Research report

Type 4 phosphodiesterase inhibition impairs detection of low odor concentrations in mice

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Abstract

The cAMP-specific phosphodiesterase PDE4A is abundant in the dendrites, soma and axons of olfactory receptor neurons of the mouse, but it is not present in the cilia, where olfactory transduction initiates. Although the function of PDE4A in mammalian olfaction is unknown, patch clamp studies on deciliated olfactory receptor cells in the newt have shown that adrenaline or cAMP analogs can increase the contrast sensitivity to current injection. We used mice to ask whether increasing the levels of cAMP in sensory neurons by inhibiting PDE4A activity with rolipram could lead to changes in the perception of odorants that correspond to the in vitro cellular responses seen in newts. In an automated olfactometer, rolipram treatment (1 mg/kg, i.p.) significantly impaired the detection accuracy of 1-propanol at relatively high dilutions but did not affect detection at lower dilutions. Meanwhile, the ability to discriminate amyl acetate alone from a mixture of amyl acetate + citronellal was not affected by rolipram at any odor dilution. In a different task in which mice were trained to discriminate between cups of scented versus unscented sand, rolipram treatment resulted in poorer discrimination at high and better discrimination at low, odor dilutions. In sum, PDE4 inhibition resulted in a consistent decrement in the ability of mice to detect low concentrations of odorants, but the effects of rolipram on detection of higher concentrations were task-dependent.

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Keywords: Rolipram; Olfactory discrimination; Olfaction; Cyclic AMP; Odor perception

1. Introduction

Olfactory transduction begins when an odorant molecule binds to receptor sites on the cilia of receptor neurons. Odorant-receptor binding activates adenyl cyclase through a second messenger cascade involving the G protein Gαolf, causing an increase in the level of adenosine 3′,5′-cyclic monophosphate (cAMP) [22]. Elevated levels of cAMP cause cyclic nucleotide gated channels to open, resulting in calcium influx and depolarization of the neuron [10,18]. Cyclic nucleotide removal is mediated by phosphodiesterases (PDEs), which consist of a large 11-family group of enzymes found in virtually all tissues [9]. The predominant PDEs that occur in the rodent olfactory epithelium are members of the calcium-dependent PDE1 and cAMP-specific PDE4 families [3,5,28]. Within olfactory receptor neurons, these enzymes are subcellularly segregated: whereas PDE1 is found in the cilia, a PDE4 isoform (PDE4A) occurs in the cell bodies, axons and dendrites but is absent from the cilia [3,4,12].

The specific role of non-ciliary cAMP in general, and PDE4A in particular, in olfactory function is unclear. One possibility is that non-ciliary cAMP may modulate sensory perception. In one study, adrenaline or cAMP analogues applied to isolated, deciliated newt olfactory receptor cells in vitro suppressed spike generation in response to weak stimuli, yet increased spike frequency in response to moderate to strong stimuli [13]. The steepened slope of the intensity-response curve reflected cAMP-induced changes in contrast sensitivity, leading to the conclusion that cAMP localized outside of the olfactory sensory neuron cilia could contribute to certain aspects of olfactory perception [8,13].
We sought to evaluate the potential involvement of PDE4A in odorant contrast by measuring olfactory discrimination in mice treated with rolipram, which is a specific inhibitor of mammalian PDE4s [23]. We asked whether increasing cAMP levels beyond the cilia by inhibition of PDE4A with rolipram could produce a behavioral manifestation of the stimulus–response properties seen by Kawai et al. [13] in olfactory neurons. Specifically, we examined whether rolipram treatment would decrease olfactory sensitivity of mice to increased dilutions of an odorant, yet enhance sensitivity at moderate to low dilutions. To investigate this question, mice were tested in two separate, appetitively motivated behavioral paradigms. In the first, an automated liquid dilution olfactometer was developed to measure the ability of water-deprived mice to: (a) distinguish a pure odorant from an odor mixture and (b) to detect increasing dilutions of a single odorant. In the second, food-deprived mice were tested in a food-motivated, two-choice discrimination task using cups of sand that were unscented or scented with different dilutions of an odorant.

