Perceptual judgements and chronic imaging of altered odour maps indicate comprehensive stimulus template matching in olfaction

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Lesion experiments suggest that odour input to the olfactory bulb contains significant redundant signal such that rodents can discern odours using minimal stimulus-related information. Here we investigate the dependence of odour-quality perception on the integrity of glomerular activity by comparing odour-evoked activity maps before and after epithelial lesions. Lesions prevent mice from recognizing previously experienced odours and differentially delay discrimination learning of unrecognized and novel odour pairs. Poor recognition results not from mice experiencing an altered concentration of an odour but from perception of apparent novel qualities. Consistent with this, relative intensity of glomerular activity following lesions is altered compared with maps recorded in shams and by varying odour concentration. Together, these data show that odour recognition relies on comprehensively matching input patterns to a previously generated stimulus template. When encountering novel odours, access to all glomerular activity ensures rapid generation of new templates to perform accurate perceptual judgements.
E ntering the relationship between stimulus-evoked activity and sensory perception is central to determining how neuronal networks construct internal representations of the external world. To examine this question, in vivo chronic imaging has proven useful for assessing the large-scale spatial dynamics of network response properties and for quantifying changes in those responses to sensory deprivation or enrichment. In parallel, behavioural and lesion experiments have been implemented to elucidate the psychophysical properties of sensory processing and the biological loci and function of neuronal elements involved.

In the olfactory system, glomeruli in the olfactory bulb receive stimulus-related information from olfactory receptor neurons (ORNs) expressing the same receptor that is then processed and integrated by bulbary circuits. The principal mitral and tufted cells that participate in glomerular circuits relay the resultant signals to olfactory cortex, where it is suggested that olfactory object templates and odour-related memories may be generated and stored. In the bulb, monomolecular odorants are known to evoke specific spatiotemporal activation sequences of glomeruli, indicating the relevance of specific patterns of glomerular activity to odour quality perception. However, the activation of many different OR types by simple monomolecular odorants.

Behavioural experiments on the other hand indicate that much of the widespread bulbary activation may not be required for olfactory perceptual judgements. Partial bulb lesions have been shown to have little or no impact on the discrimination of even highly similar odorants. Moreover, in extreme cases, where rodents have undergone double bulbectomies, and thus abolition of all glomerular-based information, newly generated ORNs that extend their axons through the cribriform plate and form contacts in the forebrain are sufficient to support odour detection and discrimination of odorants. Thus, extensive disruption of glomerular activity maps is believed to be of little or no effect on odour quality perception, indicating that such maps contain substantial redundant information that is not used for odour-related decision making.

Understanding the impact of altering odour-evoked activity on odour perception can yield information about the neuronal mechanisms the olfactory system relies on to determine the identity of an odour. Recent functional magnetic resonance imaging studies in humans have provided evidence for models where the olfactory system uses preformed odour templates to match a stimulus to a previously experienced odour. To directly test the idea of sensory redundancy and stimulus template matching in olfaction we combined chronic functional imaging of the olfactory bulb in mice and a non-surgical epithelial lesion that alters the ORN inputs that generate glomerular activity maps. This allowed us to quantify differences in functional activity maps in mice where sensory inputs to the olfactory bulb are disrupted. Psychophysical assessment of odour quality perception in these mice shows that subtle changes in odour-evoked glomerular activity impairs recognition of familiar odours and impacts the time-course of discrimination learning of novel odours compared with previously experienced odours. These effects cannot be explained by perceived reductions in odour concentration or an increased detection threshold following lesioning. Rather, we conclude that all available glomerular information is used to generate a detailed reference template and that matching glomerular activity to that template ensures accurate, rapid olfactory-related decision making.

**Results**

**Effects of ZnSO₄-induced lesions on odour recognition.** Mice were first trained to accurately discriminate monomolecular odorants (for example, amyl-acetate versus cineol; pair A discrimination task: Supplementary Figs S1 and S2) followed by a second odour pair (pair B discrimination task; ethyl-butyrate versus pentanal, Fig. 1a). After performing above criterion for pair B odours, mice were tested for recognition of odour pair A by interleaving unrewarded pair A odour trials with pair B discrimination trials (pair A recognition test, Fig. 1b, Supplementary Fig. S2c). Mice could recognize pair A odours with high accuracy, as pair A recognition test scores were not significantly different from the average of the last five blocks of pair A discrimination (pair A discrimination, 93.97 ± 0.67% versus recognition 92.23% ± 1.89, P > 0.19, n = 14 mice).

