Neuron, volume 52

## Supplemental Data

# Learning to Smell the Roses: Experience-Dependent Neural Plasticity in Human Piriform and Orbitofrontal Cortices

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## Supplemental Experimental Procedures: Complementary Study

We conducted a complementary behavioral experiment to test the reliability and durability of the perceptual learning effect. We recruited 16 healthy subjects (mean age, 26 years; 10 women) after a screening test to ensure that the qualitatively related odorants (among the original set of four odorants) were perceived to be more similar in quality, compared to other possible pairings. All subjects provided informed consent to take part in this study, which was approved by the Northwestern IRB.

In this experiment, we also included a new minty odorant (methyl salicylate; 5% diluted in mineral oil) and a new floral odorant (geraniol; 50% in mineral oil) (Amoore, 1969; Arctander, 1994), though only the original odorants (carvone, menthol, acetophenone, phenethyl alcohol) were assigned as the TG stimulus. The odor pairs included in similarity ratings comprised the six pairs made of the four original odorants and two new pairs between the TG odorant and one of the two new odorants. For example, if carvone was assigned as the TG odorant, carvone and methyl salicylate formed a new pair related in quality (TG/QR-New), while carvone and geraniol resulted in a filler pair. The TG/QR-New pair enabled us to examine whether perceptual learning could generalize to qualitatively related odorants outside of the original stimulus set. Odorant assignment was counterbalanced across conditions and subjects to exclude odor-specific artifacts.

To investigate whether exposure to the test odorants before and after habituation was critical to the learning effect, we divided the 16 subjects into two groups of eight. One group participated in the original paradigm from the main study (i.e., 14-min pre-habituation, 3.5-min habituation, 14-min post-habituation). The other group took part in a shorter version of the experiment that only involved the 3.5-min habituation phase (pre- and post-habituation sessions excluded). Similar to the main study, subjects provided ratings of pairwise odor quality similarity approximately 20 min before and 30 min after the onset of TG habituation (or 5 min before and 15 min after in the short paradigm). Subjects also provided pairwise similarity ratings at 4 hrs and 24 hrs following habituation, to characterize the time course of perceptual learning. One subject assigned to the original paradigm failed to return for the re-tests at 4 and 24 hours, and thus was not included in analysis involving these delayed post-tests.

## Supplemental Results: Complementary Study

As reported in the main text, analysis of the similarity ratings of odor quality indicated that the quality-related TG pairs (original and new) became more dissimilar following habituation, and that these effects persisted across the three post-assessments. A Friedman test including the difference scores (post – pre) comparing TG/QR (original and new collapsed) and non-TG/QR pairs at three time-points revealed no significant differences across these assessments ($\chi^2$ = 8.15; df = 5; $p$ = 0.15). Rating changes comparing TG/GR and non-TG/GR pairs were also equivalent across the three time-points ($\chi^2$ = 1.48; df = 5; $p$ = 0.92). These findings replicate our original results from the main study and demonstrate that the effect of olfactory perceptual learning persists for up to 24 hrs.

This study also allowed us to determine whether perceptual learning could generalize to novel odorants. Learning-induced changes were present at all three time-points for both the TG/QR pair and the TG/QR-New pair (no significant difference across time or between the two pairs; $\chi^2$ = 3.57; df = 5; $p$ = 0.61; Friedman test), supporting the hypothesis that olfactory perceptual learning is not restricted to odorants within the original test set. When analyzed separately, the TG/QR-New pair (vs. non-TG/QR) showed significant learning-induced increases only at 10 min and 4 hr after exposure ($p$'s $< 0.05$; Wilcoxon test, one-tailed). Finally, we note that subjects assigned to the original ($n$ = 8) or the shortened ($n$ = 8) paradigm showed similar behavioral profiles, indicating that learning occurred independently of odorant experience during the pre- and post-habituation sessions ($F_{1,13}$ = 0.318; $p$ = 0.58; repeated-measures ANOVA).

## Supplemental Discussion

This section considers two issues in greater detail that arose from the main study: (a) potential alternative explanations regarding the behavioral ratings of odor quality similarity; and (b) the relationship between perceived odor intensity and neural responses in olfactory cortex.

