Chem. Senses 32: 191–199, 2007

doi:10.1093/chemse/bjl049
Advance Access publication January 4, 2007

Asymmetric Suppression of Components in Binary Aldehyde Mixtures: Behavioral Studies in the Laboratory Rat

Ljiljana Sokolic[1], David G. Laing[2] and Iain S. McGregor[1]

[1]School of Psychology, University of Sydney, New South Wales 2006, Australia and [2]School of Women and Children's Health, University of New South Wales, New South Wales 2031, Australia

Correspondence to be sent to: Iain S. McGregor, School of Psychology, University of Sydney, New South Wales 2006, Australia. e-mail: iain@psych.usyd.edu.au

Abstract

The aim of the present study was to assess component interaction in the perception of the 2 aldehydes butanal and heptanal when presented in binary mixtures to rats. A further aim was to develop a behavioral paradigm for testing suppression of components in mixtures using rodent subjects. Thirsty rats were initially trained to discriminate between the 2 aldehydes butanal and heptanal in an olfactometer using a go/no-go discrimination task. This involved rats learning to place their noses in a sniff port where odors were presented and to lick a tube for water reward when one of the aldehydes was presented (S+) while withholding licking at the tube to the other, unrewarded, aldehyde (S−). A mixture condition was then introduced into the task, whereby a proportion of trials involved presentation of a combination of the 2 aldehydes as an additional unrewarded condition. Rats readily learned to withhold licking on trials when the mixture was presented. The concentration of the nonrewarded (S−) aldehyde in the mixture was then systematically decreased, whereas the concentration of the S+ component was held constant. This eventually caused the S+ component in the mixture to suppress detection of the S−, as shown by an increasing number of lick responses (false alarms) on trials when the mixture was presented. These suppressing effects occurred well above the detection threshold for the S− aldehyde presented alone. Results showed asymmetric suppression in the mixture condition such that butanal suppressed detection of heptanal at much lower concentrations than vice versa. A second experiment showed that when both butanal and heptanal were present in a binary mixture at the same concentration (10^{-6} volume %), then rats responded to the mixture as if only butanal was present. These findings are discussed in terms of butanal having higher mobility and being able to compete more effectively than heptanal for occupation of shared receptor sites.

Key words: aliphatic aldehydes, discrimination, odor mixtures, odor suppression, olfactometer, rats

Introduction

Most naturally occurring odors are complex blends of volatile components. The way in which they are perceived depends upon the interactions between mixture components at the level of olfactory receptors (Derby 2000) as well as the way that component signals are processed in the olfactory bulb (Tabor et al. 2004) and olfactory cortex (Wilson 2003; Zou and Buck 2006). Mixtures introduce the possibility of competitive or some other type of interaction between components (Kay et al. 2005), and characterizing these interactions using behavioral methods may provide an insight into the mechanisms underlying odor mixture perception.

Odor mixture perception is sometimes configurational where the mixture has novel perceptual qualities that are not present in the components (Wiltrout et al. 2003). However, mixture interactions can also lead to suppression effects whereby one component partly or completely reduces the perceived intensity of another in the mixture (Laing et al. 1984; Laing and Francis 1989; Linster and Smith 1999). This suppression of individual odors in mixtures is sometimes known as "odor masking" (Laing et al. 1989) or "overshadowing" (Kay et al. 2005).

Suppression may reflect competitive interaction between components at olfactory receptors, with the molecular structure of components determining the outcome of competition for occupancy of a particular receptor type (Bell et al. 1987; Ache 1989; Joerges et al. 1997; Derby 2000; Duchamp-Viret et al. 2003; Deisig et al. 2006). For example, rats have an OR-I7 olfactory receptor with high affinity for the aliphatic aldehyde octanal. Other aldehydes have affinity for this receptor, but this markedly decreases as the number of

carbon atoms in the aliphatic chain increases above the 8 carbon atoms of octanal (Araneda et al. 2000, 2004). This suggests that in binary mixtures of aldehydes, those with fewer carbon atoms may suppress detection of aldehydes with longer carbon chains. The consequence of such antagonism should be an asymmetric suppression of individual aldehydes in mixtures containing more than one aldehyde.

This hypothesis has been supported in studies with humans (Laing et al. 2002). These showed that the 3-carbon atom chain aldehyde propanal is the dominant suppressor in binary mixtures with the 7-carbon atom chain aldehyde heptanal. This study also suggested that as the difference in carbon chain length between the 2 aldehydes in a mixture decreases, the interaction systematically changes from asymmetrical to symmetrical suppression.

The present study examined whether asymmetric suppression effects can be observed in rats. To achieve this, we used a go/no-go olfactory discrimination behavioral paradigm derived from a previous study by Laing et al. (1989). These authors used a go/no discrimination task to demonstrate that limonene, carvone, and acetic acid can impair detection of propionic acid in a concentration-dependent manner. Thirsty rats were trained to lick a tube for a water reward whenever propionic acid, or mixtures involving propionic acid and one other component (e.g., limonene), was presented (S+ conditions). On trials when this other component (limonene) was presented alone, licking was not rewarded (S− condition). Rats readily learned to respond (lick) to the mixture (e.g., limonene/propionic acid) and to withhold licking on trials involving the individual component (e.g., limonene).

