

Rats can make relative perceptual judgments about sequential stimuli

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Abstract In their natural environment, animals often make decisions based on abstract relationships among multiple stimulus representations. Humans and other primates can determine not only whether a sensory stimulus differs from a remembered sensory representation, but also how they differ along a particular dimension. However, much remains unknown about how such relative comparisons are made, and which species share this capacity, in part because most studies of sensory-guided decision making have utilized instrumental tasks in which choices are based on very simple stimulus–response associations. Here, we used a two-stimulus-interval discrimination task to test whether rats could determine how two sequentially presented stimuli were related along the dimension of odor quality (i.e., what the stimulus smells like). At a central port, rats sampled and compared two odor mixtures that consisted of spearmint and caraway in different ratios, separated by a 2–4-s interval, and then entered the left or right reward port. Water was delivered at the left if the first mixture consisted of more spearmint than the second did, and at the right otherwise. We found that the difference in mixture ratio predicted choice accuracy. Control experiments suggest that rats were indeed basing their choices on a comparison of odor quality across mixtures and were not using associative strategies. This study is the first demonstration of the use of a sequential “more than versus less than” rule in rats and provides a well-controlled paradigm

for studying abstract comparisons in a rodent model system.

Keywords Abstract comparison · Two-stimulus-interval discrimination · Rat cognition · Decision making · More versus less

Introduction

The ability to learn rules that require comparing current perceptual input to remembered representations is a hallmark of higher-order cognition. Humans regularly perform such comparisons while making decisions, determining whether and how separately encountered stimuli differ from one another. Over the past several decades, many studies have shown that non-human animals can learn to determine whether or not sequentially presented stimuli are identical (e.g., Giurfa et al. 2001; Herman and Gordon 1974; Katz et al. 2002; Lu et al. 1993; Peña et al. 2006; Weinstein 1941). However, much less is known about the capacity to relate stimulus representations in more complex and useful ways than reporting whether they are the same or different.

In particular, which species are able to determine *how* a given stimulus relates to a representation of another stimulus, along some dimension, is an open question. Such judgments preserve the sign of the difference between the stimuli, while identity judgments do not and therefore convey additional relevant information about the relationship between the stimuli. Humans perform “more than versus less than” comparisons on a regular basis, such as when evaluating a series of goods with respect to some feature (e.g., nutritional value) in order to decide what to purchase. In a series of studies in non-human primates, in

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which subjects were asked to report which of the two sequentially presented vibrotactile stimuli had a higher frequency, primates were shown to be capable of such delayed comparison judgments as well (Mountcastle et al. 1990; Romo and Salinas 2003).

Animals' survival in their natural environment presumably depends on their ability to make decisions based on comparisons among multiple stimulus representations. We therefore hypothesized that the ability to perform such relative perceptual judgments would be widespread among species, including rodents. Indeed, numerous studies have demonstrated that rats are capable of learning to use comparably abstract concepts, such as numerosity (Davis and Perusse 1988), causal inference (Blaisdell et al. 2006), relational sequences (Murphy et al. 2008), generalized reciprocity on operant tasks (Rutten and Taborsky 2007), and joystick-mediated stimulus control (Washburn et al. 2004).

Rats rely largely on odor cues to guide their decisions and behavior (Doty 1986; Slotnick 2001), and the extent of olfactory sensitivity in rats has long been a topic of empirical investigation (Munn 1950). Indeed, rats were first shown to be able to discriminate between two odors associated with the location of a food reward, in a controlled experimental setting, nearly 80 years ago (Swann 1933; Brown and Ghiselli 1938). Subsequent advances in stimulus control and experimental design allowed for fine odor discrimination thresholds to be measured (Pfaffmann et al. 1958; Eayrs and Moulton 1960; Slotnick and Nigrosh 1974); it has recently been shown that rats can discriminate highly similar odors, such as enantiomers (pairs of molecules with “mirror-image,” but otherwise identical, chemical structure; Rubin and Katz 2001; Linster et al. 2001) and even binary mixtures containing similar proportions of enantiomers (Uchida and Mainen 2003). In addition, these advances allowed olfactory tasks to be used to test cognitive functions in rats (Slotnick 2001). For example, rats learn to discriminate odor pairs more rapidly after being trained to discriminate other odor pairs, demonstrating the formation of a “learning set” (Jennings and Keefer 1969; Slotnick and Katz 1974) and can apply the transitive property across pairs of odors to determine which odor in a novel pairing is associated with reward (Dusek and Eichenbaum 1997).

We therefore tested our hypothesis, in rats, by adapting the two-stimulus-interval discrimination task used in primates (Mountcastle et al. 1990; Romo and Salinas 2003) to the olfactory domain (“Methods”). As described below, we found that rats were capable of learning and applying a “more than versus less than” rule to sequentially presented stimuli, within an ethologically relevant modality.

Methods

Subjects and apparatus

Animal use procedures were approved by the University of Colorado Institutional Animal Care and Use Committee and carried out in accordance with National Institutes of Health standards. Eight male Long-Evans rats (*Rattus norvegicus*; Amarant, Blackberry, Copernicus, Darwin, Eeyore, Frith, Gali, and Hume) aged 1–2 months at training initiation, and with no prior training, performed 1 session per day. In the morning, the rats would receive water during behavioral performance (for correct choices). After training, they would receive water ad libitum in a foraging area with mild enrichment for >1 h and were then housed in their home cages with mild enrichment overnight. Rats were water-restricted from evening until morning. At the termination of the study, rats were adopted out of the animal facility.