Mice were then briefly anaesthetized with isoflurane and treated in one of three ways; with 0.9% w/v NaCl (sham), with a low dose of ZnSO₄ (three nasal flushes of 8.4% w/v ZnSO₄ per naris) or a high dose (2 × three nasal flushes per naris of 8.4% w/v ZnSO₄). Following a 24-h recovery period, mice were given 30 blocks of training in which to learn to discriminate a new odour pair (pair C). Once they reached criterion, performance of mice treated with a low dose of ZnSO₄ was not significantly different from shams (last block of pair C discrimination task, sham, 88.93 ± 4.40%, n = 7 versus low-dose ZnSO₄, 89.66 ± 2.86%, n = 6, P > 0.44, Fig. 1c). High-dose ZnSO₄-treated mice, however, discriminated at chance levels on pair C odours for all 30 blocks (all 30 blocks, 49.93 ± 6.9%, χ²-test significance from 50%, n = 5 mice, P > 0.82. Last five blocks, 49.66 ± 1.31%, P > 0.26, Fig. 1c).

Mice were next tested for recognition of the pretreatment pair B odours. Despite their accurate discrimination of pair C odours, low-dose ZnSO₄-treated animals returned subcriterion recognition scores that were on average significantly lower than shams (shams, 85.7 ± 2.97%, n = 7; low-dose ZnSO₄, 70 ± 5.47%, n = 6, P < 0.02; Fig. 1d) that is not explained by rapid extinction in the low-dose group (Supplementary Fig. S3). For recognition, high-dose ZnSO₄-treated mice performed at chance levels, with recognition scores that were significantly lower than both shams and low-dose-ZnSO₄-treated mice (53.00 ± 1.22%, n = 5 mice versus shams, P < 0.0001, versus low-dose-ZnSO₄-treated, P < 0.013).

To ensure that low-dose-ZnSO₄-treated mice could detect the previously experienced pair B odours, their ability to relearn to discriminate this odour pair (with the S + odour rewarded again) was assessed. Although low-dose-ZnSO₄-treated mice initially performed significantly worse than shams (first block, sham 87 ± 2.45%, n = 7 mice versus low-dose-ZnSO₄-treated, 71.25 ± 6.06%, n = 6 mice, P < 0.01), they quickly reached criterion on pair B odours (second block, sham, 92.86 ± 3.25%, n = 7 mice versus low-dose-ZnSO₄-treated, 92.25 ± 2.92%, n = 6 mice, P > 0.46; Fig. 1e), indicating that they could detect and discriminate the previously experienced pretreatment odours.

**Effects of lesions on glomerular activity maps.** We next recorded intrinsic signals evoked by the recognition test odours (ethyl-butyrate and pentanal) from the dorsal surface of the bulb before (imaging session one) and following (imaging session two) nasal flush treatment (see Methods and Supplementary Fig. S4). Qualitatively, although the integrity of the odour-evoked glomerular map was maintained for sham-treated mice,
low-dose-ZnSO4-treated mice revealed a phenotype ranging from very minor map changes to an almost complete abolition of odor-evoked intrinsic signal activity, they showed no detectable odour-evoked intrinsic signal activity, they were likely anosmic (at least for the odor pairs tested). Second, low-dose ZnSO4 treatment caused a significant alteration in ORN activity that is accompanied by a significant reduction in ability to recognize previously experienced odours, and likely reflects an altered percept of those odors.