The concentration of propionic acid was then systematically reduced over consecutive tests. This caused rats to increase their error rate on the trials where the individual S− odorant (e.g., limonene) was presented alone. This effect was explained as follows: the reduction of propionic acid concentration in the mixture meant that the rats were being rewarded for responding on mixture trials (limonene/propionic acid) where only the S− odorant (e.g., limonene) was detected. This caused the rats to increase their response on (unrewarded) trials where limonene was presented alone.

The procedures used by Laing et al. (1989) provided only a relatively indirect measure of mixture component suppression, with a cliff-like falloff in correctly responding to the S− odorant (and not the mixture itself) at a critical concentration. There was little indication of partial suppression that may occur as concentrations of test odorants are varied. The present study aimed to refine this behavioral paradigm to more directly assess mixture component interactions. Similar to Laing et al. (1989), our paradigm involved animals being rewarded for responding on trials where one odor was presented alone (S+) and not rewarded when the other odor was presented alone (S−). Critically, when a mixture of these 2 components was presented, responding was also not rewarded (mixture S− condition). In this way, when the concentration of the S− component is decreased to a critical level, we predicted that the S+ should suppress the S− on mixture trials and the rats will start responding on these (unrewarded) trials. This provides a direct measure of the suppression of components within binary mixtures.

We used this approach in the 2 experiments described below to determine whether asymmetric suppression occurs in mixtures of heptanal (C_7) and the smaller aldehyde butanal (C_4). Our choice of odor stimuli and its concentrations was based upon the evidence for suppression in the mixtures of aliphatic aldehydes (Laing et al. 2002) as well as the fact that aldehydes comprise an important constituent of our natural odor world that rodent subjects display excellent sensitivity toward (Laska et al. 2006).

Materials and methods

Subjects

A total of 34 inbred male Australian albino Wistar rats (*Rattus Norvegicus*) (University of Sydney, Australia), aged between 60 and 90 days (180–379 g) at the beginning of training, were used in the 2 experiments. Of 34 rats, 22 were used in Experiment 1 and 12 in Experiment 2. The rats were group housed in a temperature-controlled colony room (21 ± 2 °C) on a reverse light–dark cycle (lights off from 8 AM to 8 PM). They were maintained on a 10 ml/day water deprivation schedule with free access to water at the weekends. All training and test sessions were conducted between 10 AM and 2 PM.

All procedures were approved by the University of Sydney Animal Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1997).

Apparatus

Odors were delivered using 4 computer-controlled 12-channel olfactometers (Knosys, Tampa, FL), similar to those described by Slotnick (Slotnick and Schellinck 2001; Slotnick and Bodyak 2002). Each olfactometer had 12 independent odor channels, a test chamber, and a digital interface. Airflow was provided by individual aquarium air pumps (President Pi 8000) connected to a fritted glass particle filter containing activated charcoal. Airflow rates were controlled by Teflon and glass needle valves and calibrated flow meters. Push-button switches located in a box below the test chamber allowed manual activation of valves for maintenance and testing purposes.

Olfactory stimuli were generated by computer-controlled opening of normally closed pinch valves that were located on either side of 50-ml glass saturator bottles. The saturator bottles contained 5 ml of liquid odorant with air into and out of the saturator bottles passing through C-flex tubing when the pinch valves were opened. The odor-saturated output from the saturator bottles was added to 1950 ml/min stream of clean air, which then passed to the odor sampling port of

the test chamber. The airflow was constantly drawn under negative pressure past the odor sampling port and exhausted through tubing to the exterior of the laboratory.

The test chamber consisted of a 17 cm wide, 24 cm long, and 25 cm high Plexiglas box fitted with a stainless steel grid floor. A brushless fan was mounted on one side of the chamber for ventilation. The odor sampling port (diameter 3 cm) was located on the front wall of the chamber 14 cm above the floor. Nose pokes at this port were detected via breaks in a photo beam located across the entrance to the port.

Lick tubes, through which water rewards could be delivered, were located 3 cm to the left and 3 cm to the right of the odor sampling port and at the same height as the port. A third "central" lick tube was located inside the odor sampling port itself. The outer part of the lick tubes was made of glass, whereas the inner consisted of thin stainless steel tubing. Each time the tongue of the rat made contact with the steel inner of the lick tube, an electrical circuit was completed between the tube and the grid floor of the chambers, which was detected and recorded by the computer. The lick tubes were connected via C-flex tubing to a 20-ml syringe filled with water. Operation of a normally closed solenoid pinch valve allowed water to flow under gravitational force from the syringe reservoir down through the tubing and out of the lick tube. Opening time of the water delivery pinch valves was calibrated so that 0.05 ml water was delivered to the rat on rewarded trials. The central lick tube was used for water delivery in Experiment 1, whereas the right hand lick tube was used in Experiment 2.

A Sonalert sound generator (Med Associates, St Albans, VT, part ENV 223AM) mounted high on the front wall of the test chamber was used to provide an auditory signal (beep) during training. Cue lamps (Med Associates, part ENV-221M) mounted 2 cm above the right and left lick tubes were used to signal the intertrial intervals (ITI).

Delivery of the odor-saturated air stream to the odor sampling port was controlled by a 3-way solenoid pinch valve, known as the "final valve" (Slotnick and Schoonover 1984). The final valve diverted the odor stream away from the odor sampling port for the first 1 s that the odor was generated. This process of diverting the air stream had 2 important functions (Slotnick and Schoonover 1984). First, it acts as a cue to a rat that the odor was about to be delivered after the termination of the final valve (i.e., 1 s). Second, it allowed the odor stream to fully mix with the air stream, thus preventing variation in the concentration of the stimulus before presenting it to the rat.