The behavioral apparatus consisted of a 30-cm platform with three ports along one side, raised 6 cm above the platform (Fig. 1b, Island Motion, Tappan, NY, USA). The odor port was located in the middle of one wall, and the two water ports were located 57 mm left and right of the odor port (center-to-center). An infrared photodiode/phototransistor pair placed on either side of the port was used to determine the timing of port entry and exit, and a visible light-emitting diode at the top of each port was used to cue the rat.

Odors were mixed at desired ratios (e.g., spearmint/caraway = 65:35) and diluted 1:5 in mineral oil. Twenty μ l of the mixture was deposited on a syringe filter. One hundred ml/min of air was directed through the filter by an olfactometer (Island Motion, Tappan, NY, USA), and this odorized air was diluted 1:8 in room air before being delivered to the odor port at a total flow rate of 800 ml/min. The olfactometer, water valves, and light cues were controlled by custom Matlab software.

Initial training

Naive rats were trained to perform the task using the following criteria and sequence of steps:

- (i) Entry into either of the two reward ports (left or right) was rewarded with 15 μ l water delivery to that port (1 day of training; 1 session for all rats).
- (ii) Entry into the central odor sampling port, triggering the delivery of an odor (decanal), was required before water was available at either reward port. A light above the center port cued permission to enter the port; early entries did not trigger odor release and were penalized with a 1-s delay before the light

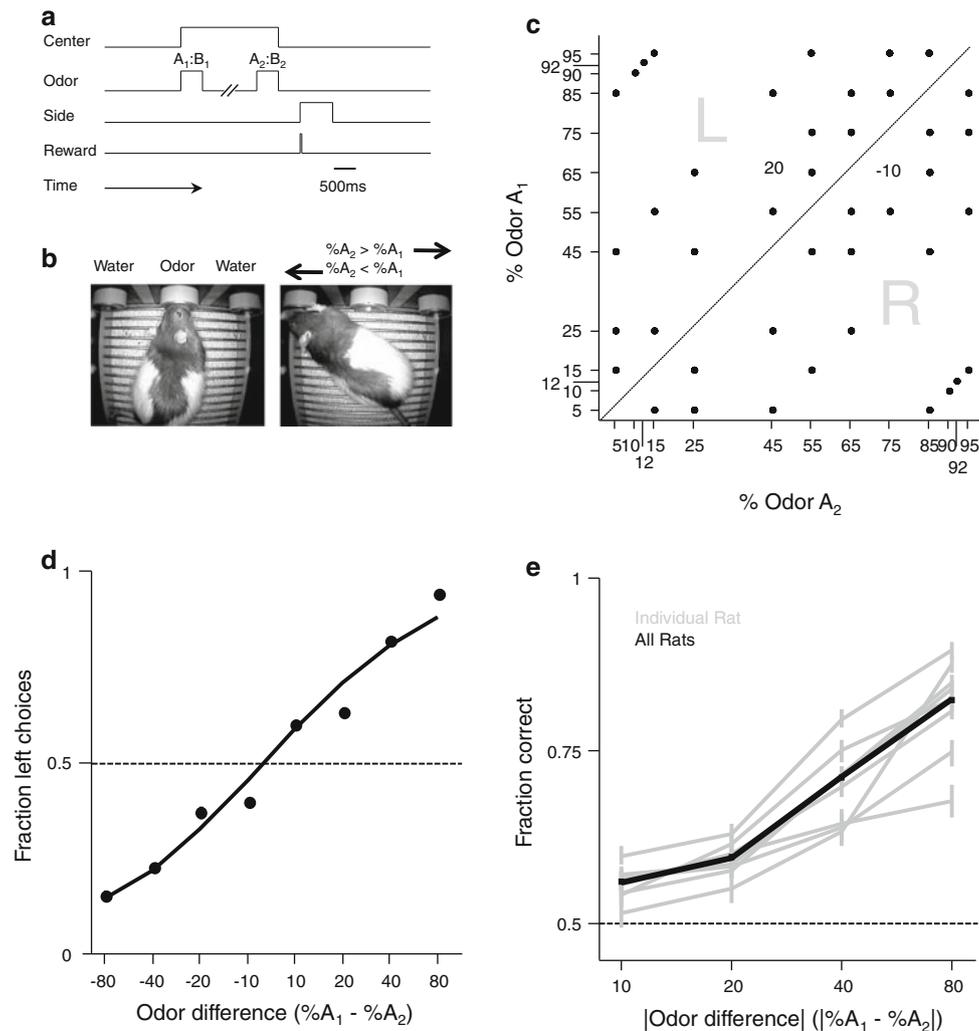


Fig. 1 Two-stimulus-interval olfactory discrimination task and behavioral performance. **a** Timing of task events. **b** The task environment, showing a rat in the odor port (*left image*) and the left reward port (*right image*). **c** Odor mixture ensemble. On each trial, the rat was presented with $A_1:B_1$ followed by $A_2:B_2$ (note that $\%A_n + \%B_n = 100$ in all trials). For example, a trial in which $\%A_1 = 65$ and $\%A_2 = 45$ results in an odor difference ($\%A_1 - \%A_2$) of 20 (shown). *Diagonal dashed line* separates odor pairs rewarded at left and right. Only odor differences of ± 10 , ± 20 , ± 40 , and ± 80

