Associative conditioning remaps odor representations and modifies inhibition in a higher olfactory brain area

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Intelligent behavior involves associations between high-dimensional sensory representations and behaviorally relevant qualities such as valence. Learning of associations involves plasticity of excitatory connectivity, but it remains poorly understood how information flow is reorganized in networks and how inhibition contributes to this process. We trained adult zebrafish in an appetitive odor discrimination task and analyzed odor representations in a specific compartment of the posterior zone of the dorsal telencephalon (Dp), the homolog of mammalian olfactory cortex. Associative conditioning enhanced responses with a preference for the positively conditioned odor. Moreover, conditioning systematically remapped odor representations along an axis in coding space that represented attractiveness (valence). Interindividual variations in this mapping predicted variations in behavioral odor preference. Photoinhibition of interneurons resulted in specific modifications of odor representations that mirrored effects of conditioning and reduced experience-dependent, interindividual variations in odor–valence mapping. These results reveal an individualized odor-to-valence map that is shaped by inhibition and reorganized during learning.

Higher brain functions depend on the interpretation of sensory information based on experience. This process involves associations between high-dimensional sensory inputs and low-dimensional, fundamental qualities such as valence. Associative computations are thought to be a main function of the piriform cortex (PC), a paleocortical area with prominent recurrent connectivity that lacks an obvious fine-scale topography. PC is one of multiple interconnected brain areas that receive sensory input from mitral cells of the olfactory bulb, where odors are represented by normalized and decorrelated activity patterns. PC is thought to establish synthetic olfactory object representations by auto-associative memory mechanisms based on activity-dependent modifications of recurrent connectivity. Consistent with such models, learning modified odor responses, pattern separation and pattern completion in PC. Moreover, optogenetic or pharmacogenetic manipulations modified associative memories, and ablation of posterior PC impaired temporally remote olfactory fear memory. Nevertheless, mechanisms of memory formation in PC are still poorly understood, and it remains unclear how odor representations are associated with behaviorally relevant qualities such as valence.

We examined the plasticity of odor representations in the zebrafish homolog of the olfactory cortex, the posterior zone of the dorsal telencephalon (Dp). Projections from the olfactory bulb to Dp lack a topographic organization, and odors evoke distributed activity patterns across Dp neurons. Repeated passive odor exposure results in an NMDA receptor-dependent adaptation and reorganization of odor-evoked activity patterns, which is consistent with activity-dependent plasticity of odor processing in Dp. Here we trained adult zebrafish in an odor discrimination task and measured activity patterns in a dorsal–posterior subregion of Dp (dpDp) that had not been characterized in detail in previous studies. Associative conditioning had pronounced effects that were partially reversed by photoinhibition of GABAergic (γ-aminobutyric acid-ergic) interneurons. These results indicate that associative conditioning remaps odor space onto a low-dimensional, behaviorally relevant representation of valence by a process that modifies inhibition.

Results
Odor representations in dpDp. We measured activity patterns in dpDp by two-photon Ca²⁺ imaging in an ex vivo preparation of the brain and nose after bolus loading of Oregon Green 488 1,2-bis-(o-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid, tetraacetoxy-methyl ester (see Methods; Fig. 1a,b). We quantified olfactory responses to four amino acids (Ala, Trp, His and Ser; 10⁻⁴ M), which are natural odorants (see Results; Fig. 1b–e). Population activity was dense (53% ± 4% of neurons active per stimulus; mean ± s.d. across odors; n = 1,790 neurons, N = 13 animals; herein, N indicates the number of animals (experiments), and unless stated otherwise, n indicates the number of neurons), but was typically dominated by a few strongly responsive neurons (population sparseness: 0.38 ± 0.09, mean ± s.d. across animals). The mean lifetime sparseness, a measure for tuning sharpness, was low (0.07 ± 0.08, mean ± s.d. across neurons). Hence neurons in dpDp were more...
**Fig. 1 | Odor representations in dpDp in NAV fish.** a. Location of dpDp (lateral view of the zebrafish brain and two coronal cross sections). Scale bars represent approximations. b, dpDp neurons loaded with OGB-1 (top) and Ca\(^{2+}\) signals (bottom) evoked in the same field of view by odor stimulation (Ala, single trial). c. Odor-evoked Ca\(^{2+}\) signals of 50 randomly selected dpDp neurons before, during and after stimulation with four different amino acid odors (10\(^{-4}\) M; average of three trials each). d. Ca\(^{2+}\) signal averaged over all trials (n = 3), odors (n = 4) and neurons (n = 1,790, from N = 13 animals). Gray shading shows s.e.m. Red bar indicates approximate duration of odor stimulation, and blue shaded area depicts the 2-s time window used for most analyses. e, Amplitude of Ca\(^{2+}\) signals evoked by different odors (median ± s.d.). Pairwise odor comparisons (paired t tests, two-sided, n = 1,790 neurons from N = 13 animals, d.f. = 1,789): His versus Ser, t = −3.70, P = 0.0002; His versus Ala, t = 0.36, P = 0.72; His versus Trp, t = 3.94, P = 8 \times 10^{-5}; Ser versus Ala, t = −4.37, P = 1 \times 10^{-4}; Ser versus Trp, t = 7.05, P = 3 \times 10^{-10}; and Ala versus Trp, t = 3.74, P = 0.0002. Box plot: center line, median; box limits, interquartile range; and whiskers, s.d. f, Pattern similarity matrix showing cosine distance (below diagonal) and Pearson correlation (above diagonal) between trial-averaged activity patterns evoked by different odor stimuli (average over N = 13 animals). Diagonal values were set to 0 (cosine distance) or 1 (Pearson correlation). g, Classification of odor identity by template matching of activity vectors. Solid colors show percentage of correctly decoded odors (10\(^{-4}\) M; average of three trials each). Cross-hatched area shows percentage of odors that could be probabilistically classified with a success rate of 80% (McNemar test for comparison against 100% correct, χ\(^2\) = 39.02, P = 4 \times 10^{-6}). NS: P > 0.05; **P < 0.01; ***P < 0.001. D, dorsal; Dc, central zone of the dorsal telencephalon; DI, lateral zone of the dorsal telencephalon; Dm, medial zone of the dorsal telencephalon; Dp, posterior zone of the dorsal telencephalon; L, lateral; NT, nucleus taeniae; P, posterior; OB, olfactory bulb; Tel, telencephalon; TeO, optic tectum; Vs, supracommissural nucleus of the ventral telencephalon.

