected to two 20-mL bottles containing either amyl acetate or cineole. The odorized air was removed from the chambers by an exhaust fan. Nose-pokes directed at the odor port and successive responses at the water port were monitored using photoelectric beams. A computer program, Med-PC, controlled the release of the odor and the sucrose solution and recorded the results of the experiments. The odors were emitted in a randomly alternating order during the sessions, which lasted for 30 minutes each. Water-cup responses made during amyl acetate trials were recorded by the computer as hits. Failure to respond during amyl acetate trials were recorded as misses. False positives were recorded when the subjects made a response during cineole trials. Lastly, when

the subjects declined to retrieve water during cineole trials, the computer recorded correct negatives.

*Startle response test.* Each rat was placed in a cylindrical Plexiglas Med Associates startle response chamber (25.5 cm in height, 8.7 cm in diameter) housed within a custom-built cage. The chamber contained a high capacity pole-fan blower, which provided background noise (70 dB) and either diffused or removed odors when appropriate. The odors used were amyl acetate and cineole, one of which would enter the chamber through an opening on the ceiling by means of a flow-dilution olfactometer. For five minutes, each rat acclimated to its surroundings without the introduction of any sound pulses or odors. After the acclimation period, olfactory conditions were introduced to the chamber in pre-programmed sequences. Condition A involved the release of an amyl acetate odor, condition B the release of a cineole odor, and condition C the lack of an odor. Conditions A and B acted as variables while condition C acted as the control. The first sequence was ABC-ABC-ABC while the second sequence was BAC-BAC-BAC. 15 seconds after the release of an odor, an auditory stimulus (sound pulse) was released. The release of odors and auditory stimuli continued and alternated in 15-second intervals for the duration of 10 pulses to create the following sequence: odor, 15 seconds, pulse, 15 seconds, odor, etc. This alternating sequence lasted for 5 minutes, ending with a pulse. The release of the odor co-terminated with the presentation of the auditory stimulus. Between each condition (A, B, and C), the rats underwent an additional 30-second rest period during which there were no pulses or odors. Each condition represented a 5.5-minute period consisting of the 5-minute odor-pulse sequence and the 30-second rest period. The entire startle test lasted for 70 minutes.

The startle-response of the rats was measured by an accelerometer and an amplifier (PCB Piezotronics, Depew, NY), which amplified the voltage signal 100 times. A microcomputer and its Recorder program (Plexon, Ft. Worth, TX) collected the results, while OfflineSorter (Plexon) and NeuroExplorer (NEX Technologies, Natick, MA) performed the analysis. The sound pulses and odors were coordinated by Med-PC so that sound pulses were 95 dB, each lasting 20 milliseconds. There was also a four millisecond rise-fall time. A Med Associates decibel meter was used to examine the sound pressure levels prior to experimentation.

## RESULTS

### Nose Poke Training

Subjects were required to perform 75 nose-pokes in at least one of two thirty-minute sessions, or, failing that, perform 100 nose-pokes in a single, 12-hour overnight session in order to proceed to the next phase of the experiment. Four subjects reached 75 nose-pokes in at least one of the first two sessions. The remaining two subjects, A17 and A18, reached 100 nose-pokes in extra overnight sessions. All six subjects proceeded to the next phase of the experiment.

### Odor Preference Tests

Figure 3a reports the ratio of the total time spent in the amyl acetate arm of the maze by a subject to the total time it spent in the cineole arm. The blue bars represent the value of this ratio from the pre-discrimination training odor preference test, while the green bars represent the post-

discrimination training ratio. All subjects A13 through 18 participated in the pre-training odor preference test, while only A14, 15, and 18 participated in the post-training test. We hypothesized that the pre-training ratios would be close to the value of 1 and the post-training ratios would be higher, since the amyl acetate odor was positively conditioned and the cineole odor was negatively conditioned.

The results were scattered. The pre-training ratios had a large range for a sample size of six subjects. Some of the subjects showed a preference for cineole and spent little or no time in the cineole-scented arm of the maze. A15 and A18 were the only subjects that explored the maze without a distinguishable odor preference.