were used. Reward was available at the *left* water port when $\%A_1 > \%A_2$ and at the *right* otherwise. **d** Choices as a function of odor difference ($\%A_1 - \%A_2$) for 1 example rat over 30 sessions. *Solid line* shows best-fit logistic function. *Dashed line* expected chance performance. *Error bars* \pm SEM across trials. **e** Performance as a function of absolute odor difference ($|\%A_1 - \%A_2|$). *Gray*, each of 8 individual rats, 15–30 sessions each. *Solid black* average across all rats, 184 sessions. *Error bars* \pm SEM across trials. *Dashed line* expected chance performance

would be turned on again. Light offset cued permission to exit the port; early exits resulted in light flashes from all ports and a 2–4-s delay before the next trial would begin. If the rat successfully waited for light offset before exiting the port, it could enter either reward port and receive 15 μ l of water. The required odor sampling duration was gradually increased from 0.01 to 4 s, on an ad hoc basis, as the rat learned to wait. Twenty-five to 36 sessions were required to reach 4 s of odor sampling duration.

- (iii) Two odor mixtures, consisting of different ratios of the enantiomers L(-)- and D(+)-carvone (odors

A (spearmint) and B (caraway), respectively), were delivered for 500 ms separated by 2–4 s (uniformly distributed). Upon delivery of the second mixture, the center port light turned off and the rat was allowed to move to one of the side ports to harvest the reward. The relative percentages of odors A and B in the two sequentially presented mixtures ($A_1:B_1$ and $A_2:B_2$) determined the location (left or right) of a water reward: Water was available only at the left reward port if $\%A_1 > \%A_2$, and only at the right reward port if $\%A_1 < \%A_2$ (Fig. 1b, c). Equivalently, since $A_n + B_n = 100\%$, rats were rewarded at the

left if $%B_1 < %B_2$; for clarity, we refer hereafter to $A:B$ mixtures only by their “%A” component. The duration of the delay period between $%A_1$ and $%A_2$ was sufficient for $%A_1$ to diffuse below detectable levels (as measured w/a photoionization detector; data not shown), ensuring that the current representation of $%A_2$ must be compared to the remembered representation of $%A_1$.

Rats were initially trained on absolute odor differences ($%A_1 - %A_2$) of 90%. After exhibiting 85% correct performance for two consecutive sessions, the next session consisted of trials of 90% and 50% absolute odor differences (pseudorandomly selected). Training proceeded with these absolute odor differences for the same number of sessions that the rat performed with 90% absolute odor differences. Fifteen and 5% absolute odor differences were introduced in subsequent sessions in the same manner; rats performed between 12 and 26 training sessions in total. Rats were allowed to continue each session until they ceased to perform any trials within 10 min. During training sessions, rats would typically perform between 300 and 400 trials, with the exact number dependent on the rat’s motivation to perform. The specific values of $%A_1$ and $%A_2$ used for training were not used in the actual task (Fig. 1c). The reward schedule was continuous for training and task.

Two-stimulus-interval task

The task was identical to the final training stage (iii), but with odor differences of ± 10 , ± 20 , ± 40 , and ± 80 , generated using the ensemble shown in Fig. 1c. Specific mixture pairs were pseudorandomly selected with uniform probability. Sessions consisted of between 145 and 543 trials (324 ± 70 , mean \pm SD).

Although in principle, the task could have employed only one odor component (A or B), and indeed, it is possible that the rats paid attention to only one of the components, we used the binary $A:B$ mixtures in order to ensure that odor quality, but not total concentration, varied across mixtures. Note that the total odor concentration was identical across mixtures because odors A and B have mirror-image chemical structure, and therefore, the total vapor pressure was identical across mixture ratios. Rats’ decisions could thus rely only on the perception of odor quality along the spearmint–caraway axis and not on odor concentration (i.e., strength).

Slope test

Data were collected and analyzed with Matlab (Mathworks). In order to determine whether performance improved as a function of increasing absolute odor

difference ($|\%A_1 - \%A_2|$), we plotted the relevant raw data as $(x$ and $y)$, where $x = |\%A_1 - \%A_2|$ and $y = \begin{cases} 0 & \text{for incorrect choices} \\ 1 & \text{for correct choices} \end{cases}$. We then calculated the best-fit line (in a mean-squared error sense) through the raw data. We tested whether the slope of this line was significantly positive, indicating improved performance as a function of increasing absolute odor difference, by shuffling the raw data a large number (5,000) of times and recalculating the resulting slopes of the best-fit lines. p values were calculated as the fraction of slopes from the reshuffled data that were larger than the slope from the actual data. Therefore, a significantly positive slope at $p < .01$ indicates a 99% chance that performance improved as a function of increasing absolute odor difference. Although these data are not necessarily linear, testing whether the slope of the best-fit line through the raw data is significantly positive suffices to determine whether performance depends on absolute odor difference (Figs. 1e, 2, 3b, and 4b).