All data acquisition and computer control was performed by custom software written in Strawberry Tree's Workbench Program running on a Macintosh computer (McGregor 1996).

Odorants

The odor stimuli were *n*-butyl aldehyde (C_4) and *n*-heptyl aldehyde (C_7) (Fluka, Sydney, Australia; 99% purity) and lemon and strawberry essences (Queen Fine Foods Pty. Ltd, Aderley, Queensland, Australia). The lemon and strawberry were used undiluted, whereas the aldehydes were diluted to various concentrations with near-odorless 1,2-propanediol (Sigma-Aldrich, Castle Hill, New South Wales, Australia; 99.5% purity).

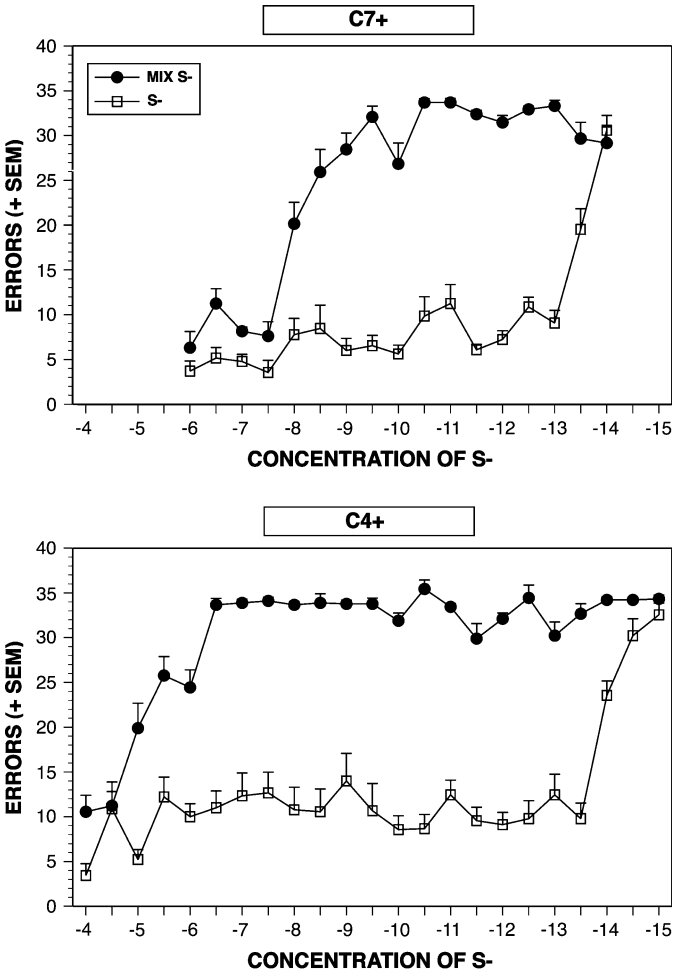

Figure 1 Number of errors in the C7+ (upper panel) and C4+ (lower panel) groups on mixture S− and S− alone trials in rats in Experiment 1. Note that there were 34–35 mixture S− trials in each session, so error levels at 34 and above indicate approximately 100% error rate. Note the high number of errors on mixture S− trials relative to S− alone trials at much higher concentrations of the S−, showing suppression of one aldehyde by the other in the mixture condition. Note also that this suppression occurred at much higher concentrations of the S− in the C4+ group than the C7+ group, indicating greater suppression of heptanal by butanal than vice versa.

Experiment 1

Experiment 1 aimed to characterize the component interactions between butanal (C_4 aldehyde) and heptanal (C_7 aldehyde) using the approach described in the Introduction. Two groups of rats were used, referred to as group C4+ (n = 11) and group C7+ (n = 11).

For group C4+, butanal served as the S+ stimulus and heptanal as the S− stimulus. For group C7+, the opposite

was the case. Both groups were initially trained on 2-odor discrimination tasks and then progressed onto a mixture task described in detail below. The mixture task involved systematic manipulation of the concentration of the S− component, whereas the S+ component was kept constant.

Procedure

Lick training

On the first day of training, rats were rewarded for licking at the central lick tube located within the odor sampling port. A lick at this tube leads to a brief beep from the sonalert (0.25 s) and delivery of a 0.05 ml drop of water down the lick tube. The cue lights then came on in the chamber to signal a 6 s ITI during which lick responses were not reinforced.

Nose poke training

On the next 4 days, rats were trained to keep their nose in the sniff port for increasing lengths of time in order to obtain a water reward. If the nose was kept in the port for the required amount of time, the sonalert sounded and any lick by the rat after this on the central lick tube was reinforced with 0.05 ml of water. Again, an ITI of 6 s, during which the cue lights came on in the test chamber, was interposed between each successive trial.

By the end of this training phase, rats had been successfully trained to keep their nose in the odor sampling port for at least 300 ms of the first 1000 ms of the odor delivery period through the odor sampling port. Because of the operation of the final valve for 1 s, odors were only delivered through the odor sampling port 1 s after odor generation started. Thus, this 300 ms poke requirement occurred in the period 1000–2000 ms after the trial was initiated. Note that during training, however, no odors were being delivered.