Figure 3b reports the ratio of the number of entrances into the amyl acetate arm made by a subject to the number of entrances it made into the cineole arm. This alternative measure of odor preference confirmed that there was not any statistically significant trend to indicate an odor bias or the lack of an odor bias in the subjects tested. The results, in the context of either form of odor preference assessment, were too sporadic to conclude that the odor discrimination training had any influence on the subjects' overall odor preference.

Figure 3a

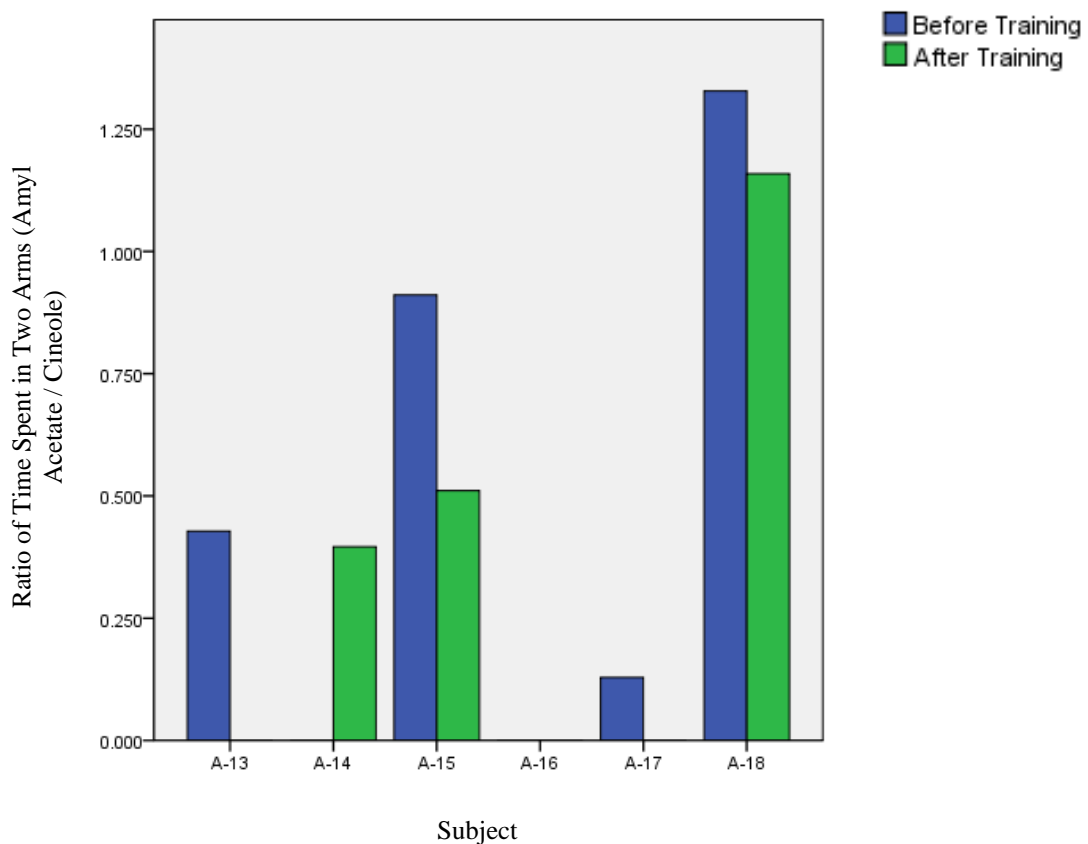


Figure 3b

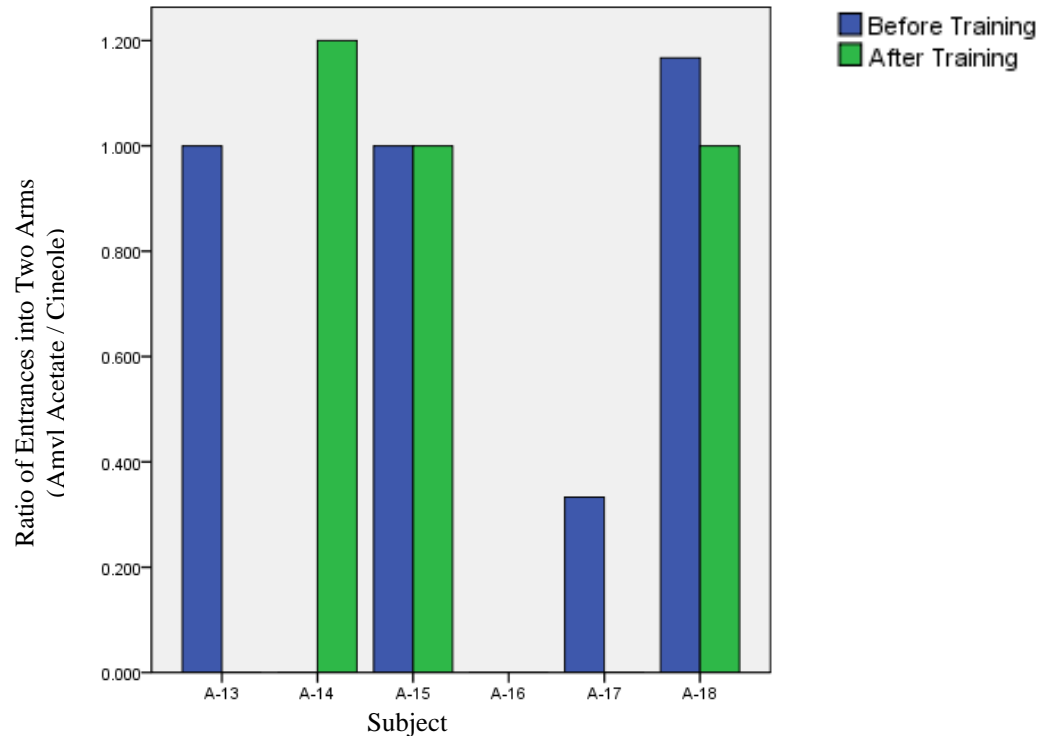


Figure 4a reports the total amount of time, in seconds, spent in the arms of the radial arm maze by each subject. Figure 4b reports the total number of threshold crossings made by each subject into the arms of the maze. A13 and A17 spent only a small portion of the 600-second test in either of the arms, and A13 and A14 made relatively few entries into the arms, making the data collected from their trials less reliable.