### ***Potential alternative explanations of the behavioral ratings data***

Although our findings were most parsimoniously explained via changes in odor quality differentiation, it was important to consider other potential explanations of these effects. First, these findings cannot be simply attributed to item-specific effects, since assignment of the four odorants was perfectly counterbalanced across condition types and across subjects. Second, it is unlikely that the changes in similarity ratings could have been made on the basis of perceived intensity differences, since by the end of the post-habituation session, odor intensity did not significantly differ across the four odorants ($p > 0.1$, Friedman test). Third, the specificity of these findings for quality- and group-related pairs (vs. the unrelated control pair) argues against possible confounds related to generalized sensory fatigue, irritation, or distortion. Fourth, measurements of sniff volume, peak amplitude, and latency were comparable across odorant conditions, making it unlikely that respiratory differences could have attributed to the neural effects. Fifth, subjective ratings of odor valence and pungency did not exhibit differential changes across the four odorant conditions as a result of TG exposure ($p$'s $> 0.1$), ruling out the likelihood that the impact of odorant exposure on odor quality differentiation was due to mere perceptual variations in these other perceptual features. Finally, similarity rating data helped to exclude the possibility that a "ceiling effect" could have prevented initially dissimilar pairs from being rated even more dissimilar, since in fact the TG/GR pair (main study) and the QR-New pair (complementary study) both showed the capacity for even greater differentiation after prolonged exposure, despite being fairly dissimilar at the initial assessment (Fig. S1).

### ***Relationship between perceived intensity and neural responses in olfactory cortex***

The habituation session (main text, Fig. 3) highlighted a close relationship between intensity ratings and piriform and OFC activation (both decreasing). This result corroborates previous findings demonstrating a dependence of piriform activity on odor concentration (Winston et al., 2005), but contrasts with previous fMRI investigations of odor habituation, which suggested a dissociation between piriform activity and perception (Poellinger et al., 2001; Sobel et al., 2000). We speculate that this discrepancy is due to our use of online intensity ratings, which offered a more sensitive perceptual index than the use of odor detection tasks (presence/absence) in these other studies.

However, the fact that the signal in piriform cortex reflects perceived intensity is still compatible with the idea that signal size may also index perceptual learning. These two mechanisms are not mutually exclusive. If learning occurs without corresponding changes in perceived intensity, it becomes relatively straightforward to interpret the fMRI changes in piriform activity. This is why, in examining the effect of learning on QR, GR, and CT, it was advantageous that there were no significant differences in perceived intensity between these stimuli. On the other hand, reduction in the perceived intensity of the TG odorant (as a result of self-habituation) likely obscured any enhancing effect that perceptual learning would have had on the neural activity evoked by this stimulus.