Results

Initial performance on the two-stimulus-interval odor discrimination task

Eight rats were trained to perform the two-stimulus-interval odor discrimination task (Methods). Rats achieved good performance on the task, choosing left more frequently when $%A_1 > %A_2$ and right when $%A_2 > %A_1$ (Fig. 1d).

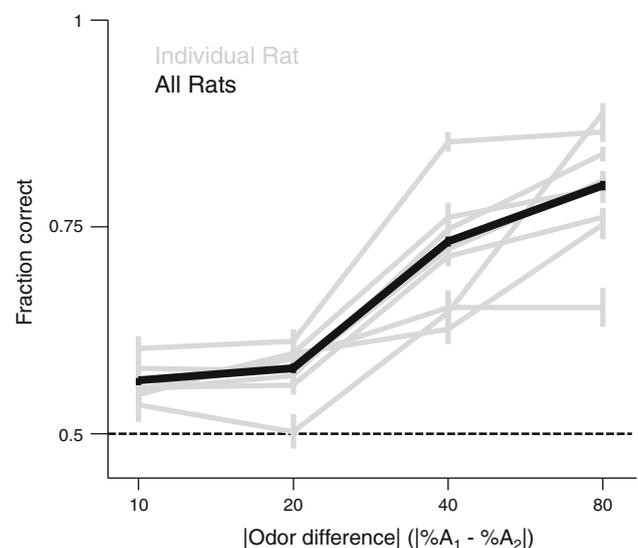


Fig. 2 Behavioral performance after discarding odor mixtures at the “edge” of the ensemble. Performance as a function of absolute odor difference, as in Fig. 1e, after discarding trials with “edge” odors ($%A_1 = 95, 5$; $%A_2 = 95, 5$). Conventions and number of sessions as in Fig. 1e. Dashed line expected chance performance

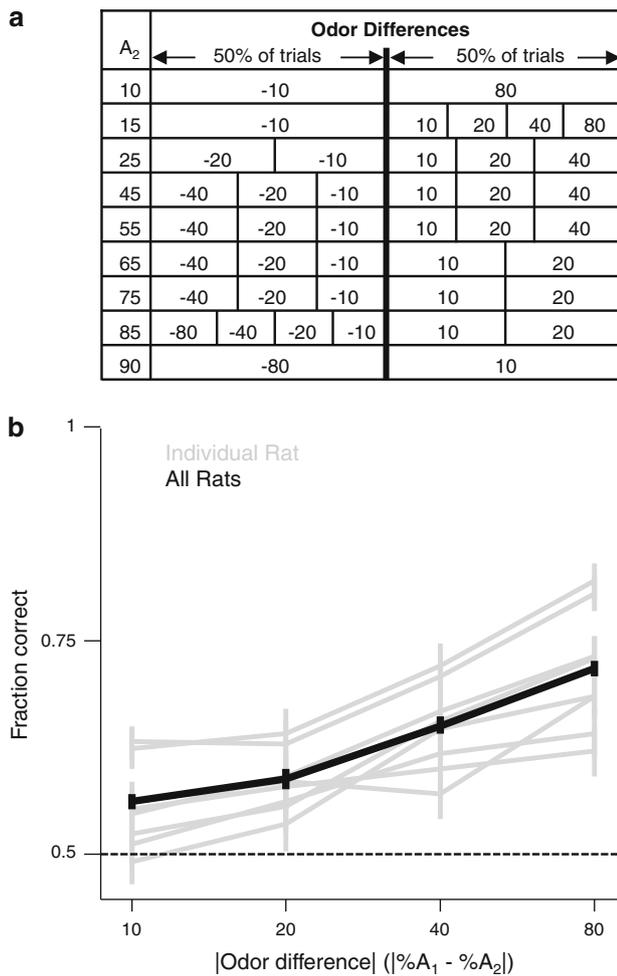


Fig. 3 Additional experiments determining whether choices depend on $\%A_1$. **a** $\%A_2$ -independent ensemble. Odor differences ($\%A_1 - \%A_2$) are shown for each $\%A_2$ used. For each $\%A_2$, odor difference was equally likely to be positive or negative. Proportion of trials with each odor difference is indicated by the width of the corresponding block. **b** Performance as a function of absolute odor differences ($|\%A_1 - \%A_2|$) on $\%A_2$ -independent ensemble (**a**). For any given $\%A_2$, left reward and right reward were equally likely. Basing choices on $\%A_2$ alone would therefore result in a fraction correct of 0.5, independent of odor difference (*dashed black line*). *Gray* each of 8 individual rats, 11–13 sessions. *Black* average across all rats, 62 sessions. *Dashed line* expected chance performance. *Error bars* \pm SEM across trials

Moreover, performance improved with increasing absolute odor differences ($|\%A_1 - \%A_2|$) (Fig. 1e; $p < .01$ for 8/8 rats, slope test [“Methods”]). These results are consistent with the idea that rats were basing their decisions on a relative comparison between the two odor mixtures.

Are rats using only one odor mixture to select their responses?

