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The Evolutionary Ability to Detect Toxins in an Odor Mixture

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Abstract

It would be adaptive for humans to identify a toxic odor in an odor mixture regardless of the number of components present. Twenty-seven undergraduate students were presented with 15 odor mixtures and asked to identify the content of each mixture. Each odor mixture contained 1 toxic odor and 1 to 5 non-toxic odors. Participants' detection of toxic odors in a mixture was not dependent upon the number of components present in a mixture. However, the higher the level of toxicity of a substance, the more easily it was identified in an odor mixture. A benefit of olfaction appears to be the ability to detect toxic odors in any given odor mixture. The results are discussed in relation to suppression, familiarity, intensity and activation of brain regions and future research is suggested.

The Evolutionary Ability to Detect Toxins in an Odor Mixture

The sense of smell is suggested to be the oldest sense because it is present in every animal, even those that lack other senses such as sight (Geldard, 1953), and olfaction was selected for very early on in animal evolution. Indeed, it may be the case that a small lump of olfactory tissue grew into a larger brain and that the cerebral hemispheres of the brain were originally buds from the olfactory stalks (Gaulin & McBurney, 2004; Geldard, 1953). There remains a lack of knowledge regarding the sense of smell because the human olfactory system, like that of any other mammal, is hidden in the backmost areas of the nose and is a complex network of components.

Humans retain the capacity to discriminate between approximately 10,000 different odorants (Reed, 1994). The process of odor identification begins when odorants bind onto olfactory receptors, known as the "lock and key" theory (Amoore, 1970). Odorants each possess different molecular features and these features bind to specific olfactory receptors which then creates a unique pattern or combination (Kajiya et al., 2001; Leon & Johnson, 2003). Olfactory receptors are only capable of encoding a single odorant at a time but may recognize more than one type of odorant. Odorants may create overlapping but otherwise distinct patterns (Malnic, Hirono, Sato & Buck, 1999) but there may also be some degree of clustering of active olfactory receptors to an odorant (Ma & Shepherd, 2000). Nevertheless, Dryer (2000) states that although olfactory receptors can recognize several different odorants, they respond maximally to only one specified odorant. The activation of an olfactory receptor further creates different patterns in the cortex.

The axons of the olfactory receptors traverse the cribriform plate and make connections with the olfactory bulb in the limbic system. Near the olfactory bulb surface there are formations known as glomeruli. Each pair of glomeruli are targeted by a specific olfactory receptor neuron creating a neural activity pattern indicating stimulation by specific odorants (Doty, 2001; Geldard, 1972). This creates an "odorant map" or combinations which are similar to the stimulus maps of the visual and auditory systems (Korsching, 2002). Zou and Buck (2006) examined the mapping effects of binary mixtures on the olfactory cortex in mice, focusing on the anterior piriform of the olfactory cortex. Some cortical neurons responded to one or both of the mixtures components while other cortical neurons responded to the odorant mixture but not to either of its components alone. Therefore, it seems that binary mixtures stimulated many cortical neurons beyond those that responded to their individual component odorants and 30% of the olfactory cortex neurons which responded to a binary mixture are not stimulated by either of the single odorants in the mix. This suggests a synthetic operation in which the features of an odorant create a different olfactory receptor pattern or combination which then activates a pattern or combination of cortical neurons in the olfactory cortex. Although the complex olfactory system is capable of identifying a variety of odors which are displayed in cortex mapping, there is evidence suggesting the sense of smell in humans has diminished over time and is continuing to diminish.

It appears that olfactory receptor genes are loosing their function due to mutation and are now being represented as pseudogenes (a gene that is non-functional although may resemble a functional gene). Olfactory receptor genes seem to be mutating into pseudogenes at random (Glusman, Yanai, Rubin & Lancet, 2001; Rouquier, Taviaux et al., 1998) resulting in only one-third of human olfactory receptor genes appearing to be functional (Glusman et al., 2001). Rouquier, Blancher and Giorgi (2000) found that from New World monkeys to hominids there is an increase in the percentage of pseudogenes. This suggests that pseudogenes are increasing in humans and will probably evolve toward a minimal set of functional olfactory receptor genes (Rouquier, Friedman et al., 1998). It appears the efficiency of the human sense of smell may currently be decreasing but the diminishing of this sense may have begun with our ancestors.