Two-odor task (lemon vs. strawberry)

Rats were then trained on a simple 2-odor discrimination task using lemon and strawberry odors. For all rats, lemon acted as the S+ odor, whereas strawberry was the S−. Rats initiated each trial with a nose poke. If the poke requirement was met (at least 300 ms poking during the first 1000 ms of odor delivery) and the odor sampled was lemon, then licking at the central lick tube was reinforced with a water reward and the trial was scored as correct (a hit). If a rat did not lick the reinforcement tube during a 8-s period following termination of the S+ stimulus, then the trial was scored as an error (a miss). If a rat made a lick response on a S− (strawberry) trial, no water was delivered and the trial was scored as an error (false alarm). If a rat did not lick the lick tube on a S− trial, the trial was scored as correct (a correct rejection). On trials where a correct response was made, an ITI of 6 s was used, whereas a longer ITI of 8 s was given after an incorrect response.

There were a maximum of 200 trials in each daily session. The sequence of trials was ordered so that in each block of 20 trials, there were 10 S+ and 10 S− odors presented, with no more than 3 S+ odors in a row. The session was terminated automatically if the rat made 17 correct responses in any 20 consecutive trials or if 200 trials had elapsed.

Two-odor task (butanal vs. heptanal)

Once rats had performed the lemon versus strawberry discrimination task with fewer than 15% errors, they were then trained on an identical 2-odor discrimination task but this time involving butanal and heptanal. The 2 odors were presented at a concentration of 10^{-6} vol% (vol% refers to odor concentration given as volume percent saturated vapor at 21 °C).

For group C4+, butanal served as the S+ stimulus and heptanal was the S−, whereas for group C7+, this contingency was reversed. After 7 days of training, rats were showing high levels of accuracy on this simple discrimination task. Again a criterion of 17/20 was in operation so that sessions automatically stopped when the rat made 17 out of 20 correct responses.

Mixture task

Rats were then switched to the mixture task. This task was similar to the heptanal versus butanal discrimination task except that additional unrewarded trials were inserted into the session involving mixtures of these 2 odors. The trials involving mixtures were called "mixture S− trials," and responding on these trials was not reinforced, with lick responses made to the mixture scored as false alarms.

The first 20 trials in the mixture task sessions involved simple presentation of the individual odors alone, with butanal and heptanal as the S+ or S− stimulus depending upon the group. In approximately one-third of the following trials (trials 21–124), a butanal/heptanal mixture was presented as an additional unrewarded S− condition. The mixture stimuli were generated by combining the liquid odorants in appropriate concentrations in a saturator bottle and were presented in a separate channel of the olfactometers. All 3 types of stimuli (S+, S−, mixture S−) were presented in pseudorandom order with no more than 3 trials of one type in succession and an approximately equal number of S+, S−, and mixture trials in each block of 20 trials. A session involved 124 trials, with 34–35 mixture S− trials and 44–45 S+ alone and 44–45 S− alone trials.

For the C7+ group of rats, for which the heptanal was the S+, performance on the mixture task was excellent when the aldehydes were each presented at a concentration of 10^{-6} vol%. For the C4+ group, however, performance on the mixture part of the task was poor with the concentrations of both aldehydes at 10^{-6} vol% When the concentration of the 2 aldehydes was increased to 10^{-4} vol%, this group exhibited greatly improved performance on mixture S− trials. This was therefore used as the baseline concentration of odorants for the C4+ group.

In general, the same identical vol% concentrations of the 2 odorants were used as a starting point to allow a clear

characterization of the magnitude of any observed suppressive interaction effect. There was little a priori reason to suspect differential sensitivity to the 2 aldehydes when presented alone given recent results from mice (Laska et al. 2006). Indeed, as shown below, the detection thresholds for the 2 aldehydes were rather similar.

Effects of varying the concentration of the S− odorant

Once rats had showed stable 85% accuracy on the mixture task, the concentration of the S− odor was systematically decreased over successive daily sessions in both the S− and mixture S− trials. Thus, for the C7+ group, the concentration of butanal (S− stimulus) was progressively reduced in test sessions by a half log unit of the preceding concentration, whereas the concentration of heptanal (S+ stimulus) was kept constant. Conversely, for the C4+ group, the concentration of heptanal (S− stimulus) was reduced, whereas the concentration of butanal (S+ stimulus) was kept constant. A single concentration decrease of a half log unit was tested in each daily session of 124 trials. Daily trials continued until the number of errors seen on S− alone trials matched that seen on mixture S− trials (see Figure 1).

Data analysis

For each subject, the difference between the number of errors on the mixture S− trials versus S− alone trials was calculated. A repeated measures analysis of variance (ANOVA) was conducted on these difference scores, with odor concentration as the within-subjects factor and group (C4+ vs. C7+) as a between-subjects factor.

Results

The results for the C7+ group are presented in Figure 1 (upper panel). At the baseline concentration of 10^{-6} vol%, the number of errors made on both S− and mixture S− trials was low. Results for S+ alone trials are not presented, as rats almost invariably made a correct response on such trials, so that accuracy can be assumed to be 100%.