Figure 4a

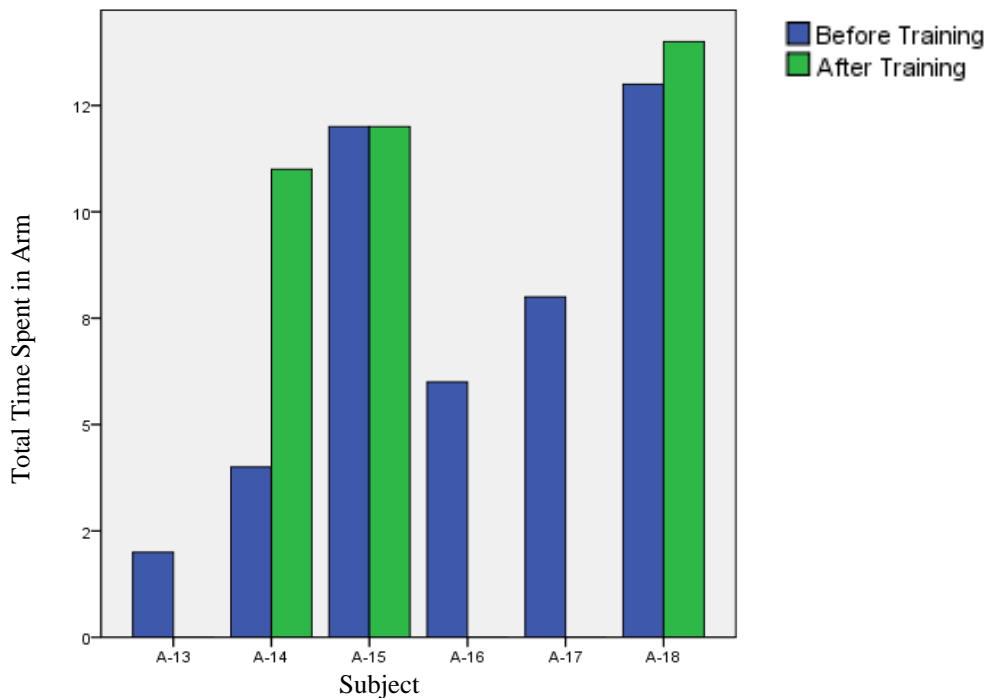
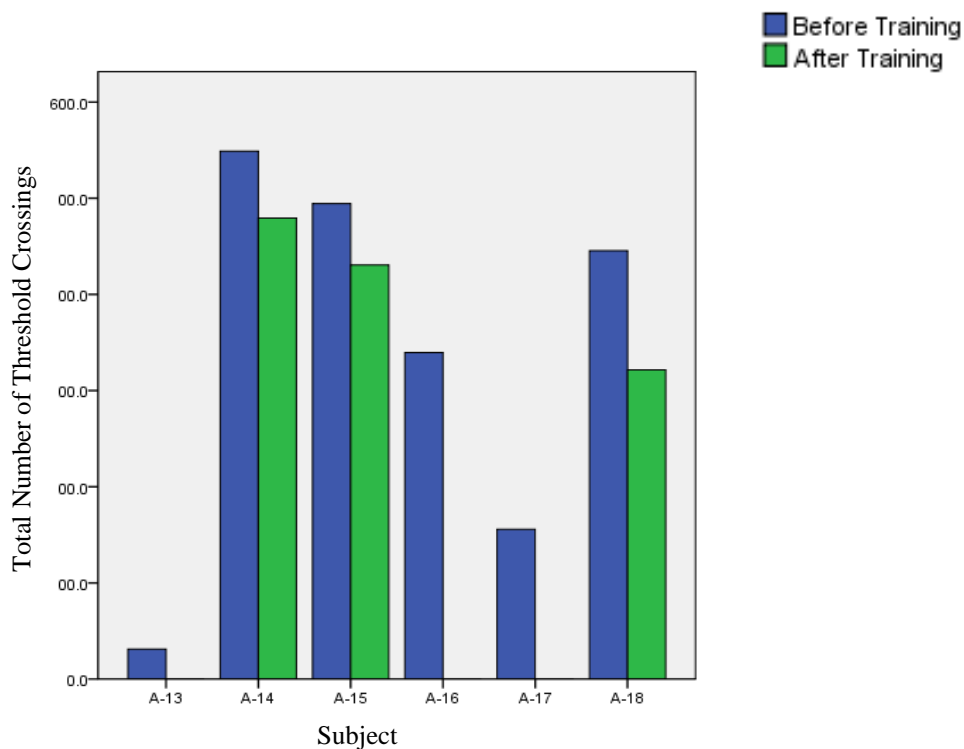




Figure 4b



### Odor Discrimination Training

Figure 5 below exhibits the learned response of discriminating between odors, amyl acetate and cineole, by the subjects. Twelve subjects proceeded to the odor discrimination phase of the experiment, A13 through 18 and A25 through 30. The subjects were divided into two groups of six, both trained in the same manner. They were given criteria of at least 75% correct responses in a trial with a minimum of 100 total nose-poke responses in order to progress to the startle portion of the experiment. Three of the subjects of the first group, A14, A15, and A18, met the criteria and clearly exhibited a learned response. The other three subjects, A13, A16, and A17, were excluded from the next phase of experiment and are therefore not included in this data. Three additional subjects that were trained using the same odor discrimination method (A26, A27 and A28) are included in the data. Also, subject A25 did not meet the criteria to proceed to the next phase of the project. A29 and A30 met the criteria provided but were used in a different experiment. All six subjects that advanced exhibited a learned response over time by poking in the nose portal at least 100 times and meeting the minimum of 75% correct responses.