Recognition of familiar odours at novel concentrations. One possible alternative explanation for the recognition scores in the low-dose-ZnSO4-treated group could be that treatment induces a homogeneous reduction in ORN input, resulting in an odorant being perceived at an apparent lower concentration during recognition testing. Although rodents have been shown to extrapolate odor identity over ~10-fold changes in concentration43, larger changes in concentration can cause them to respond as though odours appear in some way novel, that is, with reduced foraging times in associative tasks44. To therefore test the possibility that low-dose-ZnSO4-treated mice recognize previously experienced odours but do not generalize across concentrations on this task, a group of naive mice were first trained on pair A and then on pair B odours all presented at 1% v/v (Fig. 3a). Lower concentrations (0.05 or 0.01% v/v) of pair A odours were presented for the recognition test (Fig. 3b). Indeed, when test odours were reduced in concentration 20- to 100-fold from the trained pair A odour, average recognition scores were

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In a subset of mice, we were able to perform behavioural assessment, epithelial lesions and chronic imaging to determine the dependence of recognition on dissimilarity score (Fig. 2d-f). Sham-treated mice had, on average, both significantly higher recognition scores and lower dissimilarity scores than low-dose-ZnSO4-treated mice (pair B recognition scores, shams, 86.6 ± 4.41%, n = 3 versus low-dose-ZnSO4-treated, 70.6 ± 4.67%, n = 8 mice, P < 0.05; pair B dissimilarity scores, shams, –0.0378 ± 0.04, n = 10 activity maps versus low-dose-ZnSO4-treated, 0.29 ± 0.07, n = 21 activity maps, P < 0.02). High-dose-ZnSO4-treated mice also had significantly lower recognition and higher dissimilarity scores than both sham- and low-dose-ZnSO4-treated mice (pair B recognition scores, 53 ± 1.37%, n = 5 versus shams, P < 0.001, versus low-dose-ZnSO4-treated, P < 0.003). Pair B dissimilarity scores 0.56 ± 0.07, n = 15 activity maps versus shams, P < 0.02, versus low-dose-ZnSO4-treated, P < 0.01, Fig. 2d-f). The overall correlation between the dissimilarity and recognitions scores for ethyl butyrate and pentanal in this subset of animals was also significant (r^2 = 0.53, P < 0.05, n = 16 mice, 47 maps) (Fig. 2g).

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significantly lower than for control animals probed with odours presented at the previously experienced concentration of 1% v/v (1% v/v, 92.14 ± 3.02%, n = 7 versus 0.05% v/v, 78.13 ± 7.07%, n = 8, P < 0.047; 0.01% v/v, 71.67 ± 11.67, n = 3, P < 0.03, Fig. 3b).

Again, to ensure mice could detect the low concentration recognition test odours, they were retrained to discriminate pair A odours at the reduced concentration (Fig. 3c). The first and second blocks of pair A discrimination scores were significantly lower in mice presented with 0.01% v/v (second block, 1% v/v, 98.33 ± 1.67%, n = 7 versus 0.01% v/v, 73.33 ± 10.93%, n = 3, P < 0.007) but were then indistinguishable from controls (third block, 1% v/v, 96.67 ± 2.47% versus 0.01% v/v, 88.33 ± 9.28%, P > 0.13). Discrimination scores for odours presented at 0.05% v/v were not significantly different from 1% v/v odours on the first block of discrimination training trials (1% v/v, 85.00 ± 8.16% versus 0.05% v/v, 75.63 ± 6.43%, P > 0.17). Together, these data suggest that on this Go/No-Go task, mice trained to discriminate monomolecular odors at a single concentration later respond to the same odor when presented at 20- to 100-fold lower concentration as though it is being perceived in some way different.

The effect of concentration training on recognition. We therefore next sought to train mice on a range of odor concentrations so that presentation of an unfamiliar, low concentration of the same odor may be responded to as being familiar. Following the successful discrimination of ethyl butyrate and pentanal at concentrations of 0.01, 0.1, 0.25 and 1% v/v (now pair A odours; Fig. 4a–d), mice were trained to discriminate pair B odours at 1% v/v (Fig. 4e). Once they reached criterion on pair B odours, unfamiliar concentrations of ethyl butyrate and pentanal (0.05% v/v) were presented as the recognition test odours. After concentration training, mice achieved above criterion recognition scores for unfamiliar low concentrations of ethyl butyrate and pentanal, indistinguishable from those scores obtained from mice presented with the familiar 1% v/v concentration (1% v/v, 91.67 ± 2.79%, n = 6 versus 0.05% v/v, 97.14 ± 2.14%, n = 7, P > 0.07, Fig. 4f). Furthermore, recognition of 0.05% v/v odours following concentration pretraining was significantly higher than for recognition of 0.05% v/v odours in mice without concentration training (0.05%, v/v, without training, 78.13 ± 7.07%, n = 8; 0.05% v/v, trained, 97.14 ± 2.14%, n = 7, P < 0.01).