The decline in olfactory receptor genes may be explained by the relaxation of selected pressure exerted by the species. If a sense such as vision is relied upon more than olfaction for survival then the genes responsible for olfactory function will begin to mutate. Supporting this theory, there may be a correlation between the development of full trichomatic vision in primates and the decrease of the olfactory receptor genes (Gilad, Wiebe, Przeworski, Lancet, & Pääbo, 2004). Researchers hypothesize that over time the ancestors of primates and humans evolved a smaller snout and the reduced snout size allowed for the eyes to come closer together. This appeared to permit for stereoscopic vision and even color vision. As the visual system improved, the olfactory sense greatly diminished (Gribbin & Cherfas, 1982; Lewin, 1993), which may suggest that the development of sight began to dominate the sense of smell.

Stoddart (1990) discusses the evidence that brain tissue devoted to handling olfactory information decreased remarkably as the evolutionary scale was ascended. The human nose and olfactory bulbs seem to have shrunk in the evolutionary process, presumably to make room for the evolution of larger brains and to permit stereoscopic vision, seeing an object with both eyes (Ackerman, 1990). However, Laska, Seibt and

Weber (2000) suggest that although primates and humans may have reduced olfactory receptor genes, humans still possess a good sense of smell. Humans can perform just as well as primates and just as well as, or even better than, other mammals in an odor comparison task, depending on the odors. Numerous odors are also often perceived in a mixture of odors and the ability for humans to detect odors in mixture is investigated.

It has been suggested that the capacity of humans to identify individual odors in an odorant mixture is limited to mixtures containing three or four odors (Cashion, Livermore & Hummel 2006; Jinks, & Laing, 1999; Jinks & Laing, 2001; Livermore & Laing, 1996; Livermore & Laing, 1998a, 1998b). Jinks and Laing (1999) found that the odorants most readily identified in odor mixtures were mandarin, mint and sports rub while the most difficult to identify were cinnamon, almond and fish. While some of the most distinguishable substances were edible, the toxic substance, sports rub, was also readily identified. In contrast, using a variety of similar odors as Jinks and Laing, as well as numerous other odors, Cashion, Livermore and Hummel (2006) found that the toxic odor of sports rub was not identified more often than any other odors in a mixture. This may have resulted from odors activating the same olfactory receptors known as suppression.

Since humans are incapable of identifying all the odors present in a mixture, Livermore and Laing (1998b) proposed that suppression may occur when odorants share common features and these common features activate the same odorant receptors causing overlapping neural representations. The overlap may cause olfactory receptor confusion when odors are presented simultaneously. Therefore, the molecular feature is considered to play a role in the ability to identify odors in a mixture and seems to be the foundation for the cause of overlap and ultimately suppression. Jinks and Laing (2001) support the idea that molecular features amongst odorants may result in the increased competition for receptor sites which leads to suppression. However, Livermore and Laing (1998b) also suggest that the olfactory system may have evolved to identify a small number of stimuli, rather than a broad number, but the question remains which stimuli are deemed important to the olfactory system?

Hallem and Carlson (2006) found that some of the olfactory receptors of the Drosphila were not activated when presented with edible fruit. This suggests that these receptors may have evolved to detect other chemical classes such as pheromones. This finding may imply that humans have specific olfactory receptors which have evolved to detect important odors. Therefore, in beginning to answer the question as to which odors are deemed important when presented with an odor mixture, it would be logical to assume that odors which may be harmful would be deemed important to identify because this would enhance survival.