As the concentration of the S− odor (butanal) was decreased both alone and in the mixture trials across successive days, rats initially continued to make few errors. However, once the concentration was reduced to 10^{-8} vol%, the error rate on mixture S− trials increased markedly and continued to increase until reaching maximal asymptotic levels when the S− concentration reached 10^{-10} vol%. On trials on which the S− was presented alone, high error rates were not obtained until the concentration of the S− approached very low levels of 10^{-14} vol%. This presumably reflects the detection threshold for butanal for these rats.

The failure of the rats to detect butanal in mixtures at concentrations lower than the detection threshold for butanal presented alone indicates suppression of butanal by heptanal. The difference between 10^{-8} and 10^{-14} vol% ($\sim 10^{6}$ units of concentration) suggests the magnitude of the suppressive effect of heptanal (at a concentration of 10^{-6} vol%) on butanal in a binary mixture.

The results for the C4+ group are presented in Figure 1 (lower panel). For this group, the error rate on mixture S− trials rose rapidly over successive days as the concentration of the S− was decreased. Near asymptotic error levels for mixture S− trials were evident with the S− concentration at 10^{-6} vol%. In contrast, the detection threshold for the S− presented alone was approximately 10^{-14} vol%. This suggests a large magnitude suppressive effect of heptanal by butanal, in the range of $\sim 10^{8}$ vol/% concentration. This magnitude is greater than that obtained in the C7+ group and indicates that an asymmetric suppression occurred between the 2 aldehydes, with butanal being the dominant suppressor over heptanal.

Repeated measures ANOVA used to analyze the difference in errors between mixture S− and S− alone trials revealed that the main effect of odor concentration averaged across 2 different groups was statistically significant ($F(16,320) = 15.82$, $P < 0.001$). The main effect of group averaged across the different concentrations was also statistically significant ($F(1,20) = 39.38$, $P < 0.001$). The mean difference score of 21.16 (meaning that, averaged over the different concentration levels, 21.16 more errors were made for mixture S− trials than for S− trials) for the C4+ group was significantly higher than the mean error difference score of 14.81 for the C7+ group, which again illustrates the asymmetric suppressive effect.

The concentration × group interaction was also statistically significant ($F(16,320) = 9.87$, $P < 0.001$), indicating that the pattern of errors across varying odor concentrations differed between the 2 groups. Both groups retained an almost perfect level of accuracy in their response to the S+ stimulus throughout successive sessions (data not shown). As the concentration of the S+ did not vary over these sessions, this result is to be expected.

Experiment 2

Experiment 2 was designed to confirm the results obtained in Experiment 1 albeit using a slightly different experimental approach. The results of Experiment 1 suggested that group C4+ was impaired in its detection of heptanal when it was presented in a mixture with butanal and the concentration of each aldehyde was 10^{-6} vol% (see Figure 1).

In Experiment 2, we again used 2 groups that were again called group C4+ and group C7+. These rats underwent similar training to Experiment 1, initially learning to discriminate heptanal from butanal in a 2-odor discrimination paradigm. For group C4+, butanal was the rewarded odor, whereas for group C7+, heptanal was rewarded. Once high discrimination accuracy was achieved, trials involving a mixture S− were again introduced into sessions. This time the mixture S− contained a combination of the 2 aldehydes at the fixed concentration of 10^{-6} vol%.

We predicted that rats in the C7+ group would continue to show high levels of accuracy when the mixture S− trials were

introduced. For these rats, the dominant aldehyde was the S− when presented alone, and so we reasoned that the mixture, in which butanal most likely suppressed heptanal, would be treated just as butanal alone was and licking would be correctly inhibited on trials involving the mixture.

For rats in the C4+ group, however, the dominance of butanal over heptanal would lead to an opposite outcome. For these rats, the dominant aldehyde was the S+ when presented alone, and therefore, in the mixture condition, rats in this group would respond to the mixture as if it were an S+, leading to a high rate of false alarms.

Thus, when presented with exactly the same odor mixture stimulus, one group (C7+) should show very good performance, whereas the other group (C4+) should show very poor performance.

Procedures

Rats in the C4+ group (n = 7) and C7+ group (n = 5) were trained to lick and nose poke in the olfactometers as described in Experiment 1. One slight difference in Experiment 2 was the use of the right lick tube (located to the left of the sniff port) rather than the center lick tube (located within the sniff port) for the delivery of water. This procedure was adopted as intervening experiments had suggested slightly faster training of rats when a side rather than central lick tube was used.

Once rats had been shaped to nose poke and lick, they were trained on a lemon versus strawberry discrimination task as described in Experiment 1. Criterion performance for all training and testing was set at 85% correct responding in a block of 20 trials; therefore, the session was terminated as soon as the rat made 17 correct responses in any 20 consecutive trials. Criterion performance on the lemon versus strawberry task was obtained within 4 days.

Rats were then trained on the 2-odor butanal versus heptanal discrimination task as described in Experiment 1. Again, the contingency for the 2 groups was opposite on this task: for the C4+ group butanal served as the S+, whereas heptanal served as the S−. For the C7+ group, this contingency was reversed. Rats received 100 trials per day on this task over 7 consecutive days. After this, rats were moved into the test phase involving the mixture task (124 trials).

In this phase, as in Experiment 1, in addition to the single component S+ and S− stimuli, trials involving a butanal/heptanal mixture S− were introduced after the first block of 20 trials of the session. The aldehydes were delivered at a concentration of 10^{-6} vol% in propanediol. Rats received 4 identical test sessions over 4 consecutive days, with each test involving 124 trials.