broadly tuned than neurons in other subregions of Dp\(^{10}\) or in the olfactory bulb\(^{11}\).

To analyze population activity, we averaged Ca\(^{2+}\) signals during a 2-s time window after response onset and described activity patterns by vectors across neurons. Distances between patterns were quantified by the cosine distance, 1 − cos(α), where α is the angle between vectors. This measure is closely related to the Pearson correlation coefficient but independent of response intensity, which allowed us to separately analyze effects on the structure and on the intensity of population activity patterns. Pairwise distances between odor-evoked activity patterns were modest (range: 0.05–0.09), and the corresponding Pearson correlation coefficients were relatively high (range: 0.76–0.86), implying that patterns overlapped substantially (Fig. 1f). Pattern discriminability was quantified using a classifier that assigns individual trials to odors by matching activity vectors to templates constructed from other trials based on the lowest cosine distance. Using this cross-validation procedure, we found that classification success was significantly greater than chance (25%; P = 10^{-15}; Fig. 1g) but less than 100% (P = 4 \times 10^{-10}) and less than the success rate of odor classification based on activity patterns from the olfactory bulb\(^{10}\) or from other subregions of Dp. Similar results were obtained when Pearson correlation or Euclidean distance was used as a distance metric (Supplementary Fig. 1), or when linear discriminant analysis was used for classification (data not...
Associative olfactory conditioning. We next examined how odor representations in dpDp are modified by experience. We first analyzed innate behavioral responses to four amino acids (Ala, Trp, His and Ser) by infusing them into tanks containing individual naive adult zebrafish (Supplementary Fig. 2a,b). Ala evoked a transient increase in swimming speed (Fig. 2a) that was reminiscent of appetitive behavior1 (Ala versus tank water, P = 0.0006), which is consistent with a previous report that Ala is innately attractive32. Trp, His or Ser, in contrast, had no obvious effects compared with control (tank water) trials, indicating that these amino acid odors were neutral (odor versus tank water, P > 0.5 in all cases).

We then trained adult zebrafish in an associative odor discrimination task (Fig. 2b–d)11. In brief, individual fish were exposed once every 20 min to one of two conditioned odors (positively (CS+)) or negatively conditioned stimulus (CS−)). The CS+ predicted the delivery of a food reward (unconditioned stimulus) into a feeding ring 30 s after stimulus onset, whereas the CS− remained unrewarded. Fish received nine CS+ trials and nine CS− trials per day and were trained for 3 or 4 d. One set of fish (ALA) was trained on Ala as CS+ and Trp as CS−, a second set of fish (TRP) was trained on Trp as CS+ and Ala as CS−, and a third set of fish (HIS) was trained on His as CS+ and Ala as CS−. Behavioral responses were measured during the 30 s after odor onset by quantifying multiple components of appetitive behavior including swimming speed, the height in the water column, and the presence in the reward area (Supplementary Fig. 2d–f). Behavioral measures were then normalized and combined to obtain a composite behavioral score for appetitive behavior (ζ; see Methods)11. Statistically significant differences between behavioral responses to the CS+ and CS− emerged already on the first day of training and approached significance on the third day (Fig. 2c; day 1, P = 0.003; day 2, P = 1 × 10−4; day 3, P = 3 × 10−4; N = 43 animals), which is consistent with previous observations5. Five out of six individual behavioral components exhibited significant differences on the second and third days, showing that results were not dominated by a single behavioral readout (Supplementary Fig. 2f). No difference was observed between the three training cohorts (P = 0.50, analysis of variance; Supplementary Fig. 2c). Hence fish rapidly learned to establish associations between specific odors and an appetitive behavioral program.

In an additional group of fish (UNC), we temporally uncoupled the CS (Ala or Trp) and unconditioned stimulus by delivering food 15 min after odor presentation pseudorandomly in 50% of all trials. Hence fish received the same number of odor stimuli and the same number of food applications as in associative conditioning, but odor stimulation was not immediately followed by food and did not predict reward (Fig. 2c). In this control experiment, fish did not systematically develop differential appetitive responses to Ala and Trp (P ≥ 0.15 on all days; Fig. 2f).