2. Methods

2.1. Subjects

Adult male Swiss Webster mice (between 7 and 10 weeks of age at the beginning of testing) were obtained from Taconic Farms (Germantown, NY) and group-housed on a 12-h light:12-h dark cycle. On the two-choice discrimination task using scented sand, subjects (n = 9) were fed sufficient rodent chow daily to maintain 80–90% pre-testing ad libitum body weight, with feeding always following testing on test days. Water was continuously available. For olfactometer testing, a different set of subjects (n = 7) were given rodent chow ad libitum but were deprived of water during and for 30 h prior to the onset of training. Subjects that did not receive sufficient water from rewards during test trials were given up to 10 min access to water at least 4 h after completing a session. All seven subjects were tested on an odor mixtures task, and five of these mice were retrained and then tested on threshold discrimination of 1-propanol.

2.2. Materials

Light mineral oil, amyl acetate (AA) and citronellal (CIT) were obtained from Fisher Scientific (Atlanta, GA), and rolipram, cremophor and 1-propanol (PROP) were obtained from Sigma (St. Louis, MO). Rolipram was dissolved in 0.9% saline with 10% cremophor to aid solubility and was administered by intraperitoneal injection at 1.0 mg/kg for the olfactometer tasks and at 0.1 and 1.0 mg/kg in the sand-digging task. These doses are in the range typically used to produce various behavioral effects in mice (e.g., [1,11,27]). The 0.9% saline/10% cremophor solution was served as the vehicle control. Tubing, valves and connectors were obtained from Cole-Parmer (Vernon Hills, IL) and Small Parts Inc. (Miami Lakes, FL).

It is well established that rolipram produces hypolocomotor effects in rodents [1,26]. In order to prevent this effect from interfering with performance on trials, testing began 35–40 min after treatment, which provides a sufficient amount of time for mice to resume activity ([1], and unpublished observations).

2.3. Olfactometer

2.3.1. Apparatus and operation

Mice were tested on two separate tasks. The first was a complex odor discrimination task [7] in which subjects were required to distinguish AA from mixtures of AA and CIT. In the second task, threshold sensitivity to PROP was determined. Each task was conducted in a computer-controlled, two-channel fluid dilution olfactometer, which was adapted from Lipton et al. [15] (Fig. 1). A sniffing port, constructed from an odorless material (Delrin), was mounted on the outside wall of a 25 cm × 25 cm × 29 cm Plexiglas behavioral chamber. The 1.5 cm diameter port opening was located 3 cm from the floor, and a 24 V panel light was placed 6 cm above the opening. Insertion of the snout was detected by an infrared photocell unit located at the entrance of the odor port, where a stainless steel tube was also inserted to dispense water. Air was supplied to the odor port through a series of tubes (Ultra Chemical Resistant Tygon or Teflon), connectors, valves and flow meters. Operation of valves that regulated delivery of odors and water was controlled by computer.
In addition to being chemically old measurements: PROP, which is an aliphatic alcohol found in from prior conditioning with AA (an ester) and CIT (an aldehyde), extensive preliminary observations. To minimize carryover effects each odorant selected for testing were chosen based on the results of produced no obvious aversions in the mice we tested. Dilutions of the percentage of the individual odorant that was present in the 5 ml to the subjects is not known; dilutions used during testing refer to CITS were made up as 1% stock solutions in mineral oil and di- placed in the 20-ml capacity glass scintillation odor vials. AA and 2.3.2. Odorants and dilutions Five milliliters of diluted odorants or vehicle controls were placed in the 20-ml capacity glass scintillation odor vials. AA and CIT were made up as 1% stock solutions in mineral oil and dil- uted further as required to achieve the appropriate final dilution; PROP was diluted in distilled water. The actual concentration of the odorant(s) in the vial headspace that was ultimately presented to the subjects is not known; dilutions used during testing refer to the percentage of the individual odorant that was present in the 5 ml volume. Both AA and CIT are distinct, common scents (banana and lemon, respectively) with a history of use in olfactory studies and produced no obvious aversions in the mice we tested. Dilutions of each odorant selected for testing were chosen based on the results of extensive preliminary observations. To minimize carryover effects from prior conditioning with AA (an ester) and CIT (an aldehyde), an odorant from a different chemical class was selected for thresh- old measurements: PROP, which is an aliphatic alcohol found in wines and fermented fruits [24]. In addition to being chemically distinct from AA and CIT, PROP was selected because it was the first in a series of compounds that the mice were to be trained on for subsequent, unrelated experiments (unpublished data).