These data alone, however, do not rule out the possibility that the rats were using an alternative strategy, instead of

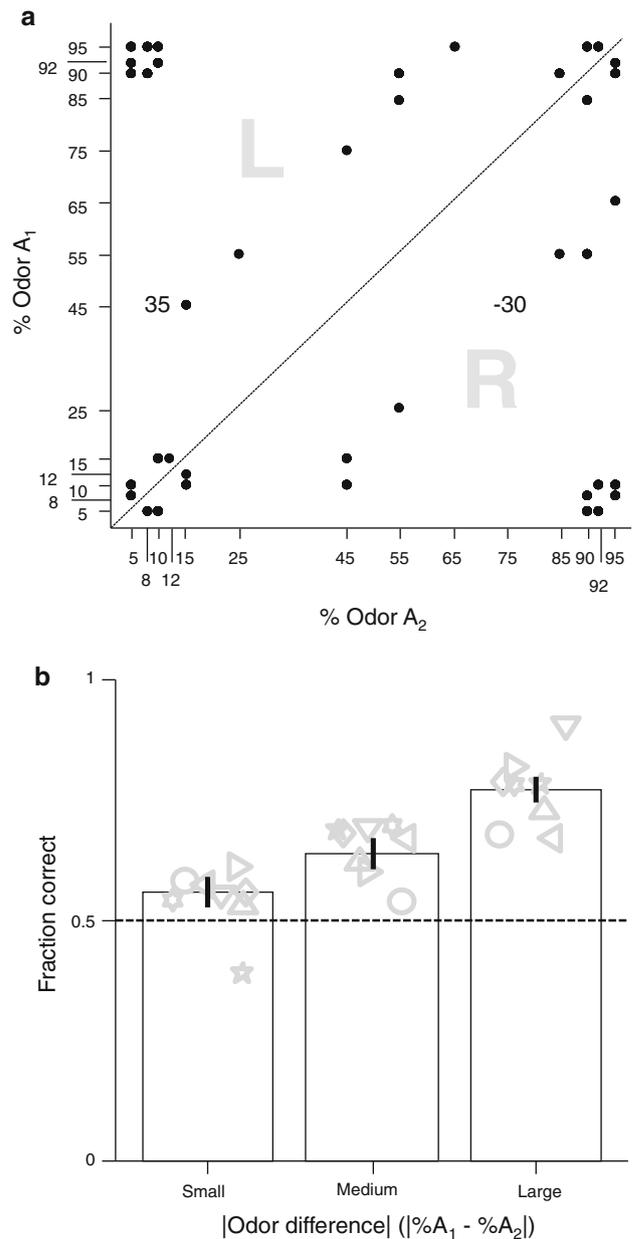


Fig. 4 Probe trials were used to determine whether rats are memorizing the responses associated with mixture pairs. **a** Probe trials ensemble, as in Fig. 1c. Probe trials were randomly interleaved during the initial task (Fig. 1), comprised 5% of the total trials, and were rewarded at the chosen side (to preclude learning). **b** Performance on probe trials. *Small* odor differences $|\%A_1 - \%A_2| < 10$, *medium* $25 < |\%A_1 - \%A_2| < 35$, *large* $|\%A_1 - \%A_2| > 80$. *Gray symbols*, each of 8 individual rats, 15–30 sessions each. *Symbols* are slightly offset for clarity of presentation. *Bars* average across all rats, 184 sessions. *Dashed line* expected chance performance. *Error bars* \pm SEM across trials

comparing the odor mixtures, in order to achieve the observed results. In particular, depending on the structure of the odor ensemble used (Fig. 1c) and the stimulus-reward contingencies, rats may have been able to achieve

good overall performance by basing their choices on only %A₁ or %A₂ and not on the difference between them. For example, since 95% was the largest %A presented (Fig. 1c), the rats could have learned that when %A₁ = 95, the reward would always be at the right side, irrespective of %A₂. In order to address this possibility, we analyzed our data to test whether the rats' choices were indeed dependent on both %A₁ and %A₂.

We first calculated rats' performance after discarding all trials in which %A₁ or %A₂ was an "edge stimulus" (i.e., 5 or 95; Fig. 1c). Performance was still significantly better for larger odor differences, even when the rat must attend to both odors ($p < .01$ for 8/8 rats, slope test; Fig. 2). However, even with these edge trials excluded, it remains possible that good performance can be achieved without attending to both %A₁ and %A₂ (cf. Hernández et al. 1997). For example, when %A₁ = 75, it was more likely that water would be delivered at the right, because %A₂ was likely to be less than %A₁ (Fig. 1c). If choices on trials where %A₁ = 75 were not affected by %A₂, then the rats cannot be said to be comparing the odor mixtures. We therefore examined whether choices depend on both %A₁ and %A₂ by fitting a binary logistic model to the trial-by-trial choice data, using a maximum likelihood procedure (glmfit within Matlab). The regression coefficients for %A₁ and %A₂, β_1 and β_2 , represent the dependence of choices on %A₁ and %A₂, respectively. We found that, for all rats, β_1 was significantly positive ($p < 10^{-7}$) and β_2 was significantly negative ($p < 10^{-7}$), indicating that choices depended on both %A₁ and %A₂ in a manner consistent with the use of the comparison rule. Thus, rats were not basing their choices on only one of the odor mixtures.