Data analysis

For each subject, the difference between the number of errors for the mixture S− trials and the number of errors for the S− alone trials was calculated. A repeated measures ANOVA was conducted on these difference scores, with test day as a within-subjects factor and group (C4+ vs. C7+) as a between-subjects factor.

Results

Figure 2 shows the responses of the 2 groups of rats over 7 days of the simple 2-odor (butanal vs. heptanal) discrimination task. As seen from Figure 2, both the C4+ and C7+ groups achieved greater than 85% accuracy by day 4 of the 7 days of the task.

The results of introducing the mixture S− are shown in Figure 3. For the C4+ group, the error rate with the mixture S− was near maximal and did not vary across the 4 test days (Figure 3). Responding on S− alone trials, however, continued to be quite accurate. This suggests significant suppression of heptanal by butanal.

In contrast, for the C7+ group, the rats made relatively few errors on mixture S− trials (Figure 3). Responding on S− alone trials in this group was accurate, and a relatively constant low error rate was maintained over days.

Repeated measures ANOVA for difference scores (mixture errors minus S− errors) across the 4 test days indicated a significant main effect of group $F(1,10) = 102.68$, $P < 0.001$, with the C4+ group having a mean difference of 19.93 and the C7+ group 1.50. There was also a significant change in the difference score averaged over groups C4+ and C7+ across the 4 test days, $F(3,30) = 5.66$, $P < 0.01$.

There was a significant linear trend over the 4 test days, $F(1,10) = 10.07$, $P < 0.05$, as well as a significant cubic trend, $F(1,10) = 16.79$, $P < 0.01$, whereas the quadratic trend was not significant, $F < 1.5$. The group × test day interaction was not statistically significant, $F < 1.5$, indicating that the pattern of errors across test days did not differ between the

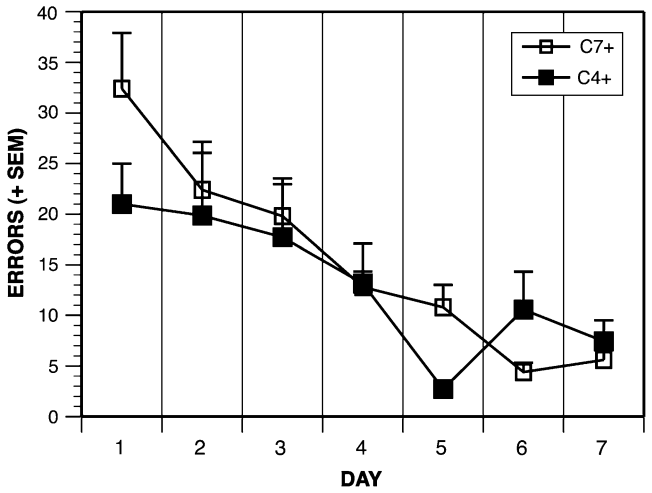

Figure 2 Number of errors made by the C4+ and C7+ groups of rats across 7 successive daily sessions of a simple butanal versus heptanal discrimination sessions in Experiment 2. Each daily session involved 100 trials with approximately equal number of S+ and S− trials.

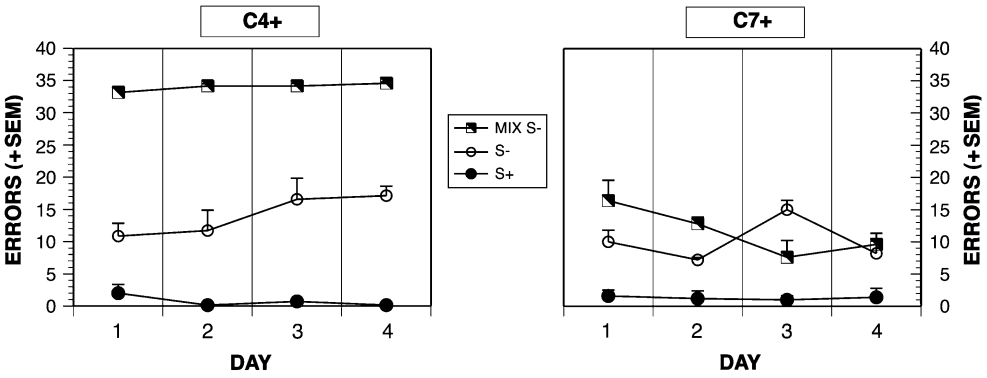

Figure 3 Number of errors made by the C4+ and C7+ groups of rats across 4 daily test sessions in which S+ alone, S− alone, and mixture S− trials were presented. Note that there were 34–35 mixture S− trials in each session, so error levels at 34 and above indicate approximately 100% error rate. Each session involved 124 trials. Note the much higher error rate for mixture S− trials in the C4+ group. In this group, presumably butanal is suppressing heptanal in the mixture so that mixture S− trials are responded to in the same way as S+ trials, causing a high rate of false alarms.

2 groups. Errors to the S+ stimulus by both groups were very low over the whole testing phase.

In summary, the results of Experiment 2 further confirm that asymmetric suppression occurs between the 2 aldehydes with butanal being a suppressor of heptanal.

Discussion

In the present study, a behavioral paradigm for assessing component suppression in binary odor mixtures in the rat was successfully developed and tested. The paradigm was used to confirm the hypothesis that asymmetric suppression effects occur in rats in the perception of binary mixtures of heptanal and butanal.