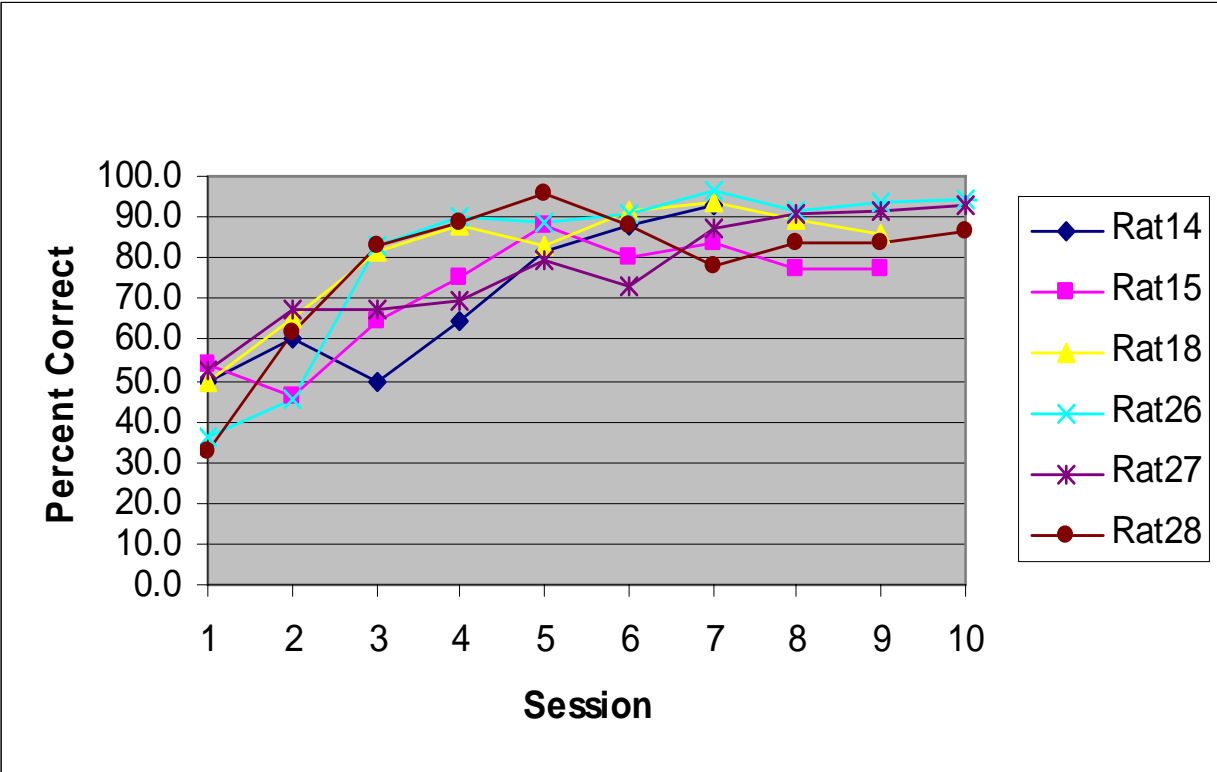


Figure 5: Odor Discrimination Training

Startle Response

Figure 6 displays the average outputs of the accelerometer during subject A14's startle response when it was exposed to amyl acetate, cineole, and no odor. All six subjects' acceleration graphs showed a peak at about 13 milliseconds and a trough at about 20 milliseconds. The peak represents the maximum force with which the subject withdraws its arms in preparation for a jump. The trough represents the maximum force with which the subject pushes down on the platform. The three lines of data coincide at the same crucial points, indicating no difference in reflex time. The pulse released during the period of no odor provoked the largest average second peak in this particular subject.