2.3.3. General procedures: training
Mice were trained to poke their nose into the odor port to sample the air stream and to receive water. If the rewarded stimulus (S+) was detected, subjects were required to maintain a nose poke for 1.5 s, after which a water reward that varied randomly between 5 and 10 μl was dispensed immediately. A new trial then followed with an intertrial interval (ITI) of 5 s. If the non-rewarded stimulus (S−) was detected, subjects were required to remove their snout from the port in less than 1.2 s, after which a 2 s ITI was imposed. Failure to remove the snout in less than 1.2 s on S− trials resulted in an extended ITI of 10 s. The panel lights were turned off during all ITIs and re-illuminated to signal the beginning of a new trial. Poking during the ITI or failing to maintain a nose poke for at least 0.3 s resulted in an aborted trial, which was not counted, and the trial was repeated.

2.3.4. General procedures: testing
On both the odor mixture and threshold discrimination tasks, each animal was tested on five blocks of 20 trials/day on a single S+/S− combination. S+ and S− trials were given in a modified random order such that they occurred in equal numbers but neither type occurred more than three times consecutively. Each test was repeated on successive days: once each with vehicle or rolipram being administered 30 min prior to testing. The order of control and rolipram tests was counterbalanced between subjects.

Performance accuracy was computed for each block of trials as the percent in which the subject responded correctly. Correct performance for a given test was defined as ≥85% accuracy on at least three of the five blocks, and failure was defined as two consecutive blocks in which the total percent correct was <85%. Overall performance accuracy at each dilution was computed for each subject from the total number of trials in all blocks completed.

2.3.5. Discrimination of odor mixtures
Animals were trained to discriminate 0.2% AA in mineral oil from 0.2% AA + 0.2% CIT until ≥90% performance accuracy on the last 100 of 200 trials/day were achieved. Subjects then were tested on successive days following vehicle or rolipram injections using 0.2% AA as the S+ stimulus and mixtures of 0.2% AA + decreasing quantities of CIT as the S− stimulus. Five tests were given with the dilutions of CIT in the S− as follows: Test 1, 0.2%; Test 2, 0.1%; Test 3, 0.02%; Test 4, 0.01%; Test 5, 0.005%. Results were analyzed in a two-way repeated measures ANOVA, with drug treatment and CIT dilution as factors (SPSS, Chicago, IL). Missing values for two animals not tested at the lowest dilution were estimated by the SPSS linear trend transformation. In this experiment and for all other ANOVAs carried out in this study, the Huynh–Feldt sphericity cor- rection (epsilon, or ε) was used to adjust degrees of freedom where necessary, and performance scores tallied as percent correct were subjected to arcsin or square root transformations before statistical analysis [25]. Control tests were given to four of the seven subjects after com- pletion of the mixtures tests. Control tests consisted of 40 trials with mineral oil in both odor vials, with one vial arbitrarily designated as the S+ stimulus. Subjects performed at chance on these tests.

2.3.6. Threshold detection of PROP
After completing the odor mixture tests, threshold detection for a different, single odorant (PROP) was assessed in mice treated with rolipram or vehicle. Training followed procedures described above with PROP diluted to 1% in distilled water serving as the S+ and distilled water alone as the S−. When ≥90% correct response was achieved after 200 daily trials of PROP versus clean air discrimi- nation, 2 additional days of 100 trial sessions were given to famil- iarize mice to descending dilutions of the odorant. Data collection then began in which mice were given up to five 20-trial blocks of a single dilution on sequential days following vehicle and rolipram treatment. The S+ consisted of 1, 0.4, 0.2, 0.1, 0.05, 0.02, 0.01 and 0.002% solutions of PROP in water. Subjects were tested at every dilution, and performance accuracies were compared in a 2 × 8 re- peated measures ANOVA with drug treatment and odorant dilution as factors. To determine whether rolipram administration resulted in non-specific changes in behavior associated with hypolocomo- tion, nose-poke durations (based on n = 4 subjects due to data being unavailable for one animal) and test session length were analyzed across dilutions using paired t-tests.