The sense of smell has served as a survival mechanism for a wide variety of species. The function of smell is to guide actions that will ultimately lead to fitness, a basic premise of the natural selection theory. The natural selection theory can help explain how our ancestors used their sense of smell to hunt, gather and mate in order to survive and pass on their genes. However, the natural selection theory also states that building and maintaining organs is metabolically expensive and the organs must provide fitness benefits to justify their costs (Gaulin & McBurney, 2004). Therefore, when presented with an odor mixture, harmful odors referred to as toxic odors should be deemed important to identify by the olfactory system because this would enhance human survival. It is proposed that the benefit of detecting toxic odors in a mixture, the enhancement of survival, would ultimately outweigh the costs of building and maintaining the olfactory system.

The present study aims to investigate how evolution plays a role in explaining the human capability of detecting toxic odors in a mixture. Therefore, it is predicted, in accordance with the natural selection theory, that humans will be capable of identifying a single toxic odor in any given odor mixture regardless of the number of odors present because doing so may contribute to survival.

Methods

Participants

Twenty-one undergraduate students in a first year psychology course participated in the study. The participants included 6 males and 15 females, between the ages of 18 and 40 years (M = 22 years, SD = 8.41). All participants volunteered to be in the study and provided written consent prior to the commencement of the study.

Materials

The stimuli used in the study included three toxic odors- gasoline, acetic acid (white vinegar) and isopropyl alcohol-and five appetitive odors- pure lemon extract, pure anise extract, pure almond extract, pure mint extract and pure cinnamon extract. A pilot study was performed prior to the experiment to obtain the proper amount of liquid substance to be used so that each odor was equally detectable. The amounts for each liquid substance used in the study were 0.5ml gasoline, 3ml white acetic acid, 1.5ml isopropyl alcohol, 1ml almond, 3ml lemon, 3ml mint, 3ml cinnamon and 1ml anise.

The odor mixtures were in small glass jars which had a lid with holes as well as a secure, solid lid. To create the mixtures, each individual toxic odor (gasoline, isopropyl alcohol and acetic acid) was separately mixed with lemon which was kept constant across all the odor mixtures. Second, each toxic odor was mixed with lemon and anise. Third, each toxic odor was mixed with lemon, anise and almond. Fourth, each toxic odor was mixed with lemon, anise, almond and mint. And finally, fifth, each toxic odor was mixed with lemon, anise, almond, mint and cinnamon. The order in which the appetitive odors were introduced to the mixture was randomly selected and kept in the same order across all toxic odors. This created five odor mixtures for each toxic odor. Overall, there were 15 odor mixtures therefore 15 jars containing mixed odors.

To prevent participants from observing the substance(s) in the jar, each jar was covered with white paper and had a 5 cm square consisting of two colors located on one side of the jar. The colors provided the experimenter with information regarding which jar contained which odor components. The color combinations were randomly assigned to each jar therefore there was no relevance in the color used on the jar and the odors present in the jar.

A booklet was given to each participant with an area to indicate the colors on each jar and checklist of odors for each jar. The checklist of odors consisted of all the odors used in the experiment as well as filler items such as onion, nail polish remover, cherry, grape, chlorine, glue, pepper, cloves and nothing.

Procedure

Two sessions were conducted per participant and each session consisted of one or two participants. In session 1, eight randomly selected odor mixtures (any of the 15 odor mixtures) were randomly placed throughout the classroom approximately 1.25 m apart. The participants were instructed to state their age, date of birth and gender in an area located on the front of the booklet. The participants were seated in front of a jar and written and verbal instructions were given to each participant. The participants were instructed to write down the colors of the jar located directly in front of them in the area provided on the booklet. Next, the participants were to remove the top lid, allowing the lid with holes to be exposed. The participants were to smell the contents of the jar and when the experimenter instructed them to stop, the participants were to securely put the lid back on the jar. Using the checklist, the participants were instructed to check off any odors they perceived in the jar. Participants were told that there may be numerous odors in one jar. When each step was completed, the participants moved to the next jar and waited for the instructor's signal to begin. The participants repeated the steps for each jar until all eight jars were completed.

Session 2 occurred within a week of the first session. The 7 remaining odor mixtures which were not previously presented in session 1 were presented in a quasi random fashion. The instructions were the same as the instructions in session 1.