Experience modifies odor responses in dpDp. To analyze the effects of learning on olfactory processing, we compared odor responses in dpDp between fish of five experimental groups: Ala (N = 12 animals), TRP (N = 16), HIS (N = 15), UNC (N = 12) and naive (NAV; N = 13) fish. The majority of fish (12 ALA, 13 TRP, 8 UNC and 9 NAV fish) originated from the same crossing (see Methods). An initial analysis of basic response properties in this subpopulation revealed that responses averaged over all odors were significantly higher than in NAV fish after training in an associative paradigm (ALA and TRP; P < 10−4), but not after uncoupled odor exposure (UNC; P = 0.30) (Fig. 3a). This increase in the mean odor response of conditioned fish (ALA, TRP) was due to an increase in the response amplitude of individual neurons rather than the fraction of odor-responsive neurons (data not shown). Moreover, changes in response amplitude relative to NAV fish were larger for the CS+ than for the CS− in ALA fish, responses to Ala were

**Fig. 2 | Innate odor preference and associative olfactory conditioning.** a. Innate behavioral responses to the four amino acid odor used in this study and tank water as control (green). Curves show mean swimming speed (boxcar smoothed) normalized to preapplication baseline. Shading shows s.e.m. Mean swimming speed averaged over 40 s (yellow rectangle) was significantly modulated by odor application (Kruskal–Wallis test, N = 141, d.f. = 4, H = 15.44, P = 0.003), differing from tank water (N = 71 animals) for Ala (N = 16, Q = 3.59, P = 0.0007), but not for other odors (His: N = 20, Q = 0.10, P = 1; Ser: N = 20, Q = 0.16, P = 1; Trp: N = 14, Q = 0.69, P = 0.93). b. Schematic of setup for associative olfactory conditioning. c. Schedule for associative conditioning. The CS+, but not the CS−, was followed by a food reward. The behavioral response was measured during the first 30 s between odor onset and food delivery (Test). d. Mean learning curves. ζ is a composite score combining multiple components of appetitive behavior. Lines and shading show the mean (± s.e.m.) of ζ for the first 3 d of training (nine trials per day). Comparisons between CS+ and CS− (Wilcoxon signed-rank test, two-sided, N = 43 animals (two fish without data on day 1), ALA, TRP and HIS): day 1, N = 41, T = 206, P = 0.003; day 2, N = 43, T = 133, P = 1 × 10−5; day 3, N = 43, T = 42, P = 3 × 10−3. e. Schedule for uncoupled odor exposure. The same odor stimuli as for associative conditioning (Ala and Trp) were applied 15 min before food presentation, which occurred with a probability of 50% on each trial, independent of the odor (see Methods). f. Mean ζ scores for Ala and Trp in UNC fish. Lines and shading show the mean (± s.e.m.). No systematic differences in appetitive responses to Ala and Trp were observed (Wilcoxon signed-rank test, two-sided, N = 12 animals): day 1, T = 25, P = 0.30; day 2, T = 20, P = 0.15; day 3, T = 33, P = 0.68. Gray dotted lines indicate zero. NS: P > 0.05; ***P < 0.01; **P < 0.001.
increased more than responses to Trp ($P = 1 \times 10^{-4}$), whereas in TRP fish, responses were increased to Trp, but not to Ala ($P = 0.005$; Supplementary Fig. 3a). As a consequence, the amplitude ratio (Ala/Trp) was significantly shifted in the direction of the CS$^+$ when compared with NAV (ALA, $P = 0.0006$; TRP, $P = 6 \times 10^{-6}$; Fig. 3b). The Ala/Trp response ratio in UNC fish, in contrast, was not significantly different from NAV ($P = 0.09$). Conditioning also changed the signal-to-background ratio (SBR) of odor responses, which is defined for each neuron as the response amplitude normalized to the signal fluctuations during spontaneous and odor-evoked activity (Supplementary Fig. 3b,c).

The remaining subpopulation of fish originated from two additional crossings and included HIS fish (4 NAV fish, 3 TRP fish, 15 HIS fish, and 4 UNC fish). The absolute amplitude of odor responses in this subpopulation was slightly but significantly different from the first subpopulation (-10% reduction in NAV fish; $P = 0.003$; Supplementary Fig. 3d), which may reflect differences in genetic background and precluded direct comparisons of response amplitudes between HIS fish and other groups. However, in HIS fish, responses to the CS$^+$ (His) were significantly increased when compared with NAV fish from the same crossings (155% ± 3% (mean ± s.e.m.) of NAV, $n = 528$; HIS, $n = 2,040$; $P < 10^{-16}$). Wilcoxon–Mann–Whitney test, two-sided; unless stated otherwise, sample means are reported ± s.e.m., which is consistent with findings in the first subpopulation. To allow for comparisons across crossings, we based further analyses on response ratios. The ratio of CS response amplitudes (Ala/His; $P = 4 \times 10^{-14}$; Fig. 3b) and the corresponding SBR (Supplementary Fig. 3c) were shifted toward the CS$^+$ when compared with NAV fish. Hence responses to the CS$^+$ and CS$^−$ were consistently shifted in the direction of the CS$^+$ in all conditioned groups.

To examine the relationship between neuronal response amplitudes in dpDp and behavioral responses to odors, we quantified odor preference at the end of training by the difference, $d$, between $ζ$ scores for Ala versus Trp or His ($d = ζ_{\text{Ala}} - ζ_{\text{Trp or His}}$; $d = 0$: no preference; $d > 0$: preference for Ala; $d < 0$: preference for Trp or His). The behavioral preference for the CS$^+$ was correlated to the relative amplitude of the CS$^+$ response after associative conditioning (ALA, TRP, HIS): stronger neural responses to the CS$^+$ predicted stronger behavioral preference for the CS$^+$ across individuals ($r = 0.37$, $P = 0.02$, $N = 43$ animals; Fig. 3c). In the same fish, the amplitude ratio of responses to odors that were not used in conditioning (Ser versus His or Trp) was not related to behavioral preference ($r = 0.00$, $P = 1$; Fig. 3d). Moreover, the Ala/Trp response ratio did not predict behavioral preference in UNC fish ($r = −0.22$, $P = 0.49$; Supplementary Fig. 3e). The relative enhancement of odor responses to the CS$^+$ therefore partially predicted behavioral preference after associative conditioning.