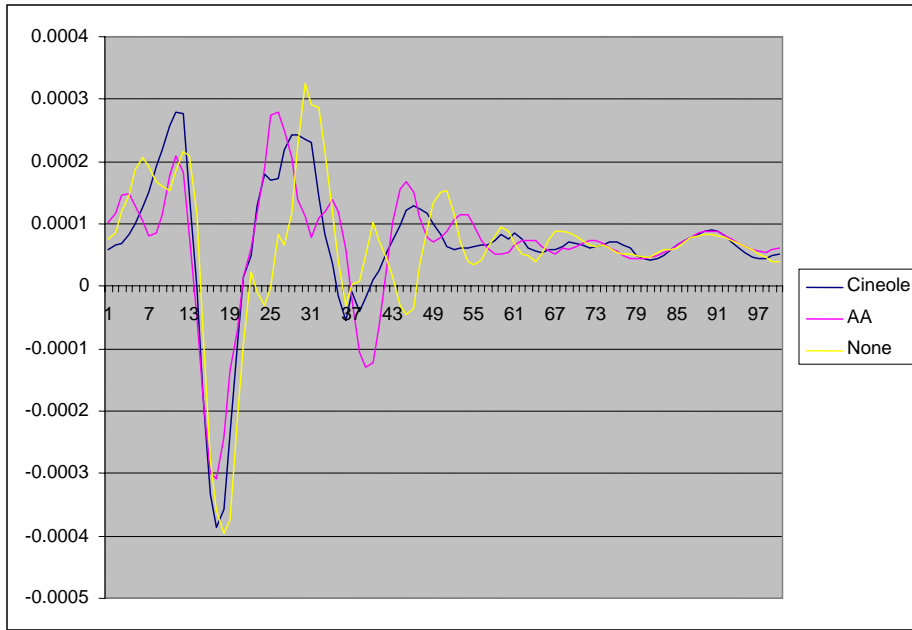


Figure 6: Startle response results for subject A14, in terms of voltage (directly proportional to force exerted by subject) reported by an accelerometer, in the presence of cineole, amyl acetate (AA), and no odor

Figure 7 displays the average difference between the initial crest and trough for subject A14. The data collected under conditions of cineole odor showed the greatest change in voltage.

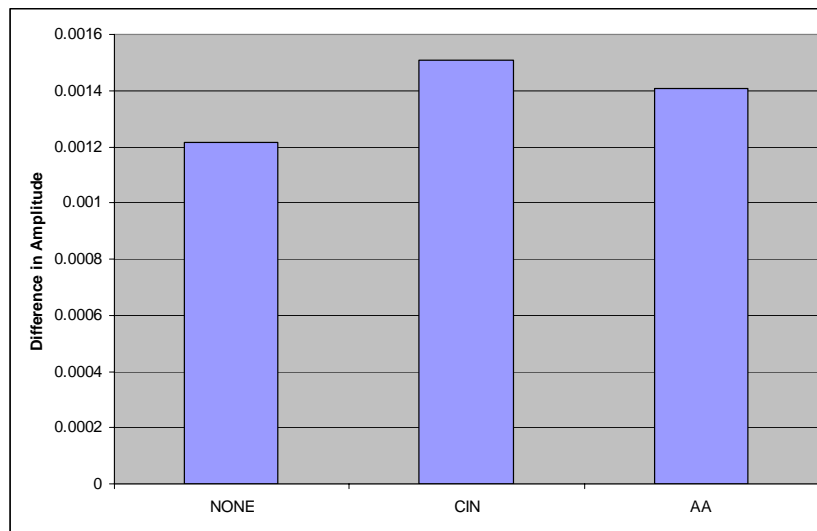


Figure 7: Startle Response results for subject A14 in terms of voltage in the presence of cineole (CIN), amyl acetate (AA), and no odor

Figure 8 displays the average of the changes in amplitude of the force exerted by the subjects' startles for pulses delivered amidst cineole odor and the amyl acetate odor, as a percent of the baseline startle. The baseline was the average startle height for the last ten no-odor pulses during the 4 minute acclimation period. The graph reveals no distinct pattern in the difference between the data collected under the conditions of different odors. The average of the percent of

baseline startle illustrates that startle amplitude under amyl acetate was slightly higher than the amplitude while in the presence of cineole. There is no conclusive evidence that the startle response can be attenuated by olfactory memory. This was supported by a paired samples t-test ( $t(5) = -0.154, p > 0.05$ ).