In both sessions, the participants smelled each jar for 10s with a 1m interval between each jar. The experimenter implemented the use of a stopwatch to time each participant.

When each session was completed, the experimenter asked the participants to perform a cognitive test to determine whether any of the mixtures may have caused any impairment. Juice and cookies were supplied to the participants at the end of each session.

Results

The Chi-Square Test of Independence was applied to determine if identification of a toxic odor in any of the 15 odor mixtures was dependent upon the number of appetitive odors. The number of correct identifications of a toxic odor in any of the 15 odor mixtures was not dependent upon the number of appetitive, χ^2 (8, N = 163) = 9.00, p = .05 (see Table 1).

A Chi-Square Test of Independence was carried out to determine if the total number of correct identifications of a toxic odor in any of the 15 odor mixtures was dependent upon the toxic odor. The number of correct identifications of a toxic odor in any of the 15 odor mixtures was dependent upon the toxic odor present, χ^2 (2, N = 163) = 103.16, p = .05 (see Figure 1).

Discussion

Consistent with the predictions, the results show that the number of odors in a given mixture has no significant effect on the ability to discriminate a toxic odor. This was demonstrated by the participants being able to identify the toxic odors at a constant rate across all odor mixtures regardless of the number of appetitive odors present in the mixture. However, there was a significant effect with the level of toxicity of the odor and the rate of correct identifications. Gasoline was identified significantly more in the odor mixtures than isopropyl alcohol and acetic acid. Gasoline may be presumed more toxic to humans because, unlike alcohol and acetic acid, humans do not consume gasoline. The more toxic, the more readily it is identified in the odor mixture.

To answer the enduring question as to which odors are deemed important to humans in an odor mixture, my study has shown that toxic odors are deemed important to humans because the toxic odors were identified at a constant rate across all mixtures. Therefore, the present study suggests that even though the human capacity to identify odors in a mixture rests at three to four, if a toxic odor were present in any given odor mixture, the toxic odor should be included as one of those three to four identifiable odors. However, the present study also found that the more toxic the odor, the more important it was to detect in the mixture. However, suppression may occur when presented an odor mixture.

In the present study, the molecular features of the toxic odors and the appetitive odors were not similar, therefore suppression presumably did not occur. This is evident as the toxic odors were identified at a fairly constant rate across all odor mixtures. If suppression had occurred, there should have been a decline in the rate of identification as the number of odors present in the mixtures increased. Suppression may have occurred within the appetitive odors since they did share similar molecular features, however the rate at which the participants could identify the appetitive odors in each mixture was not analyzed as this was not the main concern of the study. Future research could analyze if toxic odors could be suppressed by other odors which share similar molecular features. Although, in accordance to the natural selection theory, it can be predicted that suppression would not occur. However, there was an interesting finding concerning the molecular features of the toxic odors. Isopropyl alcohol and acetic acid did share similar molecular features and they were both identified less often than gasoline. The high rate of identification of gasoline and the low rate of identification of isopropyl alcohol and acetic acid may actually be related to their molecular features. The molecular features of gasoline may have allowed for faster perception of the odor in the mixture.

Some odors can potentially be processed at a faster rate which may be explained by the natural selection theory. When inhaling an odor mixture, different odors can differ greatly in the time they take to stimulate the olfactory receptors (Laing, Eddy, Francis & Stephens, 1994). This difference in the processing of certain odors is proposed to be temporal processing and this may also limit the ability of humans to identify odors in a mixture. From an evolutionary perspective, odors which are deemed important for survival may be perceived at a faster rate than odors of less importance. This corresponds with the findings of the present study in which the toxic odors were identified at the same rate across all odor mixtures. Gasoline was presumably more toxic compared to isopropyl alcohol and acetic acid and may have been processed faster leading to higher identification. Toxic odors may actually be the "fast" odors but the rate at which they are identified may correlate to their level of toxicity. Therefore, important odors may be processed faster by the olfactory cortex which can be explained by the natural selection theory.