However, the results of this analysis suggest that %A₂ may have exerted more influence over choices than did %A₁ ($-\beta_2 > \beta_1$, 8/8 rats). These results leave open the possibility that in some instances, some rats may be relying only on %A₂. In order to completely exclude the possibility that rats could perform well while attending only to %A₂, we designed a new %A₂-independent odor mixture ensemble in which it would not be possible for the rats to achieve good performance by using %A₂ alone. Specifically, we ensured that for any %A₂, there was an equal probability of reward at the left and the right (Fig. 3a). Thus, if rats' relied entirely on %A₂, they would choose the correct reward port on 50% of trials, independent of the difference between %A₁ and %A₂. The rats were tested on the %A₂-independent ensemble without any prior exposure to it; the first session with the new ensemble (Fig. 3a) followed the last session with the original ensemble (Fig. 1c). The task was otherwise identical to that previously described (Fig. 1). Performance on the %A₂-independent ensemble was similar to that described above

(Fig. 1e); rats performed better on those trials with greater difference between mixtures (Fig. 3b; $p < .01$ for 8/8 rats, slope test). Indeed, even in the first session of exposure to the %A₂-independent ensemble, most rats performed better on trials with greater odor difference ($p < .01$ for 6/8 rats, slope test, "Methods"). Since the rats could not have learned, within the first session, to change their strategy based on the structure of the new ensemble, these results indicate that they were not attending only to %A₂ in the previous sessions with the original ensemble (Fig. 1c). Together, our analyses and new experiments suggest that in this task, rats base their choices on both %A₁ and %A₂, specifically on the difference between them.

Are rats memorizing the response associated with each mixture pair?

Although we have established that rats are using both odor mixtures to solve the task, it is possible, in principle, that the rats performed the task by memorizing specific combinations of odor mixtures and their corresponding reward location and not by comparing %A₁ to %A₂. For example, they may have learned that reward would be at the right when %A₁ = 25 and %A₂ = 65, and when %A₁ = 5 and %A₂ = 15, without utilizing the %A₁ versus %A₂ comparison rule at all. This strategy would require memorizing a very large number of contingencies (44 in the original ensemble; Fig. 1c), which rats have not been shown to be capable of, and it is thus not likely that rats utilize this strategy. However, in order to entirely exclude this possibility in our experiments using the original ensemble (Fig. 1), rats were occasionally and unpredictably presented with "probe trials." These trials comprised 5% of the total number of trials, were rewarded at whichever port was selected, and consisted of novel pairs of odor mixtures that were not presented during standard (i.e., non-probe) trials (e.g., %A₁ = 65, %A₂ = 90; Fig. 4a). Thus, if the rats were using a memorization strategy, they could not have associated the reward side with these mixture pairs by trial and error. Despite both choices being equally rewarded, the rats' choices were consistent with the comparison rule: they selected the left reward port when %A₁ > %A₂ and the right reward port otherwise. Furthermore, they performed better on probe trials with larger than smaller odor differences (Fig. 4b; $p < .01$ for 7/8 rats, slope test), similar to their performance on standard trials (Fig. 1e). These results demonstrate that rats are not memorizing the reward side associated with specific mixture pairs, and provide further evidence that they are basing their decisions on a comparison of the second stimulus with the remembered representation of the first stimulus.

Discussion

We have shown that rats are capable of using a relative sensory comparison to guide their behavioral choices. We first demonstrated, using a two-stimulus-interval task, that rats' choices depended upon which of the two binary odor mixtures contained more of each component (Fig. 1), suggesting the use of a $%A_1$ versus $%A_2$ comparison rule. Several alternative hypotheses explaining their behavioral performance were then tested, with additional experiments and analyses, and rejected. Specifically, although on some trials rats may have attended more to one mixture than the other, decisions were not based on only one mixture, and as we discuss below, it is unlikely that the rats formed rote associations between the mixture pairs and reward sides. Thus, our results suggest that rats are able to select their responses based on the relationship between the two stimuli.

Our analysis of responses to probe trials (Fig. 4) demonstrates that the rats are not memorizing the reward locations associated with specific odor mixtures. However, is it possible that the rats could have performed the task by learning to associate a subset of stimuli with the correct response and generalizing that association with other stimuli (McLaren and Mackintosh 2002), without invoking the use of the comparison rule? Clearly, rats cannot have generalized learned associations between single odor mixtures (e.g., $%A_1 = 55$) and reward side (e.g., left): by doing so, they would not have been able to achieve the level of performance observed; furthermore, our binary logistic regression analysis demonstrated that their choices depended on both mixtures. A generalized association strategy therefore requires that the rats formed associations between specific *pairs* of mixtures (e.g., $%A_1 = 45$, $%A_2 = 5$) and reward side, which they then generalized to similar mixture pairs. While this strategy would be viable for restricted sets of mixture pairs (e.g., the association of $%A_1 = 90$, $%A_2 = 10$ with left reward could be successfully generalized to $%A_1 = 85$, $%A_2 = 5$), it is less clearly applicable to the sets of mixture pairs closer to the left/right decision boundary (diagonal line in Fig. 1c). For example, while the mixture pair $%A_1 = 25$, $%A_2 = 15$ is relatively similar to the pair $%A_1 = 15$, $%A_2 = 25$, the rats learned to respond to them, and to other mixture pairs along the decision boundary, differently ($p < 10^{-7}$ for choices on +10% versus -10% odor differences, χ^2 test, 7/8 rats). Thus, in order for the rats to have used a generalized association strategy, they must have learned to associate several specific mixture pairs with reward side such that those associations could be successfully generalized to other similar mixture pairs in order to yield the correct response. While our data cannot categorically rule out the use of such a strategy, the fact that it would require

associations between reward side and a number of strategically placed mixture pairs (within the stimulus space defined in Fig. 1c) suggests that its use is less likely than the use of the decision rule.