Figure 2 | Effects of epithelial lesioning on odour-evoked intrinsic optical images and odour recognition. (a) Example scatter plots of ROI correlations for odour-evoked intrinsic signal activity maps recorded within sessions (session one, orange boxes; session two, yellow boxes) and across sessions (purple boxes) after sham treatment. Values for each ROI (open green circles) are expressed as normalized pixel value (NPV). r-values displayed are averages of all ROI correlations for each block comparison. The total number of data points plotted correspond to nROIs x pixels/ROI for one odour. Concomitant activity maps are displayed to the left of NPV correlations. NPV range, 0.9965–1.0027. Scale bar, 500 μm for all images. (b) Example scatter plots of ROI correlations (grey) for odour-evoked intrinsic signal activity maps within sessions (session one, orange boxes; session two, yellow boxes) and across sessions (purple boxes) after low-dose ZnSO4 treatment. (c) Bar graph showing dissimilarity score of intrinsic signal activity maps (evoked by ethyl-butyrate and pentanal) from sham (n = 27 activity maps, green open box) and low-dose-ZnSO4-treated animals (n = 32 activity maps, grey-filled box). NPV range, 0.996302–1.000254. Dissimilarity scores evaluated by Mann-Whitney U-test (*P < 0.001). (d) Left, example odour-evoked activity maps from before (top, session one) and after (bottom, session two) nasal flush for sham-treated mice. Right, average dissimilarity score for pair B odour-evoked activity maps (left axis) and recognition scores for pair B odours (right axis) for shams. (e,f) As above for low- (e) and high-dose (f) ZnSO4-treated mice. Data represent mean ± s.e.m. (g) Recognition accuracy plotted against dissimilarity score for ethyl butyrate and pentanal from all mice in D1-D3. Pearson’s correlation value, P < 0.05.
If low-dose ZnSO₄ treatment results in recognition test odours being perceived as though presented at a reduced concentration, then pre-training low-dose-ZnSO₄-treated mice on a range of concentrations would be expected to improve recognition scores, as demonstrated above. However, despite being trained to discriminate a range of concentrations (0.01, 0.1, 0.25 and 1% v/v) of ethyl butyrate and pentanal (Fig. 5a–f), and being able to learn to discriminate pair C odours (shams, 96.75 ± 1.35%, \( n = 5 \), low-dose-ZnSO₄-treated, 96.67 ± 2.47%, \( P > 0.48 \)), low-dose-ZnSO₄-treated animals continued to perform below criterion for recognition of ethyl butyrate and pentanal (presented at 1% v/v) and were significantly worse than shams (sham, 1% v/v, 96.00 ± 1.87%, \( n = 5 \) versus low-dose ZnSO₄, 1% v/v, 65.00 ± 8.56%, \( n = 6 \), \( P < 0.008 \), Fig. 5g). Again, to determine whether low-dose-ZnSO₄-treated mice retained the ability to detect the unrecognized odours, mice were retrained to discriminate this odour pair (at 1% v/v). As above, although low-dose-ZnSO₄-treated mice initially performed significantly worse than shams, they quickly learned to discriminate unrecognized odours within two blocks (first block, sham, 93.00 ± 3.74% versus low-dose-ZnSO₄-treated, 64.17 ± 8.89%, \( P < 0.02 \); second block, sham, 97.00 ± 2.00% versus low-dose-ZnSO₄-treated, 89.17 ± 8.00%, \( P > 0.2 \), Fig. 5h). Relearning of discrimination of unrecognized odours was also not significantly different between concentration-trained and non-concentration-trained low-dose-ZnSO₄-treated mice (first five blocks, block by block Student’s t-tests, all comparisons, all \( P \)-values: \( P > 0.12 \); \( F_{(1.88)} = 1.7 \), \( P = 0.19 \), Fig. 5i). This data thus suggests that low-dose ZnSO₄ treatment alters apparent odour identity rather than its perceived concentration.