Another area to be of concern from the present study is the rate of familiarity of the odors. It has been found that familiarity of an odor allows for easier odor identification (Rabin, 1988). The present study has found contrasting results. It would be assumed that people would be more familiar with isopropyl alcohol and acetic acid because they are consumed. This is especially true for the participants in my study because they are undergraduate students who may consume alcohol on a regular basis. However, gasoline was identified the most often in the mixtures; therefore familiarity probably had little relevance in the identification of toxic odors in a mixture. This finding also supports the natural section theory. Another aspect of the toxic odor identification which should be discussed is the intensity of each odor.

In the present study, care was taken to make sure that all odors were of equal intensity which allowed for each odor, toxic and appetitive, to have an equal chance of being identified. Other studies have demonstrated that increasing or decreasing the intensities of odors in a mixture had substantial effects on identifications (Jinks & Laing, 1999; Laing, 1994; Laing, Panhuber, Willcox & Pittman, 1984). Therefore, the ability for participants to identify the toxic odors throughout all the mixtures at a fairly constant rate was most likely not due to intensity because all odors were of equal intensity. This finding lends support to the natural selection theory because regardless of the intensity, toxic odors are still deemed important to identify in a mixture.

Although it was previously discussed that the human sense of smell is diminishing, this study demonstrates that humans are capable of using their sense of smell to detect danger when presented in odor mixtures. Perhaps the human sense of smell is diminishing but maybe it is becoming specially tuned for specific odor identification such as toxic odors rather than broad odor identification. For instance, humans no longer need their noses for hunting so maybe the human sense of smell is becoming more accustomed to relevant information, such as detecting danger, rather than irrelevant information. The function of smell is ultimately to guide actions which will lead to survival and previously our ancestors needed their sense of smell to detect prey. Nowadays, humans no longer need their sense of smell to detect prey therefore the sense of smell may be diminishing in many ways but the function of smell still remains because detecting toxic odors in a mixture enhances survival. Utilizing brain imaging techniques may help increase understanding of the ability of humans to detect toxic odors in a mixture.

Techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) can be used to view olfactory mapping and have demonstrated that different odors can elicit distinctly different patterns of activity in the brain (Buck, 2004; Royet & Plally, 2004; Savic, 2002; Zald & Pardo, 2000; Zou, Li & Buck, 2005). Studies using neuroimaging to map aversive and appetitive olfactory stimuli have found that the amygdala was of primary focus for the perception of olfaction of appetitive and aversive stimuli (Royet et al., 2000; Zald & Pardo, 1997). The direct activation of the amygdala to aversive stimuli may allow for an immediate autonomic, preconscious and nonconscious emotional response (Zillmer & Spiers, 2001). From an evolutionary perspective, autonomic responses to an aversive odor, such as fight or flight responses, may act as a survival mechanism against ingesting toxic substances. Since it is suggested that primary emotions such as fear, disgust and anger are innate (Zillmer & Spiers, 2001), it can be hypothesized that the ability to detect an aversive stimuli that would elicit an emotion such as disgust may also be an innate ability. Therefore, toxic odor detection in an odor mixture may be a natural response thus supporting the natural selection theory.

Some limitations of the study should be addressed. The checklist provided to the participants may have had priming effects. For example, seeing the word of an odor may have primed them to think they smelled that specific odor. Although distractor items were used to decrease priming effects, priming may still have occurred. Also, odors were manually mixed and glass jars were used to present the odor mixture to the participants whereas in most other studies olfactometers are used. Olfactometers automatically mix the odors and present them in the specified quantities but olfactometers were not used in the study because they were not readily available.

Future research could replicate the current study using wide a variety of toxic and appetitive odors to observe if toxic odors will still be readily identified at a fairly constant rate across odor mixtures. Doing so may provide more knowledge as to which odors are deemed important in an odor mixture as well as information regarding olfactory suppression of toxic odors. Furthermore, the minimum intensity required for toxic odor detection could be investigated. This could offer understanding as to how finely tuned humans are at identifying toxic odors in a mixture in addition to the amount of toxic odor needed for humans to deem it an important odor to identify.