These findings build upon those of several previous studies. First, rats have been shown to associate the relative ratios of the components of odor mixtures (i.e., odors *A* and *B*) with the spatial locations of rewards (Uchida and Mainen 2003) even when the decision boundary is not set at $%A = %B$ (Uchida and Mainen 2007). The first mixture in our two-stimulus-interval task can be thought of as setting the decision boundary for the trial; our results thus demonstrate that rats can perform the comparison task even when this boundary shifts across trials. In agreement with some studies in the auditory and visual system, the use of a “roving” decision boundary (i.e., the first mixture) appeared to make the task more difficult than those with a fixed boundary (Bull and Cuddy 1972; Zhang et al. 2008): even on the trials with the largest odor differences, average performance did not exceed about 85% correct. Second, previous studies have shown that rats can use a “same versus different” rule to guide responses to olfactory cues (Lu et al. 1993; Peña et al. 2006), similar to performance on matching-to-sample tasks in other species and sensory domains (e.g., Giurfa et al. 2001; Herman and Gordon 1974; Katz et al. 2002; Weinstein 1941). Our results demonstrate that rats also comprehend the more complex concept of how odors are related along the dimension of odor quality. Finally, the task most structurally similar to ours is the two-stimulus-interval vibrotactile discrimination task, used in primates (Romo et al. 1999; Romo and Salinas 2003). While our study owes much to this behavioral paradigm, it is the first to show that rodents can learn and utilize this type of relative comparison, in any modality.

It is worth noting that research examining counting in animals suggests that some species are capable of determining which of two groups contains more items, a form of “relative numerosness judgment” (Davis and Perusse 1988; Reznikova and Ryabko 2011). While at first glance, this work may appear similar to ours, the comparison required in our study was fundamentally different from such judgments of “greater versus fewer” numbers of stimuli, in several ways: First, the concept of numerosity (i.e., counting) is distinct from that of stimulus magnitude (i.e., $%A$, in our case) (Davis and Perusse 1988). Indeed, studies of numerosity generally control for all non-numerical differences between the alternatives, such as stimulus magnitude (Davis and Perusse 1988; Reznikova and Ryabko 2011). Second, to our knowledge, no studies along these lines have examined delayed comparisons of sequentially presented stimuli.

Along with other previous studies that have suggested that rats are capable of making decisions based on abstract

rules (Blaisdell et al. 2006; Davis 1996; Murphy et al. 2008), our results provide further evidence that the capacity of rats for higher-order cognition is greater than has been traditionally assumed (Slotnick 2001). Moreover, our two-stimulus-interval task can be extended to examine other complex cognitive functions in rodents, the study of which often requires that the formation of simple stimulus–response associations be a non-viable strategy for achieving good performance (Dwyer et al. 2009; Smith et al. 2008). In addition, it is straightforward to record and manipulate neural activity during the behavior required by this task (Felsen and Mainen 2008; Jaramillo and Zador 2011; Erlich et al. 2011). Thus, future studies can build upon ours to examine higher-order decision making, and its neural bases, in rodents.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of the country in which experiments were performed.