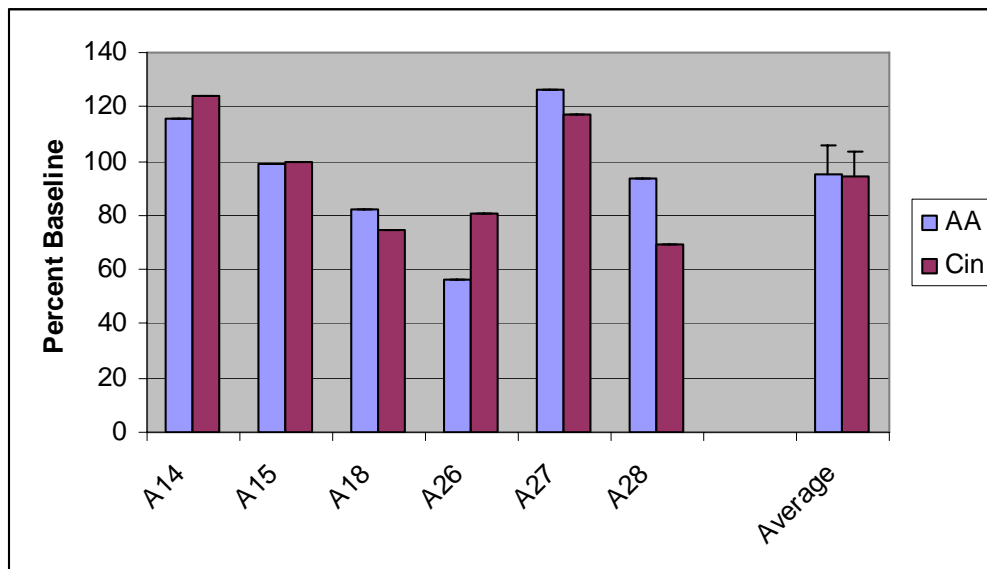


Figure 8: Startle response results for qualified subjects in terms of percent baseline in the presence of amyl acetate (AA) and cineole (Cin)

## DISCUSSION

### Odor Discrimination

In order to effectively perform a “go/no-go” test, a method of training rats to learn when to perform an action and when to restrain from acting, the rats were required to first participate in training sessions to learn how to “nose poke” for a reward [2]. Each rat was individually trained to discriminate between amyl acetate and cineole by pairing amyl acetate (the odor associated with an appetitive stimulus) with a sugar water reward before a five second interval, and pairing the cineole (the odor associated with an aversive stimulus) with a punishment of a longer period of darkness. Eventually, three of the six subjects in each of the two groups exhibited a clear ability to discriminate between the two scents—discriminating and responding correctly approximately 85-90% of the time after a few training sessions. With such data, it seemed reasonable to assume that these select rats had indeed associated each scent with a specific mentality.

### Startle Response

Since the subjects seemed to possess the ability to differentiate between odors and to correlate each scent with an emotional state of mind, they were then tested to see if a specific odor, and therefore a specific mindset, would affect the degree to which the rat startled. The subjects’ startle amplitude resulting from a sudden noise was recorded in the presence and absence of an amyl acetate odor as well as a cineole odor within the startle chamber. If the

original hypothesis made was correct, attenuation of startle when the rat was in the presence of the amyl acetate odor would have been witnessed in comparison to the startle amplitude produced in the presence of no odor and the cineole odor. It was also conjectured that the startle of the rats in the presence of the cineole odor might be increased compared to rats in the presence of no odor, but overall, similar amplitudes of each odor were expected since neither odor was charged with an appetitive or aversive emotion. However, only one subject's data for each odor supported our hypothesis, with the startle amplitude, from highest to lowest, being in the presence cineole, then no odor, and then amyl acetate. Other subjects displayed the highest amplitude of startle in the presence of amyl acetate and still others in the presence of no odor. This data exemplifies that even though one rat followed our hypothesis, the data was not significant enough to draw any single conclusion, and the results may have been merely that of chance. When all of the data is compared, it seems that no conclusive evidence can be extrapolated because the results are extremely inconsistent. As a whole, there appears to be no evidence that supports a relationship between the mentality of the rat, due to environmental factors such as odors previously associated with an appetitive or aversive mindset, and an decrease or increase, respectively, in the rat's startle amplitude.

Even though this experiment's data does not exhibit a direct relationship between an appetitive and pleasurable state of mind and the attenuation of acoustic startle, recent research done by Schneider and Spanagel clearly proves the existence of the correlation. Therefore, the only reasoning available to explain why these results differed from the prior experiment is because of the variances in the conditioning procedures. The present experiment's rats spent more time in training and underwent more sessions than the rats in Schneider and Spanagel's research [8]. It is likely that our experiment did not emotionally charge the odors, but rather elicited a cognitive or instructional mentality. Also, this experiment involved the use of two odors; one odor was conditioned to be associated with an appetitive state and the other was associated with aversive feelings resulting from the longer time-out period. It is possible that the rats were unable to "switch" back and forth between the two odors in such short time periods [8].