2.4. Two-choice discrimination task
Procedures were adapted from [16]. Mice were presented with cups filled with unscented and scented sand and were required to
choose the scented sand cup for the reward—an approximately 10–20 mg piece of Hershey's semi-sweet chocolate, which appeared to be a more effective stimulus than others we sampled (e.g., cereal), allowing for more trials to be conducted per test session. Testing was conducted in a (29 cm × 19 cm × 13 cm) Plexiglas cage. Screw-top plastic bottle caps (3 cm × 1.3 cm) from beverage containers were filled with approximately 11 g of playground sand (Toys "R" Us, Paramus, NJ) and lowered into the cage on a Plexiglas platform. A Plexiglas divider separated the two cups, which were secured to the platform with mounting putty. The stimuli were prepared by mixing 500 g of sand in mineral oil only (S−) or with AA diluted in 0.2 ml of mineral oil (S+). The range of dilutions used during testing refers to the percent of AA in the 200 µl oil mixture. Sand and mineral oil were shaken for 90 s prior to use to distribute the mixture uniformly. Batches of odor/sand/sand mixtures were stored in airtight plastic containers for up to 1 week and new batches were made as needed. Between trials, a Plexiglas barrier was placed in the cage to prevent the mouse from approaching the platform before the designated start of the trial.

2.4.1. Training
Mice received step-wise training each day that began with exposure to chocolate buried in cups of scented sand. Cups with scented or unscented sand were then presented together on the divided Plexiglas platform and mice were rewarded for approaching and digging in the cup with scented sand. After mice learned to approach and dig in the scented cup, chocolate rewards began to be delivered by forceps lowered into the cage rather than burying the reward in the sand. This alteration in procedure was implemented to minimize physical contact of subjects with the odor source, to prevent chocolate buried in the sand from being detected directly and to prevent sand from being strewn about the cage. A choice was then defined as the initiation of digging in a cup. Mice that chose the scented cup correctly on 9/10 trials given on 2 consecutive days were considered trained. Four mice that did not reach this criterion after 5 consecutive days of training were excluded, leaving nine animals for testing.

2.4.2. Testing
Mice were tested once per day. Before each test, animals received rolipram (0.1 or 1.0 mg/kg) or vehicle. Testing began 30 min later with a 5 min habituation period after which mice were given 15 trials separated by a 20 s ITI. Trials commenced when the platform containing the sand cups was lowered into the cage and the barrier was lifted. The latency to approach a cup was measured, and if mice chose the S+ cup a reward was given, the platform was removed, and a correct response was recorded. If mice chose the S− cup or if no choice occurred within 2 min, the platform was removed and an incorrect response was recorded.

Five dilutions of AA (0.1, 0.001, 0.0002, 0.0001% and 0.00001%) were selected based on results of preliminary testing. All mice were tested at each combination of the five dilutions and three drug concentrations for a total of 15 tests. The order of testing was pseudo-randomized for each mouse using a random number table. The order was adjusted if necessary to minimize repeated testing of subjects at dilutions near the threshold of detection (no more than 2 consecutive days of 0.00001% or 3 consecutive days of 0.0001% were allowed) and to avoid development of decreased sensitivity to rolipram from repeated exposure (no consecutive days of the high dose).

The percent of correct responses in 15 trials was determined for each mouse for each test. Means of subjects' scores were then computed for each odorant dilution by rolipram dose combination and compared in a two-way repeated measures ANOVA. Because a dose × dilution interaction is predicted by the hypothesis that drug treatment enhances odorant detection at low to moderate dilutions yet decreases detection at higher dilutions, single degree of freedom contrasts for this interaction were conducted that examined performance accuracy at the three lowest versus the two highest dilutions. These comparisons were computed for the control versus the highest dose of rolipram and for the control versus the high and low rolipram doses.

To provide a measure of the hypolocomotor effects of rolipram, as well as to reveal whether tolerance or sensitization developed from repeated rolipram administration, latency to approach the cups was measured on each trial. Latency was defined as the time elapsed after the barrier was lifted for subjects' paws to touch the platform, and scoring for each test was the percent of trials (out of 15) in which latency was ≥ 1 s. For this analysis, changes in approach speed across the increasing number of rolipram injections was of greatest interest, so latency scores were compared in a two-way repeated measures ANOVA, with rolipram dose (0, 0.1 and 1.0 mg/kg) and injection order (1–5) as factors. A priori contrasts for dose were computed to allow individual comparisons of control versus low, and control versus high rolipram dose.