Further analyses showed that the mean lifetime sparseness of odor responses in ALA, TRP, HIS and UNC fish was significantly
The success of odor identification by template matching of odor-evoked activity patterns was not significantly different between any of the experimental groups (Fig. 7d). Hence experience did not significantly facilitate or impair odor identification by a simple classifier, supporting the notion that precise odor identification is unlikely to be a primary function of dpDp.

**Mapping odor space onto a representation of valence.** Associative conditioning and uncoupled odor exposure affected not only responses to conditioned odors but also modified responses to other odors in an odor- and task-dependent fashion, raising the possibility that experience-dependent modifications of odor representations generalize according to a global logic. We thus asked whether the organization of odor representations in dpDp could be mapped onto a low-dimensional structure that captures variations across individuals and experimental groups. In each fish, we described the organization of odor representations by the six pairwise cosine distances between the four odor-evoked activity patterns (Ala, Trp, His and Ser). The resulting six-element vector therefore characterized the organization of the olfactory coding space and is referred to as the ‘coding structure’ (Fig. 5a). Coding structures of individual fish from all groups (NAV, ALA, TRP, HIS and UNC) were pooled and then analyzed by principal component analysis.

The first two principal components (PC 1 and PC 2) represented 62% and 15% of the variance, respectively (Fig. 5b). In the space defined by these PCs, coding structures of ALA fish were close to those of NAV fish but partially separated from those of TRP and HIS fish. Coding structures of UNC fish were similar to those of NAV fish, with few exceptions. To further simplify this analysis, we focused on PC 1, which included high loadings (weights) on a subset of distances (Fig. 5c), implying that it did not represent the distance between a single odor pair or the global distance between all odor pairs. After projection onto PC 1, coding structures of TRP and HIS fish were separated significantly from those of NAV fish (TRP, \( P = 0.03 \); HIS, \( P = 0.03 \); Fig. 5d), whereas coding structures of ALA and UNC fish were not significantly different from NAV (ALA, \( P = 0.98 \); UNC, \( P = 0.30 \); Fig. 5d). Similar results were obtained when the coding space was not characterized by distances between odors of defined identity (for example, Ala versus Trp) but by distances between stimuli representing task-relevant categories (for example, CS\(^+\) versus CS\(^-\); Supplementary Fig. 4a). These results show that effects of different behavioral manipulations can be represented to a large extent by modifications of coding structures along a single dominant dimension.

We next examined the relationship between the organization of coding space and behavioral odor preference. In UNC fish, which had not learned to associate specific odors with reward, behavioral odor preference \( (d = \zeta_{\text{ Ala }} - \zeta_{\text{ Trp }}) \) was significantly correlated to the PC 1 score \( (r = 0.74, P = 0.006; \) Fig. 5c). Hence a high PC 1 score predicted a preference for Ala, whereas a low PC 1 score predicted a preference for Trp. In fish that were trained in an associative paradigm (ALA, TRP, HIS), behavioral preference values were distributed over a broader range. Nevertheless, the correlation between the behavioral odor preference \( d \) and the PC 1 score remained significant across all experimental groups \( (r = 0.52, P = 4 \times 10^{-5}; \) Fig. 5f; Supplementary Fig. 4). Correlations also remained significant when scores were obtained by projecting coding structures from a subset of the experimental groups onto PC 1 extracted from the other experimental groups (Supplementary Fig. 5). Shuffling of coding structures, however, reduced or abolished this correlation (Supplementary Fig. 4c,d). Hence the PC 1 score consistently predicted behavioral odor preference independent of the specific associations between odor and reward in different experimental groups. This indicates that PC 1 represents appetitiveness, and that experience modified the representation of odors along this

**Effects of experience on neuronal population activity.** Models of the olfactory cortex suggest that information storage involves the strengthening of recurrent excitatory connections among specific neurons\(^4\), which may increase the correlation of spontaneous and odor-evoked activity among neuronal subsets. To test this hypothesis, we quantified pairwise correlations between activity traces of simultaneously recorded neurons in the absence of stimuli (spontaneous correlation). On average, spontaneous correlations were positive in NAV fish and significantly increased after associative conditioning or uncoupled odor exposure \( (P < 10^{-15} \text{ for all groups}; \text{Fig. 4a}) \). These observations cannot be explained by chance effects because shuffling of trials abolished correlations. Similarly, correlations between tuning curves (signal correlations) of dpDp neurons in the same individuals were positive in NAV fish and further increased by experience \( (P < 10^{-15} \text{ for all comparisons}; \text{Fig. 4b}) \). Across individuals, signal correlations were at chance level in NAV fish \( (P = 0.89; \text{Fig. 4b}) \) but became positive after associative conditioning or uncoupled odor exposure \( (P < 10^{-15} \text{ for all groups}; \text{Fig. 4b}) \). These effects cannot be explained by a general increase in activity after conditioning (Supplementary Fig. 3f,g). Associative conditioning and uncoupled odor exposure therefore strengthened pairwise neuronal correlations, which is consistent with models of auto-associative memory\(^4,9\).