The key characteristic of the behavioral paradigm was the maintenance of one component of a binary mixture as the S+ stimulus at a fixed concentration while varying the concentration of the other component, the S− stimulus, in the mixture and when presented alone. This paradigm provided good control of the behavior of the animals, with incorrect responses only occurring when the S− component was either suppressed in the mixture or when the threshold for this component was approached. It is likely that the paradigm could be employed successfully to study component interactions across a wide range of odors.

A second feature of behavioral paradigm used in Experiment 1 was the determination of the threshold of the S− component, both when presented alone or in a binary mixture, and this allowed quantification of the measured extent of suppression. The quantitative index of suppression is the difference between the threshold for S− stimulus and its concentration in the mixture when suppressed by the S+. This appears to be the first behavioral study to provide a quantitative measure of the magnitude of suppression in mixtures.

Methods used in other studies with the rat involving mixtures precluded such a measure (Laing et al. 1989; Linster and Hasselmo 1999; Linster and Smith 1999; Kay et al. 2003). In the study by Laing et al. (1989), the animals unexpectedly and incorrectly transferred their responses from the S− stimulus to the S+ stimulus when suppression occurred, and the threshold had to be determined using a different paradigm. Other studies (Linster and Hasselmo 1999; Kay et al. 2003, 2005) involving mixture suppression have used a digging task where rats learn to discriminate the location of a buried food reward by odor cues in the sand. This approach has the advantage of requiring only simple equipment but lacks precise control over the timing, duration, and concentration of odor delivery. To avoid this problem, Kay et al. (2006) introduced an operant lever press task, which allowed better control of odor delivery. However, the generalization paradigm used in that study is more suitable for assessing odorant similarities rather than directly measuring mixture component suppression across the range of different concentrations.

The results obtained in the present study clearly demonstrated that butanal was the dominant suppressor in mixtures with heptanal. This was very clearly seen in Experiment 2, where rats presented with a mixture of these 2 aldehydes at equal concentrations (10^{-6} vol%) treated the mixture as if it only contained butanal. Thus, when butanal was the S+ and butanal/heptanal mixture was an S−, rats in this experiment made very high levels of false alarms to the mixture S− suggesting little if any detection of heptanal. On the other hand, when butanal was the S− and the butanal/heptanal mixture also an S−, the mixture was responded to appropriately with few errors, again suggesting dominance of butanal over heptanal.

The results of Experiment 1 also indicate complete suppression of heptanal by butanal when they are present in a mixture at concentrations of $10^{-6.5}$ and 10^{-4} vol%, respectively, whereas complete suppression in the opposite direction only occurred with heptanal at 10^{-6} vol% and butanal at $10^{-10.5}$ vol%. Taken together, these results support the "asymmetric suppression" hypothesis discussed in the Introduction and

importantly define the conditions required for suppression of each odor by the other. Such information might be used in future physiological studies to determine whether the suppression originates at the receptor level in the olfactory epithelium or centrally, for example, at the glomerular level in the olfactory bulb (Linster and Cleland 2004).

The dominance of butanal over heptanal supports the view that butanal may act as an antagonist at the receptors for heptanal. The smaller molecular size of butanal compared with heptanal favors an easier access to the receptor site(s) for heptanal than vice versa. The findings for the molecular structure–receptor activity interaction in the perception of aliphatic odorants suggest that there is a significant negative correlation between discrimination performance and structural similarity of aliphatic odorants, with carbon chain length as one factor, which determines an odor–receptor interaction (Laska et al. 1999; Linster and Hasselmo 1999). Because the present study used aldehydes with different carbon chain length, that is, butanal and heptanal, the results provide good evidence that this molecular feature plays a significant role in determining interaction with an olfactory receptor. This proposal is also supported by studies of the specificity of receptor cells for the aliphatic aldehydes (Araneda et al. 2000, 2004), which indicate that differences of several carbon atoms in the aliphatic chains of 2 aldehydes markedly change their ability to activate common receptor cells.

The asymmetric suppression between the aldehydes is similar to that observed with humans (Laing et al. 2002) and provides further evidence for similarities in the functioning of the olfactory system across different mammals (Eayrs and Moulton 1960; Moulton and Eayrs 1960). Thus, not only do humans and rats show similar changes in sensitivity to aliphatic odorants as the aliphatic chain varies but also they show similarities in their perception of the components of mixtures. For example, in a human psychophysical study, an asymmetric suppression was found to occur between propanal and heptanal similar to that reported here (Laing et al. 2002). In addition, Bell et al. (1987) used the 2 deoxyglucose imaging technique to provide physiological evidence at the level of the olfactory bulb of asymmetric suppression of propionic acid by limonene in the rat, an effect that was first observed in psychophysical studies with humans.

In conclusion, the present study has demonstrated that it is possible to investigate the perception of odor mixtures in the rat by using a paradigm allowing determination of the magnitude of the suppression of one component by the other. The procedure allows determination of the concentrations at which suppression occurs so that physiological studies of mixture phenomena can be conducted and the underlying mechanisms resolved. In addition, the paradigm should allow comparative studies on the perception of odor mixtures by humans and rats, which because of the similar outcomes may provide insight into the mechanisms that underlie human responses to odor mixtures.

Acknowledgements

Research was supported by a Strategic Partnership with Industry–Research and Training grant from the Australian Research Council. We thank Darek Figa and Debbie Brookes for animal care and Raja Vijayenthiran for technical assistance with olfactometers. We are also very grateful to Dan Costa for statistical advice.