**Effects of odour concentration and lesioning on activity maps.**

If low-dose ZnSO₄ treatment results in a previously presented odour being perceived as novel and not merely reduced in concentration, one would expect the glomerular activity map altered by low-dose ZnSO₄ treatment to be changed in a manner that does not reflect map changes observed by varying odour concentrations. First, we tested this assumption by using correlations to compare intrinsic signal maps evoked by S+ and S− odours presented at varying concentrations. Intrinsic-signal activity maps recorded at 1% v/v odour concentrations in session one were compared with various concentrations recorded in
session two. Over the range of concentrations recorded, dissimilarity scores for maps evoked by 1% v/v odours in session one and those evoked by varying odour concentrations in session two were not significantly different from dissimilarity scores for maps evoked by 1% v/v odour presentation in both sessions (Supplementary Fig. S6).

Activity maps were next analysed to determine whether low-dose ZnSO₄ treatment altered the intensity of ROIs relative to one another (see Supplementary Methods). For all odour-evoked activity maps that contained ROI activity, we observed no reliable changes in the relative intensities of ROIs across concentrations (1% v/v session one – 1% v/v session two, interquartile range = 0.20, n = 7; session two, 0.1% v/v, 0.17, n = 5, P > 0.60; session two, 0.05%v/v, 0.19, n = 7, P > 0.97; session two, 0.01% v/v, 0.25, n = 7, P > 0.83, Fig. 6d). This is entirely consistent with experiments that show relative glomerular intensity remains constant over a moderate range of concentrations. In contrast, low-dose ZnSO₄ treatment produced a marked change in relative ROI intensity across sessions compared with that of shams (sham, interquartile range Q1–Q3 = 0.24, n = 15 versus low-dose ZnSO₄ treatment, 0.48, n = 9, P < 0.0004, Fig. 7a–g).

**The effect of lesions on odour discrimination.** To establish the degree of congruence between the sham and low-dose treatment groups in their ability to learn to discern odours, we pooled data from concentration- and non-concentration-trained mice to first assess whether there was an overall impact on the number of trials (that is, exposures) required to achieve criterion for (i) novel (pair B) odours before treatment, (ii) novel (pair C) odours after treatment and (iii) previously experienced but no longer recognized ethyl butyrate versus pentanal odours after treatment.
Figure 6 | Effects of varying odour concentrations on ROI intensities. (a) Left, example bar graphs of ranked average pixel intensity for each ROI evoked in session one by 1% v/v odours. ROI intensity is normalized to the maximum ROI in session one. Right, example bar graph of ranked average pixel intensity for each ROI evoked in session two by either 1, 0.1, 0.05 or 0.01% v/v odours. ROI intensity is normalized to the maximum ROI from session two. (b) Subtraction of session two ROI intensity values from session one values. (c) Average ROI pixel intensity map; large blue spots have the highest average pixel intensity, small, orange spots have the lowest average pixel intensity. Change in average pixel intensity rank between session one (left) and session two (right) is indicated by change of size and colour of an ROI spot. (d) Normalized ROI intensity change for subtraction values of all ROIs. Medians have been normalized to 0. Boxes indicate the first (bottom) and third (top) quartile. Whiskers extend to the most extreme data values within the range q1 - 1.5 × (q3 - q1) to q3 + 1.5 × (q3 - q1), where q1 and q3 are the 25th and 75th percentiles, respectively.
Before treatment, there was no significant difference between the discrimination scores during discrimination training for novel pair B odours (repeated measures analysis of variance (ANOVA) for the first five blocks, shams versus low-dose-ZnSO4-treated, F(1,4) = 0.8, P > 0.52, Fig. 8a). This was also the case when block scores from treated animals were aligned to the first block when shams, on average, reached criterion (Fig. 8b). However, for discrimination learning of novel set C odours ZnSO4-treated mice performed significantly and consistently worse than shams for the first 20 blocks (ANOVA, F(1,19) = 3.49, P < 10^{-4}, Fig. 8c) requiring more trials to reach criterion (n = 12 shams, n = 12 low-dose-ZnSO4-treated mice, Fig. 8d).