To further analyze the effects of experience on the structure of population activity patterns, we subtracted the mean matrix of cosine distances between activity patterns in NAV fish \( (\text{Fig. 11}) \) from the distance matrices of other experimental groups. The resulting difference matrices \( (\text{Fig. 4c}) \) reflect effects of experience on the similarity of odor representations, independent of effects on response amplitude. We found that ALA training had small and heterogeneous effects on pairwise pattern distances. On average, differences in cosine distances between ALA and NAV fish were indistinguishable from variability across individual NAV fish \( (\text{Fig. 4d}) \). TRP and HIS training, in contrast, significantly increased the mean distance between odor representations \( (\text{TRP, } P = 0.02; \text{HIS, } P = 0.02; \text{Fig. 4d}) \). This increase was observed for all odor pairs and was particularly pronounced between representations of the CS\(^+\) (Trp or His) and other stimuli \( (\text{Fig. 4c}) \). UNC fish showed a trend toward increased pattern distances that was, however, not significant when compared with NAV fish \( (P = 0.33; \text{Fig. 4d}) \).

To test for a more specific reorganization of pattern distances, we summed absolute distance values in each individual distance matrix, after centering each matrix on the group-specific mean and subtracting the mean centered distance matrix of NAV fish. This measure detects differences in specific pattern distances even in the absence of a change in the mean cosine distance and was also significantly increased in TRP and HIS fish \( (\text{TRP, } P = 0.0004; \text{HIS, } P = 0.02; \text{Fig. 4e}) \), but not in ALA and UNC fish \( (\text{ALA, } P = 0.97; \text{UNC, } P = 0.20) \), when compared with NAV fish. Hence associative conditioning had specific effects on the structure of population activity patterns in dpDp that depended on the association between specific odors and reward. This reorganization of the structure of odor representations was not significant when an intrinsically appetitive odor (Ala) was chosen as CS\(^+\) and the CS\(^-\) was intrinsically neutral (Trp or His), but it was pronounced when the CS\(^+\) was intrinsically neutral (Trp or His) and the CS\(^-\) was intrinsically appetitive (Ala). Hence the amount of reorganization of odor representations may be related to changes in odor–value associations.

higher than in NAV fish \( (P < 10^{-15} \text{ for all comparisons}; \text{Fig. 3e}) \). Consistent with this observation, the slope of tuning curves constructed by rank ordering of odor responses in individual neurons was significantly increased \( (\text{Fig. 3f}) \). Neurons in dpDp therefore became more sharply tuned after associative conditioning and uncoupled odor exposure.
Fig. 4 | Experience strengthens pairwise correlations and modifies neuronal population activity in dpDp. a. Correlation of spontaneous activity after associative conditioning and uncoupled odor exposure was significantly higher than in NAV fish (data; Kruskal–Wallis test, n = 8,991, d.f. = 4, H = 431.64, P < 10^{-15}). Nonparametric multiple comparisons against NAV, two-sided: ALA, Q = 17.33, P < 10^{-15}; TRP, Q = 12.43, P < 10^{-15}; HIS, Q = -4.88, P = 2 × 10^{-4}; and UNC, Q = -14.76, P < 10^{-15}. Shuffling of time bins abolished correlations (data versus shuffled; paired t test, two-sided, d.f. = n−1). NAV, t = 79.16, P < 10^{-15}; ALA, t = 72.86, P < 10^{-15}; TRP, t = 80.06, P < 10^{-15}; HIS, t = 58.90, P < 10^{-15}; and UNC, t = 76.33, P < 10^{-15}). Number of neurons is as in Fig. 3e. Box plot: center line, median; box limits, interquartile range; and whiskers, s.d. b. Correlation of odor tuning curves (signal correlation) after associative conditioning and uncoupled odor exposure was significantly higher than in NAV fish, both within the same fish (left; Kruskal–Wallis test, n = 6,202, d.f. = 4, H = 332.18, P < 10^{-15}) and across fish (right; H = 785.84, P < 10^{-15}). Only neurons that responded to at least one odor were included in this analysis. Nonparametric multiple comparisons against NAV within fish (n = 1,322), two-sided: ALA, Q = 14.18, P < 10^{-15}, t = 1.325; TRP, Q = 16.86, P < 10^{-15}, n = 1603; HIS, Q = -12.14, P < 10^{-15}, n = 992; and UNC, Q = -10.45, P < 10^{-15}, n = 960. Nonparametric multiple comparisons against NAV across fish (n = 1,322), two-sided: ALA, Q = -14.38, P < 10^{-15}; TRP, Q = -14.95, P < 10^{-15}; HIS, Q = -26.57, P < 10^{-15}; and UNC, Q = -5.31, P = 2 × 10^{-4}. Correlations were abolished after shuffling of stimulus labels (data versus shuffled, paired t test, two-sided, d.f. = n−1). Within fish: NAV, t = 31.68, P = 9 × 10^{-20}; ALA, t = 48.93, P = 3 × 10^{-7}; TRP, t = 48.68, P = 4 × 10^{-7}; HIS, t = 39.17, P = 2 × 10^{-12}; and UNC, t = 38.59, P = 2 × 10^{-12}. Across fish: NAV, t = -0.14, P = 0.89; ALA, t = 14.28, P = 4 × 10^{-4}; TRP, t = 19.55, P = 2 × 10^{-12}; HIS, t = 19.55, P = 2 × 10^{-12}; and UNC, t = 4.57, P = 5 × 10^{-4}. The box plot is as in a. c. Effects of experience on the cosine distance between odor-evoked activity patterns. The triangles above and below the diagonal contain the difference between each matrix and the distance matrix of NAV fish (Fig. 1f). Increases (decreases) in pattern distance are depicted by blue (red) colors. The number of animals is as in a. d. Mean pairwise cosine distance of activity patterns (pattern separation) was increased in TRP and HIS fish (Kruskal–Wallis test, N = 68 animals, d.f. = 4, H = 10.71, P = 0.03). Nonparametric multiple comparisons against NAV, two-sided: ALA, Q = -0.51, P = 0.96, N = 12; TRP, Q = -2.35, P = 0.02, N = 16; HIS, Q = -2.59, P = 0.02, N = 15; and UNC, Q = -1.38, P = 0.33, N = 12. The box plot is as in a. e. Mean absolute difference of centered distance matrices to the mean matrix of NAV fish was increased in TRP and HIS fish (Kruskal–Wallis test, H = 17.84, P = 0.0008). This measure quantifies the reorganization of the structure of distance matrices (see Methods) relative to the mean NAV matrix, even in the absence of a change in mean pattern distance. Nonparametric multiple comparisons against NAV, two-sided: ALA, Q = -0.49, P = 0.97; TRP, Q = -3.71, P = 0.0004; HIS, Q = -2.50, P = 0.02; and UNC, Q = -1.65, P = 0.20. The number of animals and d.f. are as in d. The box plot is as in a. Gray dotted lines indicate zero, and black dotted lines indicate the median of NAV fish. NS: P ≥ 0.05; *P < 0.05; ***P < 0.001.