3. Results

3.1. Olfactometer
3.1.1. Odor mixtures
Mice treated with rolipram or vehicle readily distinguished AA-only from AA mixed with any of the three lowest dilutions of CIT (Fig. 2). However, performance began to decline under both rolipram and vehicle treatment once the dilution of CIT in the S− was brought to 0.01%, as indicated by two animals that failed to pass criterion on both the rolipram and vehicle tests. At the highest CIT dilution tested, all animals failed on both treatment conditions. Repeated measures ANOVA corroborated these observations, revealing a significant effect of CIT dilution on performance (F(1,24) = 20.6, ε = 0.47, p = 0.001). However, there was no effect of rolipram on ability to discriminate odor mixtures (F(1,6) = 0.49, p > 0.5) nor was there an interaction between drug treatment and dilution. Therefore, there was neither an enhancement nor a decrement in odor discrimination due to rolipram.

3.1.2. Detection of PROP
Rolfipram- and vehicle-treated mice easily discriminated the weakest dilution of PROP (1%) from clean air, but beginning at the 0.4% dilution and through the lowest dilution tested (0.002%), the mean % of correct trials achieved following rolipram was lower than corresponding performance after vehicle administration (Fig. 3). Overall, there was a significant decrement in performance due to rolipram (F(1,28) = 7.75, p < 0.05), as well as with increasing odorant dilution (F(3,28) = 24.1, ε = 0.87, p < 0.001); however, there was no interaction between the two main effects (F(2,28) = 0.58, ε = 0.81, p > 0.7). The reduced ability to detect the odorant
In this and subsequent graphs, the effect of dilution, performance as CIT dilutions increased (repeated measures ANOVA, main effect of dilution). Whereas there was a significant decline in performance with increasing dilutions, mean performance accuracy of subjects after receiving vehicle was significantly greater than performance following rolipram (93 ± 0.88% versus 51 ± 5.6% correct, t2 = 6.94, p < 0.025). In contrast, when scores from the three lowest dilutions were compared, mean performances following receipt of vehicle or rolipram injections did not differ significantly (95 ± 0.68% correct on vehicle versus 88 ± 2.6, t4 = 2.40, p > 0.05). Thus, the effect of rolipram was most pronounced on the detection of the highest PROP dilutions.

3.2. Two-choice discrimination task

As expected, animals exhibited a better ability to discriminate scented from unscented sand at the weaker odorant dilutions (F3,12 = 13.7, p < 0.0001) (Fig. 4), but neither the main effect for rolipram dose nor the overall rolipram dose × odorant dilution interaction was significant (F2,16 = 0.77, p > 0.45; F1,16 = 1.365, p > 0.2). However, single degree of freedom a priori contrasts for the interaction that compared performance at the moderate to low dilutions of odorant (0.0002, 0.001, 0.01%) against the two highest dilutions (0.0001 and 0.00001%) were statistically significant for control versus the high-dose rolipram treatment (F1,8 = 7.15, p < 0.03), and nearly so for control versus high- and low-dose rolipram combined (F1,8 = 4.98, p = 0.056). Both contrasts indicate that rolipram reduced responsiveness to higher odorant dilutions, but facilitated responsiveness to lower dilutions. We also noted that rolipram significantly delayed the time to approach cups at the onset of a trial (Fig. 5; main effect of dose, F2,16 = 18.45, ε = 0.54, p < 0.02). A priori contrasts showed that when mice received the high rolipram dose they...
Fig. 4. Mean ± S.E.M. performance accuracies (% correct) are shown in tests for which chocolate rewards were given to mice for approaching cups containing sand scented with different dilutions of amyl acetate (AA). Mice (n = 9) were given 15 trials at each of five dilutions and three rolipram doses (0 (vehicle control), 0.1 and 1.0 mg/kg), and the percentage of trials in which the scented cup was chosen over an unscented cup was computed for each odontant dilution/drug dose combination. Mean performance accuracy declined with increasing dilution (repeated measures ANOVA, main effect of dilution, p < 0.05). Specific contrasts of the dilution × rolipram dose interaction that compared performance at high vs. low odorant dilutions indicated that rolipram enhanced detection at lower, but reduced detection at higher dilutions (p < 0.05).

3.3. Non-specific effects of rolipram on performance

Because systemic rolipram administration can produce motor disturbances, such as head twitches, hypothermia and hypolocomotion [26], several measures that could be affected by motor deficits were compared after rolipram versus control treatment. For example, latency to approach the sand cups in the two-choice discrimination task was significantly increased in animals receiving the high dose of rolipram. Nevertheless, this effect appeared not to impair overall the ability to identify the cup with scented sand because, although subjects performed more poorly at high odorant dilutions, they performed as well or better at low dilutions.