## Supplemental Figures

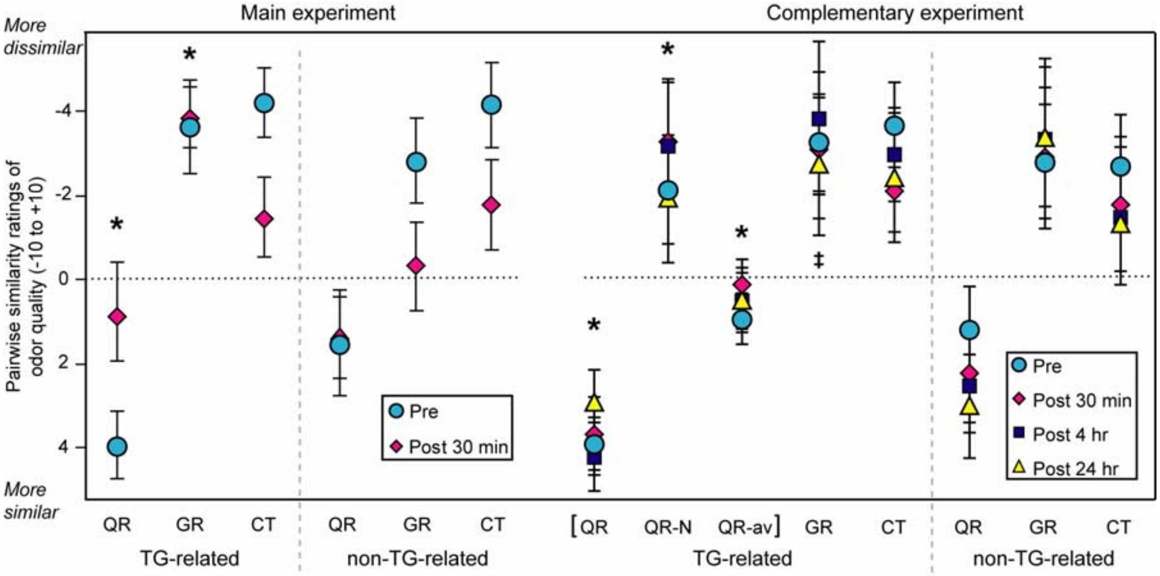

**Figure S1.** Pairwise similarity ratings of odor quality before and after habituation. (Left) Main study, ratings for the six possible odorant pairs, collected 20 min before and 30 min after habituation. (Right) Complementary study, ratings for the two TG quality-related pairs (QR and QR-N), their averaged rating (QR-av), and ratings for the five other pairs, collected 20 min before habituation, and 30 (or 10) min, 4 hr, and 24 hr after habituation. QR-N = pairwise similarity rating of TG and QR-New; QR-av = ratings averaged across TG/QR and TG/QR-New. (*) Significant compared to non-TG pairs; $p < 0.05$. **(‡)** Significant compared to the unrelated control pair; $p < 0.05$.

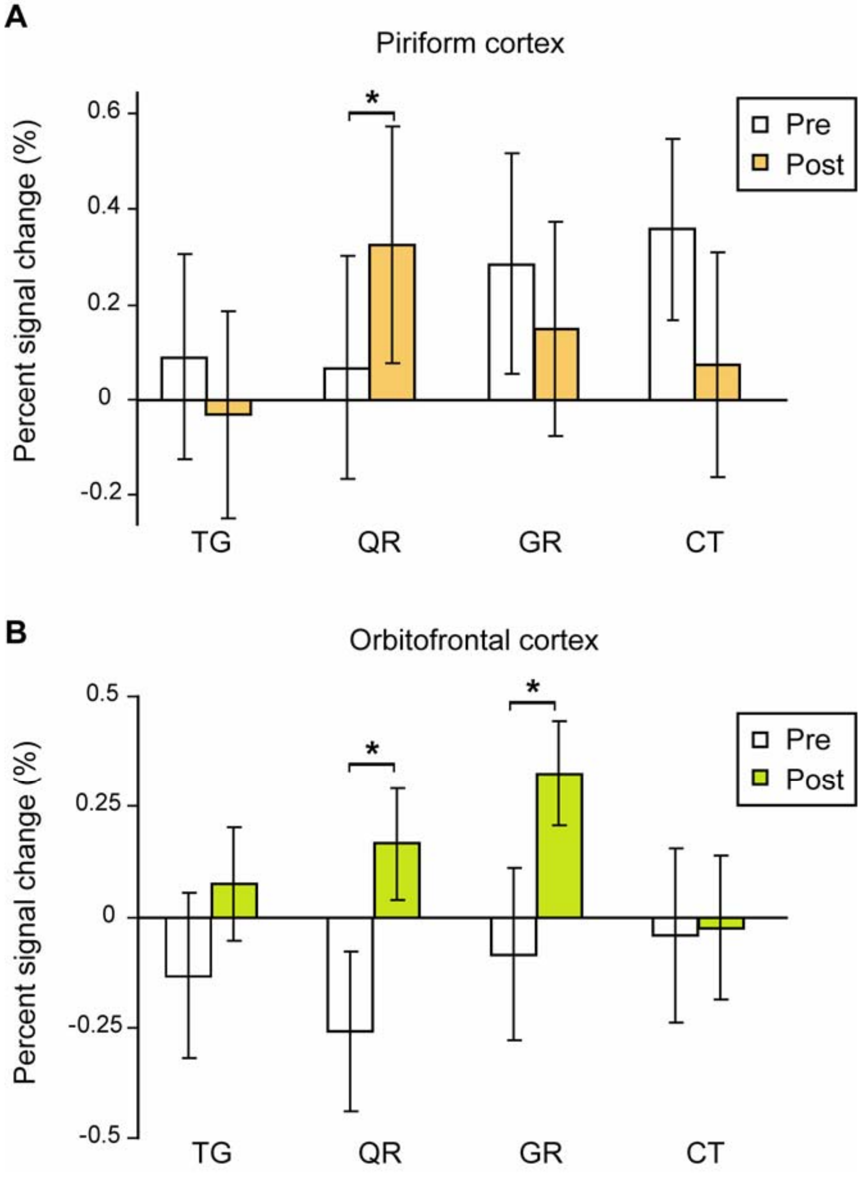

**Figure S2.** Neural responses to all test odorants at pre- and post-habituation sessions. (A) Right posterior piriform cortex showed increased activity to QR at post-habituation, despite a general trend of decreased activity for other odorants, likely due to progressive olfactory adaptation following prolonged TG exposure.

(B) OFC activity increased for TG, QR and GR odorants, though only significantly for the latter two. (*) Significant increase from pre- to post-habituation, relative to changes in CT (at $p < 0.05$, SVC).

## Supplemental References

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