In summary, the present study has revealed the ability of humans to identify toxic odors in a mixture is not dependent upon the number of odors present in a mixture. However, the level of toxicity of the odors does have an effect of the rate of detection with the most toxic odors being identified more often than the less toxic odors. These findings are consistent with the natural selection theory which states that the function of olfaction is survival but the benefits must outweigh the costs. The ability for humans to detect toxic substances in a mixture regardless of the number of odors in the mixture enhances survival and thus survival outweighs the costs of maintaining the sense of smell.

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Table 1.

The number of correct identifications of each toxic odor in appetitive mixtures

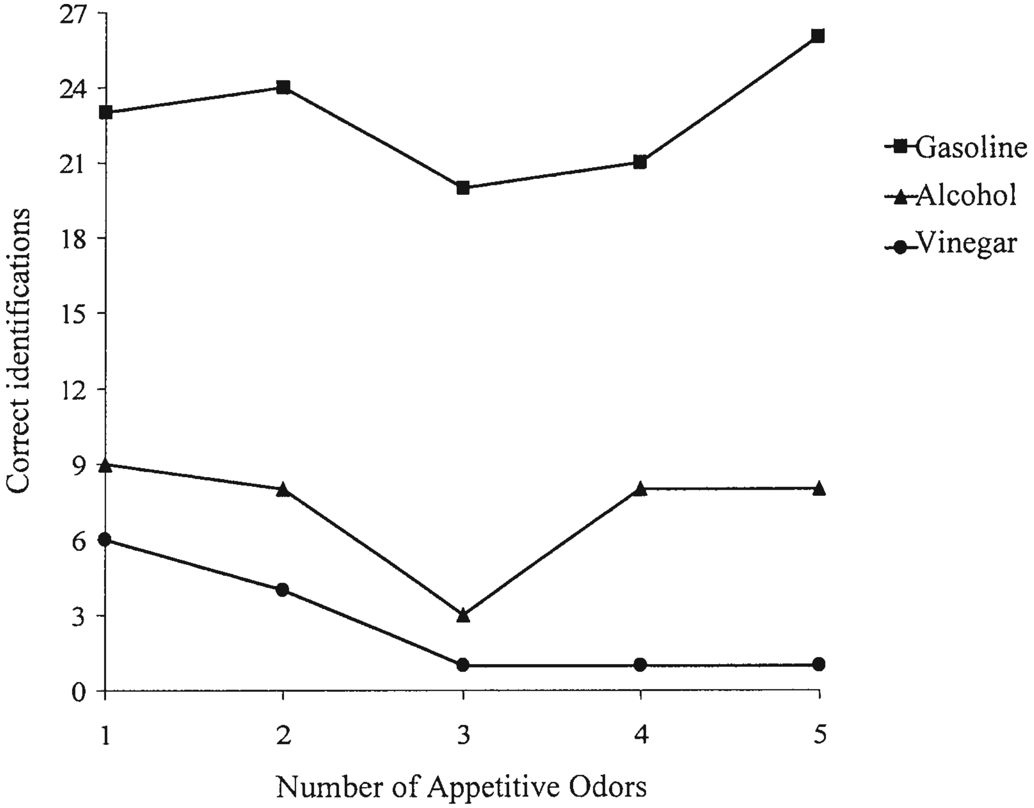
Appetitive odors[a]	Gasoline	Isopropyl alcohol	Acetic acid	Total
1	23	9	6	38
2	24	8	4	36
3	20	3	1	24
4	21	8	1	30
5	26	8	1	35
Total	114	36	13	163

[a] Number of appetitive odors in a mixture

Figure Caption

Figure 1. Frequency of correct identifications of toxic odors in odor mixtures containing different numbers of appetitive odors.

Figure 1.


27
24
21
18
15
12
9
6
3
0
Correct identifications
1
2
3
4
5
Number of Appetitive Odors
■ Gasoline
▲ Alcohol
● Vinegar