To examine whether the detrimental effect of rolipram on detection of PROP in the olfactometer could be related to hypolocomotor effects of rolipram, nose-poke durations were measured in S− trials, in which subjects were required to remove their nose from the port in <1.2 s. Although the mean ± S.E.M. time taken to withdraw the nose was slightly greater for rolipram- than vehicle-treated subjects (1.36 ± 0.027 versus 1.19 ± 0.067), this difference was not statistically significant.

Table 1

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>F-value</th>
<th>Level of significance (p-value)</th>
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</thead>
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<td>0.45</td>
</tr>
<tr>
<td>Odontant dilution</td>
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<td>13.7</td>
<td>&lt;0.0001</td>
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<td>1.37</td>
<td>0.23</td>
</tr>
<tr>
<td>Error (dose × dilution)</td>
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</table>

A priori contrasts for dose × dilution:

1. High rolipram vs. control (3 lowest vs. 2 highest dilutions) | 1 | 7.15 | 0.028 |
2. High + low rolipram vs. control (3 lowest vs. 2 highest dilutions) | 1 | 4.98 | 0.056 |

* p ≤ 0.01 or 1.0 mg/kg.
* p ≤ 0.01, 0.001, 0.002, 0.0001, or 0.00001% in mineral oil.
* These values are for F1,8.
Electrophysiological observations on cAMP-induced spiking in newt olfactory sensory neurons led us to examine whether treatment of mice with the PDE4 inhibitor rolipram could increase the contrast between high and low odor concentrations. Kawai et al. [13] found that adrenaline could reversibly suppress responses of deciliated cells in vitro under weakly stimulated conditions, and strengthen responses to moderate and higher levels of input. These responses were mimicked by application of the adenylyl cyclase activator forskolin, or the cAMP analog 8-bromo-cAMP, suggesting that adrenaline acts via the cAMP second messenger system to modulate signaling during olfactory transduction. We asked whether cAMP produced by inhibition of PDE4s could increase the contrast between high and low odor concentrations. Overall, our findings provide partial support for the idea that increasing non-ciliary cAMP in olfactory receptor neurons may be a cellular mechanism by which the contrast between odors is regulated in olfactory perception.

Our results are compatible with a recent report in mice in which intact noradrenergic inputs from the superior cervical ganglia to the olfactory epithelium were shown to decrease the sensitivity of olfactory neurons to higher dilutions of some urinary odors, yet enhance the ability to detect other mildly diluted urinary odors [21]. In the present study, we suggest that accumulation of cAMP due to inhibition of PDE4A by rolipram may, in certain tasks, similarly increase the contrast between odors. Variation in PDE4 activity, therefore, could be a means by which the olfactory system modulates signals during transduction. Currently, however, we do not know if the level of PDE4 protein in the olfactory epithelium varies within individuals over time, or if PDE4 levels and/or activity can be regulated, for example, by changes in behavioral state, hormones, or environmental conditions.

Despite effects of rolipram in the tests of threshold sensitivity using single odorants, rolipram had no effect on discrimination in the odor mixtures task. In this task, mice were asked to detect the presence of one odorant (CIT) against a background of another (AA). In no case were the odorants in this task used at levels anywhere near threshold. In fact, the highest dilution of CIT (0.01%)—which when mixed with AA could not be discriminated from AA alone—was readily detected by mice and even the human investigators in this study when presented by itself (unpublished data). This suggests that the inability of mice to distinguish 0.01% CIT + AA from AA alone was not because CIT was presented at levels below the threshold of olfactory sensory neuron activation. One possibility is that inability to make this discrimination could reflect a failure in central perceptual mechanisms that must distinguish between the population of sensory neurons corresponding with the observation that fewer spikes were generated by low levels of current injection in the presence of cAMP activators, mice receiving rolipram would perform more poorly at low odor concentrations than they would after getting vehicle. In addition, in correspondence with the relative enhancement of spike frequency by cAMP at moderate to high levels of stimulation, rolipram-treated animals would perform better than controls when testing with higher odor concentrations.