Experience may also modify odor representations along other dimensions, but we did not observe a significant correlation between other principal components and behavioral odor preference (Supplementary Fig. 4g). Consistent with the low dimensionality of dpDp representations, the variance represented by PC 1 remained high in a dataset containing responses to eight odors (28-dimensional coding structures; NAV fish; Supplementary Fig. 4f), but experiments using additional odors are required to assess the precise dimensionality of behaviorally relevant dpDp representations. In summary, dpDp maps odor representations onto an
Articles which is consistent with previous observations38. To hyperpolarize but scattered GFP-positive somata were found throughout Dp, responses was significantly higher after associative conditioning or among fish from the same crossing, the PIN-induced increase of responses (Fig. 6c–e and Supplementary Fig. 6b). When compared to control conditions and during photoinhibition of interneurons (PIN).

Experience-dependent modification of inhibition. Memory storage depends not only on modifications of excitatory interactions but also includes inhibitory interactions that may shape neuronal tuning curves and activity patterns. To examine potential functions of inhibition, we targeted halorhodopsin–yellow fluorescent protein (YFP; eNpHR3.0YFP)36 to GABAergic interneurons using transgenic (Tg) line that contained a Tg(gad1b:Gal4) driver and a Tg(UAS:eNpHR3.0YFP) responder (see Methods). In adult fish, eNpHR3.0YFP was detected in scattered somata and fibers throughout Dp (Fig. 6a and Supplementary Fig. 6a). Because the membrane association of eNpHR3.0YFP complicated the detection of somata within the densely labeled neuropil, we also examined a Tg(gad1b:GFP) line that expressed cytosolic green fluorescent protein (GFP) under the control of the same promoter37. GFP-positive somata were particularly dense below the anterior nucleus taeniae, but scattered GFP-positive somata were found throughout Dp, which is consistent with previous observations38. To hyperpolarize interneurons in a subset of trials, we directed orange light (594 nm) at Dp through an optical fiber (Fig. 6b) for 6.2 s, starting approximately 500 ms before the onset of odor stimulation. This approach allowed us to compare activity across the same neurons under control conditions and during photoinduction of interneurons (PIN).

PIN significantly increased spontaneous activity and odor responses (Fig. 6c–e and Supplementary Fig. 6b). When compared among fish from the same crossing, the PIN-induced increase of responses was significantly higher after associative conditioning or uncoupled odor exposure than in NAV fish ($P < 10^{-12}$, for all groups; Fig. 6f). These observations show that experience increased inhibitory components of odor responses.

We next examined subtractive and divisive effects of inhibition. Uniform subtractive inhibition decreases all responses by a constant amount, which can sharpen tuning and sparsify activity patterns. This form of inhibition has been observed in PC upon silencing of somatostatin-expressing interneurons34. Uniform divisive inhibition, in contrast, decreases activity by a constant factor and scales activity patterns without modifying their structure or sparseness. To determine whether effects of inhibition are better described by a subtractive or by a divisive model, we fitted a linear function to all responses of individual neurons (Fig. 6g). In NAV fish, the slope of the linear fit was significantly greater than unity ($r = 0.905 \pm 0.03$, mean $\pm$ s.d.), which is consistent with primarily divisive inhibition.

Further analyses showed that PIN reduced the SBR (Supplementary Fig. 6d) and significantly reduced the mean lifetime sparseness of odor responses (NAV, $P = 2 \times 10^{-11}$; ALA, $P = 8 \times 10^{-47}$;
In fish that underwent associative conditioning (ALA, TRP, HIS), the relative amplitudes of responses to CS\(^+\) and CS\(^-\) during PIN remained correlated to behavioral odor preference in individual fish (r = 0.35; P = 0.02; Fig. 7b). No systematic relationship was observed between the amplitude ratio of responses to nonconditioned odors (Ser versus His or Trp) during PIN and behavioral odor preference (r = −0.09, P = 0.58; Fig. 7c). The CS\(^+\)/CS\(^-\) response ratio during PIN therefore still predicted behavioral odor preference after conditioning, as observed under control conditions. Classification of odor-evoked activity patterns by template matching was not significantly different during PIN in any of the experimental groups (Fig. 7d).