References

Ache BW. 1989. Central and peripheral basis for mixture suppression in olfaction: a crustacean model. In: Laing DG, McBride RL, Ache BW, editors. Perception of complex smells and tastes. Sydney (Australia): Academic Press. p. 101–114.

Araneda RC, Kini AD, Firestein S. 2000. The molecular receptive range of an odorant receptor. Nat Neurosci. 3:1248–1255.

Araneda RC, Peterlin Z, Zhang X, Chesler A, Firestein S. 2004. A pharmacological profile of the aldehyde receptor repertoire in rat olfactory epithelium. J Physiol (Lond). 555:743–756.

Bell GA, Laing DG, Panhuber H. 1987. Odour mixture suppression: evidence for a peripheral mechanism in human and rat. Brain Res. 426:8–18.

Deisig N, Giurfa M, Lachnit H, Sandoz JC. 2006. Neural representation of olfactory mixtures in the honeybee antennal lobe. Eur J Neurosci. 24:1161–1174.

Derby CD. 2000. Learning from spiny lobsters about chemosensory coding of mixtures. Physiol Behav. 69:203–209.

Duchamp-Viret P, Duchamp A, Chaput MA. 2003. Single olfactory sensory neurons simultaneously integrate the components of an odour mixture. Eur J Neurosci. 18:2690–2696.

Eayrs JT, Moulton DG. 1960. Studies in olfactory acuity. I. Measurements of olfactory thresholds in the rat. Q J Exp Psychol. 12:90–98.

Joerges J, Küttner A, Galizia CG. 1997. Representations of odours and odours mixtures visualized in the honeybee brain. Nature. 387:285–288.

Kay LM, Crk T, Thorngate J. 2005. A redefinition of odor mixture quality. Behav Neurosci. 119:726–733.

Kay LM, Krysiak M, Barlas L, Edgerton GB. 2006. Grading odor similarities in a go/no-go task. Physiol Behav. 88:339–346.

Kay LM, Lowry CA, Jacobs HA. 2003. Receptor contributions to configural and elemental odor mixture perception. Behav Neurosci. 117:1108–1114.

Laing DG, Francis GW. 1989. The capacity of humans to identify odors in mixtures. Physiol Behav. 46:809–814.

Laing DG, Jinks AL, Segovia C, Hutchinson I. 2002. Systematic changes in the types of odor interactions are related to the molecular structure of the components. 24th Annual Meeting Association for Chemoreception Sciences; 2002 Apr 24–28; Sarasota, FL.

Laing DG, Panhuber H, Slotnick BM. 1989. Odor masking in the rat. Physiol Behav. 45:689–694.

Laing DG, Panhuber H, Willcox ME, Pittman EA. 1984. Quality and intensity of binary odor mixtures. Physiol Behav. 33:309–319.

Laska M, Joshi D, Shepherd GM. 2006. Olfactory sensitivity for aliphatic aldehydes in CD-1 mice. Behav Brain Res. 167:349–354.

Laska M, Trolp S, Teubner P. 1999. Odor structure-activity relationships compared in human and nonhuman primates. Behav Neurosci. 113:998–1007.

Linster C, Cleland TA. 2004. Configurational and elemental odor mixture perception can arise from local inhibition. J Comput Neurosci. 16: 39–47.

Linster C, Hasselmo ME. 1999. Behavioral responses to aliphatic aldehydes can be predicted from known electrophysiological responses of mitral cells in the olfactory bulb. Physiol Behav. 66: 497–502.

Linster C, Smith BH. 1999. Generalization between binary odor mixtures and their components in the rat. Physiol Behav. 66:701–707.

McGregor I. 1996. Using Strawberry Tree Workbench Mac and Workbench PC software for data acquisition and control in the animal learning laboratory. Behav Res Methods Instrum Comput. 28: 38–48.

Moulton DG, Eayrs JT. 1960. Studies in olfactory acuity. II. Relative detectability of n-aliphatic alcohols. Q J Exp Psychol. 12:99–109.

Slotnick BM, Bodyak N. 2002. Odor discrimination and odor quality perception in rats with disruption of connections between the olfactory epithelium and olfactory bulbs. J Neurosci. 22:4205–4216.

Slotnick BM, Schellinck H. 2001. Methods in olfactory research with rodents. In: Simon SA, Nicolelis M. editors. Frontiers and methods in chemosenses. Washington (DC): CRC Press. p. 21–61.

Slotnick BM, Schoonover FW. 1984. Olfactory thresholds in unilaterally bulbectomized rats. Chemical Senses. 9:325–340.

Tabor R, Yaksi E, Weislogel JM, Friedrich RW. 2004. Processing of odor mixtures in the zebrafish olfactory bulb. J Neurosci. 24:6611–6620.

Wilson DA. 2003. Rapid, experience-induced enhancement in odorant discrimination by anterior piriform cortex neurons. J Neurophysiol. 90:65–72.

Wiltrout C, Dogra S, Linster C. 2003. Configurational and nonconfigurational interactions between odorants in binary mixtures. Behav Neurosci. 117:236–245.

Zou Z, Buck LB. 2006. Combinatorial effects of odorant mixes in olfactory cortex. Science. 311:1477–1481.

Accepted December 5, 2006