In low-dose-ZnSO4-treated animals, discrimination of previously experienced but not recognized odours (ethyl butyrate versus pentanal) was initially impaired compared with shams, but improved quickly (first block, shams, 89.5 ± 2.20%, n = 12, low-dose-ZnSO4-treated, 67.71 ± 5.06%, n = 12, P < 0.001; second block, shams, 94.58 ± 2.08%, low-dose-ZnSO4-treated, 90.83 ± 4.35%, P > 0.22, Fig. 8e). Despite not accurately recognizing ethyl butyrate and pentanal, the post-treatment discrimination scores for these odours over the first five blocks were significantly improved compared with the scores for the same odours presented before treatment (ANOVA, F(1,119) = 12.68, P < 0.0005, Fig. 8e). Thus, after lesioning ZnSO4-treated mice require more trials than shams to reach criterion on novel odours, but their discrimination learning of the unrecognized ethyl butyrate versus pentanal pair is significantly better than for their initial training period on the same odour pair before lesioning.

Taken together, our data show that low-dose ZnSO4 treatment of the epithelium causes maps to be altered sufficiently such that previously experienced odours are not recognized. Despite being altered, these odour maps remain similar enough to pretreatment maps such that previously encountered odours may be ‘relearned’ and discerned more quickly than genuine novel odours.

Discussion

In contrast to previous studies, we have combined chronic functional imaging with peripheral olfactory epithelial lesions to directly examine the relationship between glomerular map functionality and odour-quality perception. We show that treatment of the epithelium with two different doses of ZnSO4 results in two distinct olfactory behavioural phenotypes. High-dose treatment prevents recognition and discrimination of both familiar and novel odours. In support of this observation, we find no detectable odour-evoked signals on the dorsal surface of the olfactory bulb, consistent with an anosmic phenotype.

Low-dose ZnSO4 treatment, on the other hand, resulted in mice that could not recognize previously presented odours, although they could detect and eventually learn to discriminate both previously presented and novel odour pairs. In contrast to the high dose, our intrinsic-signal imaging data revealed that low-dose ZnSO4 treatment results in a modest functional alteration of the glomerular map. This suggests that the coding requirements for correct odour discrimination and recognition are sensitive to relatively minor changes to input patterns that is in contrast to previous suggestions.

One parsimonious explanation for our observations could be that low-dose ZnSO4 treatment simply results in a homogenous reduction in the strength of input to the bulb. In this scenario, low-dose ZnSO4 treatment would impair recognition by causing previously presented odours to be perceived at a novel, reduced concentration. Alternatively, ZnSO4 treatment may cause mice to perceive previously presented odours as having distinctly unique
qualities. In agreement with others\textsuperscript{43,44,53}, our results show that in untreated mice trained on single concentrations, large differences in the training versus test odour concentration can cause mice to respond as if test odours appear in some way novel. We show that this is not the case when naive mice are pretrained on a series of test odour concentrations. However, despite being concentration pretrained, low-dose-ZnSO\textsubscript{4}-treated mice do not recognize familiar odours presented at familiar concentrations. Previous studies in mouse show that the regenerative process following complete ORN transection results in substantial numbers of spared axons intermingled with those receiving sparse or no innervation\textsuperscript{52}. We therefore suggest that the non-homogenous effect of low-dose ZnSO\textsubscript{4} treatment on map activity impacts the integrity of the glomerular representation such that odour-quality perception is significantly impaired.