References

- Blaisdell AP, Sawa K, Leising KJ, Waldmann MR (2006) Causal reasoning in rats. *Science* 311:1020–1022. doi:10.1126/science.1121872
- Brown CW, Ghiselli EE (1938) Subcortical mechanisms in learning. IV. Olfactory discrimination. *J Comp Psychol* 26:109–120. doi:10.1037/h0062529
- Bull AR, Cuddy LL (1972) Recognition memory for pitch of fixed and roving stimulus tones. *Percept Psychophys* 11:105–109. doi:10.3758/BF03212696
- Davis H (1996) Underestimating the rat's intelligence. *Cogn Brain Res* 3:291–298
- Davis H, Perusse R (1988) Numerical competence in animals: definitional issues, current evidence, and a new research agenda. *Behav Brain Sci* 11:561–579. doi:10.1017/S0140525X00053437
- Doty RL (1986) Odor-guided behavior in mammals. *Experientia* 42:257–271
- Dusek JA, Eichenbaum H (1997) The hippocampus and memory for orderly stimulus relations. *Proc Natl Acad Sci USA* 94:7109–7114
- Dwyer DM, Starns J, Honey RC (2009) “Causal reasoning” in rats: a reappraisal. *J Exp Psychol Anim Behav Process* 35:578–586. doi:10.1037/a0015007
- Eays JT, Moulton DG (1960) Studies in olfactory acuity: I. Measurement of olfactory thresholds in the rat. *Q J Exp Psychol* 12:90–98
- Erlich JC, Bialek M, Brody CD (2011) A cortical substrate for memory-guided orienting in the rat. *Neuron* 72:330–343. doi:10.1016/j.neuron.2011.07.010
- Felsen G, Mainen ZF (2008) Neural substrates of sensory-guided locomotor decisions in the rat superior colliculus. *Neuron* 60:137–148. doi:10.1016/j.neuron.2008.09.019
- Giurfa M, Zhang S, Jenett A, Menzel R, Srinivasan MV (2001) The concepts of ‘sameness’ and ‘difference’ in an insect. *Nature* 410:930–933. doi:10.1038/35073582
- Herman LM, Gordon JA (1974) Auditory delayed matching in the bottlenose dolphin. *J Exp Anal Behav* 21:19–26. doi:10.1901/jeab.1974.21-19
- Hernández A, Salinas E, García R, Romo R (1997) Discrimination in the sense of flutter: new psychophysical measurements in monkeys. *J Neurosci* 17:6391–6400
- Jaramillo S, Zador AM (2011) The auditory cortex mediates the perceptual effects of acoustic temporal expectation. *Nat Neurosci* 14:246–251. doi:10.1038/nn.2688
- Jennings JW, Keefer LH (1969) Olfactory learning set in two varieties of domestic rat. *Psychol Rep* 24:3–15
- Katz JS, Wright AA, Bachevalier J (2002) Mechanisms of same-different abstract-concept learning by rhesus monkeys (*Macaca mulatta*). *J Exp Psychol Anim Behav Process* 28:358–368. doi:10.1037/0097-7403.28.4.358
- Linster C, Johnson BA, Yue E et al (2001) Perceptual correlates of neural representations evoked by odorant enantiomers. *J Neurosci* 21:9837–9843
- Lu XC, Slotnick BM, Silberberg AM (1993) Odor matching and odor memory in the rat. *Physiol Behav* 53:795–804
- McLaren IPL, Mackintosh NJ (2002) Associative learning and elemental representation: II. Generalization and discrimination. *Animal Learn Behav* 30:177–200. doi:10.3758/BF03192828
- Mountcastle V, Steinmetz M, Romo R (1990) Frequency discrimination in the sense of flutter: psychophysical measurements correlated with postcentral events in behaving monkeys. *J Neurosci* 10:3032–3044
- Munn NL (1950) Handbook of psychological research on the rat: an introduction to animal psychology. Houghton Mifflin, Oxford
- Murphy RA, Mondragón E, Murphy VA (2008) Rule learning by rats. *Science* 319:1849–1851. doi:10.1126/science.1151564
- Peña T, Pitts RC, Galizio M (2006) Identity matching-to-sample with olfactory stimuli in rats. *J Exp Anal Behav* 85:203–221
- Pfaffmann C, Goff WR, Bare JK (1958) An olfactometer for the rat. *Science* 128:1007–1008. doi:10.1126/science.128.3330.1007
- Reznikova Z, Ryabko B (2011) Numerical competence in animals, with an insight from ants. *Behaviour* 148:405–434. doi:10.1163/000579511X568562
- Romo R, Salinas E (2003) Flutter discrimination: neural codes, perception, memory and decision making. *Nat Rev Neurosci* 4:203–218. doi:10.1038/nrn1058
- Romo R, Brody CD, Hernandez A, Lemus L (1999) Neuronal correlates of parametric working memory in the prefrontal cortex. *Nature* 399:470–473. doi:10.1038/20939
- Rubin BD, Katz LC (2001) Spatial coding of enantiomers in the rat olfactory bulb. *Nat Neurosci* 4:355–356. doi:10.1038/85997
- Rutte C, Taborsky M (2007) Generalized reciprocity in rats. *PLoS Biol* 5:e196. doi:10.1371/journal.pbio.0050196
- Slotnick B (2001) Animal cognition and the rat olfactory system. *Trends Cogn Sci* 5:216–222. doi:10.1016/S1364-6613(00)01625-9
- Slotnick BM, Katz HM (1974) Olfactory learning-set formation in rats. *Science* 185:796–798
- Slotnick BM, Nigrosh BJ (1974) Olfactory stimulus control evaluated in a small animal olfactometer. *Percept Mot Ski* 39:583–597

- Smith JD, Beran MJ, Couchman JJ, Coutinho MVC (2008) The comparative study of metacognition: sharper paradigms, safer inferences. *Psychon Bull Rev* 15:679–691
- Swann HG (1933) The function of the brain in olfaction. I. Olfactory discrimination and an apparatus for its test. *J Comp Psychol* 15:229–241. doi:[10.1037/h0074623](https://doi.org/10.1037/h0074623)
- Uchida N, Mainen ZF (2003) Speed and accuracy of olfactory discrimination in the rat. *Nat Neurosci* 6:1224–1229. doi:[10.1038/nn1142](https://doi.org/10.1038/nn1142)
- Uchida N, Mainen ZF (2007) Odor concentration invariance by chemical ratio coding. *Front Syst Neurosci* 1:3. doi:[10.3389/neuro.06.003.2007](https://doi.org/10.3389/neuro.06.003.2007)
- Washburn DA, Rulon MJ, Gullledge JP (2004) A new breed of computer users: rats control a cursor via joystick manipulation. *Behav Res Methods Instrum Comput* 36:173–179. doi:[10.3758/BF03195562](https://doi.org/10.3758/BF03195562)
- Weinstein B (1941) Matching-from-sample by rhesus monkeys and by children. *J Comp Psychol* 31:195–213
- Zhang J-Y, Kuai S-G, Xiao L-Q et al (2008) Stimulus coding rules for perceptual learning. *PLoS Biol* 6:e197. doi:[10.1371/journal.pbio.0060197](https://doi.org/10.1371/journal.pbio.0060197)