We found that in mice trained to select a cup of scented sand for a food reward, a high dose of rolipram significantly decreased the detection of a highly diluted odorant relative to controls while improving detection of less diluted odorant. And, in tests conducted in an olfactometer, rolipram similarly decreased the ability to detect highly diluted PROP, but it had no significant effect at lower dilutions. The differential effects of rolipram across behavioral paradigms indicates that the perceptual consequences of elevating cAMP through PDE4 inhibition may be task-dependent. Similarly for rats, whether chemically different odors are perceived similarly depends strongly on the type of behavioral test being used [6].

4. Discussion

Electrophysiological observations on cAMP-induced spiking in newt olfactory sensory neurons led us to examine whether treatment of mice with the PDE4 inhibitor rolipram could increase the contrast between high and low odor concentrations. Kawai et al. [13] found that adrenaline could reversibly suppress responses of deciliated cells in vitro under weakly stimulated conditions, and strengthen responses to moderate and higher levels of input. These responses were mimicked by application of the adenylyl cyclase activator forskolin, or the cAMP analog 8-bromo-cAMP, suggesting that adrenaline acts via the cAMP second messenger system to modulate signaling during olfactory transduction.
activated by the mixture versus the single odorant. On the other hand, it has been demonstrated that one odorant in a binary mixture can suppress the response to the other odorant at the level of the sensory neurons themselves[2,20], so, it may be that AA-antagonized interactions of CIT with olfactory receptors such that 0.01% CIT produced much less sensory activation mixed with AA than when presented alone. Whether rolipram might alter responsiveness to mixtures of other odorants remains to be determined.

The specific mechanism by which rolipram altered performance of mice in our study is not known. It is possible that decrements in performance could have been due to non-specific consequences of rolipram administration on behavior. In tasks conducted in the olfactometer, rolipram significantly increased the time required for mice to complete all five blocks of trials, a measure that may reflect compromised motor function. Nevertheless, rolipram treatment had no effect on olfactory discrimination in the odor mixtures task, so it seems less likely that side-effects of rolipram administration contributed to the poor performance of mice in the detection of high dilutions of PROP. Other possibilities are that odor detection was impaired by rolipram-induced hypothermia[26], or that reported effects of rolipram on synaptic plasticity, learning and long-term memory e.g.,[1,11,19] could have influenced, for example, conditioned aspects of task performance in unpredictable ways. However, our results indicate that performance was impaired by rolipram at higher dilutions in the PROP discrimination task, not affected at all in the odor mixtures task, and enhanced at higher odorant concentrations in the two-choice discrimination task. It seems unlikely that non-specific effects of rolipram could have produced this pattern of results.

If one accepts the alternative explanation that effects of rolipram in this study were specific to olfactory processing, the question of whether rolipram acted centrally or peripherally remains. Although the predicted enhancement in odorant contrast may have been due to a rolipram-dependent increase in cAMP in olfactory sensory neurons, PDE4s are also widely expressed throughout the brain, both in the olfactory system structures like the olfactory bulb, anterior olfactory nuclei and piriform cortex, as well as in many other brain regions[4]. It is, therefore, possible that the antagonistic effect of rolipram on PROP detection observed in this study was due to hindrance of the processing and perception of odors by systemic rolipram acting on PDE4s that occur in higher olfactory centers. As rolipram did not affect performance in the odor mixtures task, this explanation would require that disruption of perception by rolipram was selective for threshold detection of a single odorant, but not the ability to make a more complex discrimination between odor mixtures. It is also unclear how, in the two-choice discrimination task, rolipram acting centrally could produce enhanced performance at high, yet poor performance at low concentrations of odorant.

Although other explanations are possible, our results are consistent with the conclusion that rolipram affected olfactory function by a direct action on the olfactory receptor neurons in which PDE4A is abundant. In Kawai et al.[13], it was concluded that increased somal cAMP in newt olfactory neurons caused: (1) inactivation of T-type calcium currents, which are responsible for setting the threshold for firing, and (2) enhancement of sodium current, which represents the primary component of receptor-neuron spiking. Both effects were dependent on the activation of protein kinase A (PKA) by cAMP. More recently, using slices from olfactory bulbs of both rats and mice, cyclic nucleotides were found to modulate neurotransmission by depressing olfactory nerve excitability at high concentrations, while increasing spontaneous transmission at lower concentrations[17]. These effects were due to activation of cyclic nucleotide gated channels, but were PKA-independent. Although we do not know which, if either, mechanism is affected by rolipram, it now seems clear that alterations in the levels of non-ciliary cAMP can have distinct effects on signaling in olfactory receptor neurons, as well as on olfactory perception.

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