Although the training procedure is the most significant factor attributing to the unexpected results, other aspects of the experiments could have contributed to the inconclusive data. The rats could have also been habituated to the loud noises, which would result in a decrease in startle amplitude. Habituation occurs when the animal becomes accustomed to the external stimulus and is no longer frightened to the same degree as it had been initially. However, habituation is unlikely, considering that there was a thirty-second interval between sounds; a gap of this length generally limits the effects of habituation [2]. Nonetheless, it is reasonable to believe that habituation could have been a factor since the rats received many impulses within a brief time period. No evidence can be drawn that would justify the neglect of this possibility.

The time constraints under which the experiment was conducted greatly influenced the final outcome. In the case of the odor discrimination test, the time restraint resulted in less time available to devote to training the rats to identify odors with states of mind. In some cases, more than one training session was required to ensure that the rats were able to learn to nose poke only when amyl acetate was released into the chamber. The lack of time could explain why three of the rats were unable to learn how to accurately discriminate between odors. Three of the rats did

learn to nose poke and these three rats were included in the group that was tested for startle attenuation in the presence of the odors. These rats, even though they demonstrated a high level of accuracy in nose poking, might not have associated the amyl acetate with a positive state of mind. This shows that due to the limited time available, the rats may not have learned to associate the odor with a state of mind, but just associated the smells in the nose poke chambers with the rewards.

Using sugar water as a reward created an appetitive motivation rather than motivation to be “happy.” This could have potentially created variable results since the rats’ actions were dependant on multiple factors such as hunger and energy level. The original intention of this experiment was to examine the relationship between a positive emotional state of a rat and its startle reaction, but if the positive pleasurable mental state was never truly created, this correlation obviously cannot be recorded. One must recognize that an appetitive state in which the animal is craving an edible reward is not necessarily synonymous with a pleasurable state in which the animal is generally content and happy. Therefore, it is difficult to determine if the results were due to the absence of relation between a positive mentality and decrease in startle amplitude, or if the results were simply due to the lack of a positive state altogether.

Inherent preferences for specific odors may have contributed to the rats’ performance, which was contrary to the experiment’s hypothesis. The odor preference test provided a control to compare the initial behavior to the final behavior after training, in order to determine whether or not the rat truly learned the association of the smells with the mentality, or if it simply preferred the odor naturally. In most cases, the rats preferred the cineole to the amyl acetate. However, the neutral odor in the chambers was always cineole and the positive or appetitive odor was always amyl acetate. Since the rats were intrinsically inclined to choose the neutrally associated odor before training, it seems plausible to assume that the rats retained that bias throughout the testing, which could have possibly affected not only their learning of association, but also their mentality during the startle test. If this is the case, the rats might not have been in a truly pleasant state of mind, which would severely alter the data.

Overall, this experiment has released no results that can directly connect the emotional state of a rat to the attenuation of its startle response. Different regions of the brain control different expressions, and although emotions are generally grouped together, we are not able to conclude that an exact area is responsible for all induced feelings. Fear is easily measured and witnessed, but pleasurable or appetitive states are much more difficult to declare.

Most research regarding brain regions involved in olfactory learning in rats generally focuses on odor-induced fear. In this experiment, appetitive odor-cue conditioning was used to test for a relationship between a pleasurable mentality and amplitude of startle response. For future studies, experiments can be conducted that will better allow the scientific community to understand regions activated when rats are feeling emotions other than fear, such as pleasure. Hopefully, with extensive research, similar experiments will enable scientists to more fully understand how the human brain works in regard to drug addictions and withdrawal. Drug addictions are maintained due to the pleasurable state of mind resulting from chemicals released in the brain, and when this pleasure is removed, a person experiences great pain and suffering.

Both stages, of pleasure and suffering, may prove to be relevant to experiments such as the present one, but as of now, no direct correlation can be made.

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