Inhibition may contribute to systematic modifications of odor-evoked activity patterns after experience. If so, inhibitory components of odor responses should have two properties. First, inhibition in individual neurons should not be uniform across odors but exhibit nonuniform components that shape odor tuning. Second, effects of experience on the tuning of inhibition should be coordinated across different neurons to shape population activity patterns in a systematic fashion.

Consistent with nonuniform inhibition in individual neurons, linear models of inhibition did not fully explain the effects of PIN on odor responses, as indicated by the substantial scatter around linear fits (cf. Fig. 6g). More detailed analyses of individual neurons showed that effects of PIN on odor responses were nearly uniform in some neurons but highly odor dependent in others (Fig. 7e). To further characterize nonuniform inhibition, we measured the relative PIN-induced response change for each neuron–odor pair by a change index (ChI) that varies between −1 and 1 (see Methods). A low variation of the ChI across odors implies that the effects of PIN are largely uniform (divisive), whereas a high variation implies nonuniform inhibition. We therefore define the s.d. of the ChI as a ‘nonuniformity index’ for each neuron. As a control, we used the
Fig. 7 | Nonuniform effects of inhibition in dpDp. a, PIN decreased tuning sharpness, and this effect was enhanced after associative conditioning. Box plots quantify differences in lifetime sparseness of the same neurons under control conditions and during PIN (Kruskal–Wallis test, n = 8,991, d.f. = 4, H = 167.75, P < 10−10). Comparisons of PIN versus control (paired t test, two-sided): NAV, t = 7.09, P = 2 × 10−12; ALA, t = 18.12, P = 8 × 10−10; TRP, t = 20.09, P = 2 × 10−6; HIS, t = 13.74, P = 4 × 10−4; and UNC, t = 2.92, P = 0.004. Nonparametric multiple comparisons against NAV, two-sided: ALA, Q = 7.51, P = 1 × 10−3; TRP, Q = 9.30, P < 10−6; HIS, Q = 4.87, P = 2 × 10−4; and UNC, Q = −1.68, P = 0.18. The number of neurons and d.f. are as in Fig. 3c. Box plot: center line, median; box limits, interquartile range; and whiskers, s.d.

b, Relative response amplitudes to training odors (ALA versus Trp or His) remained correlated to behavioral odor preference in individual ALA, TRP and HIS fish during PIN. Pearson correlation: r = 0.35 (t test of null hypothesis of r = 0, two-sided, t = 2.40, P = 0.02). Kendall’s rank correlation: r = 0.30 (test of null hypothesis of r = 0 using a normal approximation, two-sided, z = 2.79, P = 0.005). The number of animals and d.f. are as in Fig. 3c. c, Relative response amplitudes to neutral odors (Ser versus His or Trp) remained uncorrelated to behavioral odor preference in individual ALA, TRP and HIS fish during PIN. Pearson correlation: r = −0.09 (t test of null hypothesis of r = 0, two-sided, t = −0.56, P = 0.58). Kendall’s rank correlation: r = −0.07 (test of null hypothesis of r = 0 using a normal approximation, two-sided, z = −0.68, P = 0.49).

Number of animals and d.f. are as in Fig. 3c. d, PIN did not affect odor identification by template matching. Bars show percentage of correct odor identifications under control conditions (open bars) and during PIN (orange bars). Control versus PIN comparisons, McNemar test, d.f. = 1: NAV, χ² = 0, P = 1, n = 156 trials; ALA, χ² = 0.25, P = 0.62, n = 144; TRP, χ² = 0.17, P = 0.68, n = 192; HIS, χ² = 0.5, P = 0.48, n = 180; and UNC, χ² = 0.5, P = 0.48, n = 144). Multiple comparisons against NAV (Pearson χ² test, d.f. = 1), control responses: ALA, χ² = 0.01, P = 0.90; TRP, χ² = 0.58, P = 0.45; HIS, χ² = 4.5, P = 0.03; and UNC, χ² = 1.06, P = 0.30. Multiple comparisons against NAV (Pearson χ² test, d.f. = 1), PIN responses: ALA, χ² = 0.50, P = 0.48; TRP, χ² = 0.25, P = 0.62; HIS, χ² = 0.38, P = 0.54; and UNC, χ² = 0.07, P = 0.80. e, Odor responses of two individual neurons under control conditions (different colors) and during PIN (orange). Top: PIN-induced disinhibition was nonuniform across odors. Bottom: PIN-induced disinhibition was largely uniform. Numbers below traces show odor-specific change indices. SDinh shows s.d. across the four change indices, a measure of the nonuniformity of inhibition. Vertical scale bars: 20% ΔF/F; horizontal scale bars: 10 s. f, Nonuniformity of inhibition. Filled bars (orange; CP) show mean nonuniformity of inhibition, calculated by comparing control with PIN trials. Hollow black bars (CC) show the same analysis comparing control trials with each other. Hollow orange bars (PP) show the same analysis comparing PIN trials with each other. Comparison of CC versus CP (paired t test, two-sided): NAV, t = −31.78, P = 4 × 10−66; n = 1,433; ALA, t = −30.05, P = 1 × 10−50; n = 1,363; TRP, t = −9.37, P = 2 × 10−20; n = 1,650; HIS, t = −10.09, P = 3 × 10−21; n = 1,740; and UNC, t = −22.53, P = 2 × 10−11; n = 1,151. Comparison of PP versus CP (paired t test, two-sided): NAV, t = 35.78, P = 7 × 10−20; ALA, t = 36.72, P = 1 × 10−20; TRP, t = 42.99, P = 2 × 10−20; HIS, t = 41.14, P = 6 × 10−20; and UNC, t = 28.72, P = 4 × 10−10. Only PIN-modulated, responding neurons were considered for this analysis (see Methods). The number of animals is as in Fig. 4d. The box plot is as in a. Gray dotted lines indicate zero, except in panel d, where it indicates chance level (25%); and black dotted line indicates the median of NAV fish. NS: P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001.

same procedure to compare successive trials with the same odors under the same conditions (control–control or PIN–PIN). The non-uniformity index was significantly higher than the control indices in all experimental groups (P < 10−10 for all comparisons; Fig. 7f), confirming that inhibition had significant nonuniform effects on individual neurons.