Previous studies in mouse show that the regenerative process following complete ORN transection results in substantial miswiring between the receptor neuron axon and the target glomerulus\textsuperscript{84}. Several months after transection when this regenerative process is completed, deficits in odour recognition have been observed in the hamster\textsuperscript{55}. In our study we have assessed odour perception and map integrity within ten days post treatment, well before ORNs are believed to regenerate\textsuperscript{50,56}. Although low-dose ZnSO\textsubscript{4} treatment perturbs input to the bulb, significant glomerular activity is spared. We find that this rather arbitrary manipulation impairs olfactory performance, whereby...
treated mice require significantly more trials to learn to discern novel odours compared with shams. In the case of previously presented odours not recognized after treatment, the impact of ZnSO4 treatment on their discriminability is more subtle. Low-dose-ZnSO4-treated mice discriminate with the same accuracy as shams already after only one block of training. This rapid relearning of non-recognized odours is indeed faster than for the learning curve observed for mice when they are first exposed to the same odour pair before lesioning.

This observation shows that after lesioning, the olfactory system does not process the unrecognized and novel odours in entirely the same way. The fact that low-dose-treated mice learn to discern non-recognized odours more quickly than novel odours indicates that odour stimulus templates stored before treatment remain relevant to the non-recognized odours and may be rapidly modified and used during post-treatment retraining. Along these lines, theoretical models suggest that predictive neuronal codes generated in advance of a stimulus enhance the speed and accuracy of perceptual judgements. In this model, a pre-existing odour template and incoming sensory information interact to rapidly generate a sensory percept. 

In accordance with our results, recent behavioural and/or imaging experiments indicate that activity patterns in piriform cortex may be the locus of such odour stimulus templates.

In summary, we show for the first time that perceptual correlates of odour concentration and other elements of odour identity are separable in rodents. We use this paradigm to determine that the effects of nasal–epithelial lesion with moderate concentrations of ZnSO4 differ from reduction in concentration in that such lesions induce a unique change in the relative intensity of glomerular maps. Our data contrast previous work, indicating widespread lesions of the bulb have little or no impact on odour quality perception that has led to the suggestion that the spatially organized input to the bulb contains significant redundant information. Our results support theoretical work and functional magnetic resonance imaging studies in humans that show that a cortical prestimulus template is used by the olfactory system to match incoming odour information to perform accurate perceptual judgements. We show that even relatively modest deviations from these templates can cause previously experienced odour stimuli not to be recognized. This suggests that coding in the olfactory bulb is non-redundant and that significant amounts of all available information is acquired to generate and match templates to perform rapid and accurate olfactory perceptual judgements.

**Methods**

**Animals.** All procedures and experiments were carried out on male C57Bl/6 mice over the ages of 4 weeks, in accordance with the UK Home Office and the Animals and Scientific Procedures Act, 1986.

**Behavioural training.** Mice were given restricted access to water for 48 h before behavioural training. After habituation to the olfactometer environment, they were trained to discriminate between two odours. Infrared beam interruption triggered presentation of either an S+ (rewarded) or S− (unrewarded) odour. Lick patterns were recorded for the duration of the stimulus across four 350 ms time bins (Supplementary Fig. S2a, top). Responses were deemed correct if the mouse licks for three or more time bins during presentation of the S+ odour, or if they licked for two or fewer time bins during the S− odour. Between 8 and 12 S+ and 12 and 8 S− odour trials were presented in a pseudo random order in blocks of 20 trials. Percentage of correct responses for S+ and S− trials was averaged over 20 trials, giving an overall percentage for each block (Supplementary Fig. S2b). The mouse was classed as discriminating correctly when the average of each of five consecutive blocks first reached a criterion (≥ 80% correct, Supplementary Fig. S2b). Mice usually performed between 20 and 30 blocks.

Those mice trained on a range of concentrations were first trained to discriminate pair A odours at a concentration of 1% v/v. Next, pair A odours at lower concentrations were interleaved among higher concentrations forming pseudo blocks constituting, for example, 55 + trials of 0.01% v/v, 55 + trials of 1% v/v, 55 – trials of 0.01% v/v and 55 – trials of 1% v/v. 

**Nasal flush.** At least 1 day before treatment, mice were given free access to water. Animals were anaesthetized with isoflurane (Abbott Laboratories, UK) and each car was flushed separately with 3 ml of either NaCl (9%, 263 mosm, sham) or ZnSO4 (8.4% ZnSO4, 275 mosm) using a micropipette (Gilson, USA). This was repeated two more times in immediate succession such that mice received a total of three flushes per nares within 5 min. Mice treated with the high-dose ZnSO4 were given a recognition test consisting of a further 5 blocks, each comprising 16 pair B odour trials (8 S+ and 8 S− pseudo-randomly interleaved with four pair A odour trials (that is, recognition test odours; two unrewarded S+ and two S− trials) (Supplementary Fig. S2c). The recognition test S+ odour was unrewarded to ensure that mice could not rapidly relearn to discriminate odour pair A.

**Data analysis.** Images were first 2×2 binned (WinMix BlockConverter, Optical Imaging Ltd., USA), then those frames recorded during stimulus presentation (frames 35–65) were divided by the average of prestimulus baseline frames (frames 10–50). All intensity activity maps were aligned to the first map recorded in session one using software custom written in Matlab (Mathworks, USA) and Labview (NI) (Supplementary Fig. S4a).

Using Imaging (NIH, Bethesda), circular ROIs 75 μm in diameter were positioned over areas with odour-evoked signal in the first activity map recorded in session one during presentation of 1% v/v odours. The same set of ROIs was imposed upon aligned images from all activity maps and session one and two. For determining relative ROI intensity, activity maps that had within-session correlation coefficient r-values lower than 0.999, pixel intensity values were normalized by dividing the average pixel intensity of the whole image. The mean normalized pixel value of each ROI was then determined and averaged over 40 stimulus frames (frames 10–50 after odour onset) and subtracted from 1. Pixel intensity values for each ROI were correlated with the concomitant ROI recorded in other activity maps recorded in the same bulb for the same odour. For each image frame, all pixel intensity values were normalized by dividing by the average pixel intensity of the whole image. The mean normalized pixel value of each ROI was then determined and averaged over 40 stimulus frames (frames 10–50 after odour onset) and subtracted from 1. 

For determining relative ROI intensities, activity maps that had within-session correlation coefficient r-values lower than 0.5 and ROIs whose normalized average pixel intensity did not on average drop below 0.9990 during odour presentation in both sessions one and two, were removed to ensure that only ROIs that were responsive to both odours in both session were included in analyses. The number of ROIs removed from activity maps of sham and low-dose-ZnSO4-treated mice following thresholding was not significantly different (median of ratio of number of ROIs before filtering to after filtering, sham, 1.83 ± 1.5, n = 15 activity maps, low-dose-ZnSO4-treated, 1.19 ± 1.5, n = 9 activity maps). For activity maps generated from different concentrations, the mean ratio of number of ROIs obtained from activity maps evoked by lower concentrations in session two were not significantly different from the ratio obtained from 1% v/v presented in both sessions (1% v/v session one versus 1% v/v session two, median ratio, 1.27 ± 0.28, n = 7; session two, 0.1% v/v, median ratio, 1.65 ± 1.27, n = 5; P > 0.1; session two, 0.05% v/v, median ratio, 1.19 ± 0.67, n = 7, P > 0.1; session two, 0.01% v/v, median ratio, 1.57 ± 0.89, n = 7, P > 0.1).

After thresholding, intensity values of each ROI were averaged across blocks and then ranked. Session two ROIs were ordered according to their rank in session one. Correlation values for session one and two were then normalized to 1 and session two values were subtracted from session one values to determine the extent of change in relative ROI intensity across sessions for the whole activity map. Subtraction values for all ROIs were then compiled and the population statistics determined. A higher variance of subtraction values indicated a higher change in relative intensity. ROI intensity subtraction values were compared using Mann–Whitney’s U-tests.

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Author contributions
E.F.B. performed all of the experiments. B.P. wrote acquisition and analysis software. A.T.S. provided advice on the design and implementation of the behavioural training apparatus. D.H.W. and B.P. wrote image alignment software. E.F.B., B.P. and T.W.M. analysed the data. T.W.M. conceived the project. E.F.B., B.P. and T.W.M. wrote the manuscript.

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