Remapping of odor–valence relationships involves inhibition. To explore whether nonuniform inhibition is coordinated throughout the population, we examined the effects of PIN on cosine distances between odor-evoked activity patterns. If inhibitory components of odor representations are uncoordinated, effects of PIN are expected to be unspecific and global. However, we found that effects of PIN systematically depended on the odor and task. PIN significantly decreased cosine distances between odor-evoked activity patterns in all groups except NAV (Fig. 8a,b). As a consequence, differences in pattern distances between experimental groups disappeared (Supplementary Fig. 6e,f). Furthermore, the pattern of PIN-induced distance changes was similar in structure, but opposite in sign, to the pattern of distance changes induced by associative conditioning or uncoupled odor exposure in each experimental group. Consistent with this observation, PIN-induced distance changes
were negatively correlated to experience-related distance changes across all fish and odor pairs (ALA, $r = -0.77$; TRP, $r = -0.80$; HIS, $r = -0.81$; UNC, $r = -0.50$; Fig. 8c). These results show that inhibitory response components systematically depended on associations between odors and reward, implying that the effects of inhibition were coordinated across the population of neurons in dPdP.

The observation that the effects of PIN on odor representations in dPdP were mirror symmetric to the effects of experience (Fig. 8b,c) indicates that changes in inhibitory response components contributed significantly to the reorganization of odor representations during associative conditioning. To corroborate this conclusion, we projected coding structures onto the first two principal components and found that PIN reduced distances between coding structures from different experimental groups (Fig. 8d and Supplementary Fig. 6g). This observation cannot be explained by a change in response amplitudes because coding structures were defined by cosine distances. Moreover, in other distance metrics, a nonspecific increase in response amplitude would be expected to increase, rather than decrease, differences between activity patterns.

Hence PIN had specific effects on coding space that were opposite to those of experience, which is consistent with the hypothesis that experience-dependent reorganizations of coding space included coordinated plasticity of inhibitory response components.

This conclusion was further supported by the observation that PIN-induced changes in PC 1 scores were negatively correlated to the PC 1 score under control conditions: a large initial PC 1 score predicted a large PIN-induced change of opposite sign (Fig. 8e; over all fish, $r = -0.74$, $P = 4 \times 10^{-13}$, $N = 68$ animals; see also Supplementary Fig. 6h). Hence PIN systematically reduced interindividual variations in odor–valence mappings across all experimental groups. Nevertheless, PIN did not fully abolish differences in PC 1 scores between experimental groups. Moreover, the PC 1 scores during PIN, although reduced, still showed a residual positive correlation to behavioral odor preference (Supplementary Fig. 6j,k).
These observations indicate that the observed modifications of excitatory response components (cf. Fig. 3a) also contribute to interindividual variations in odor–value maps. Our results therefore indicate that experience results in coordinated modifications of excitatory and inhibitory interactions that both contribute to the remapping of odor space onto an axis of valence in dpDp.

**Discussion**

Our results revealed multiple effects of experience on odor-evoked activity in dpDp that collectively had two major consequences. First, associative conditioning enhanced the representation of the CS+ relative to the representation of the CS−. Second, manipulations of odor–reward associations remapped representations of odors along a behaviorally relevant axis of valence in an experience-dependent fashion. Remapping of odor representations involved a global increase in inhibition and specific effects on inhibitory components of neuronal tuning curves, as revealed by PIN. Hence modifications of inhibitory response components play an instructive role in the experience-dependent reorganization of odor–valence associations in dpDp.

**Experience-dependent remapping of odor representations.** Unlike odor-evoked activity in other subregions of Dp26,27 and in subregions of the PC4,5,7,39, odor responses in dpDp were dense and not very odor selective. Activity in dpDp represented a combination of sensory and task-related information, which is generally consistent with neuronal activity in the posterior PC33,42; the phylogenetic relationships, however, between subregions of Dp and the mammalian olfactory cortex remain to be clarified. To manipulate odor–value associations, we conditioned a familiar feeding behavior on olfactory cues31. Although activity was measured in an ex vivo preparation after conditioning, multiple features of neuronal activity were highly correlated to interindividual variations in odor preference. Activity patterns therefore reflected behaviorally relevant variations in neuronal circuit function.

Associative conditioning and uncoupled odor exposure enhanced correlations of spontaneous activity and odor responses between individual neurons, which is consistent with predictions of auto-associative memory models. However, most memory models consider a regime of high dimensionality and high memory capacity2. The high pattern and signal correlations in dpDp, in contrast, indicate that activity in dpDp is low-dimensional and not optimized for high storage capacity, which is consistent with the hypothesis that dpDp maps odor responses onto a low-dimensional output.

One effect of associative conditioning was a general enhancement of odor responses that was more pronounced for the CS+ than for the CS−. Enhanced representations of rewarded stimuli have also been observed in other brain areas including the auditory and visual cortex43–45. In these brain areas, conditioning usually increases the discriminability of stimulus representations, suggesting that plasticity supports perceptual learning. In dpDp, in contrast, experience did not affect pattern discriminability, suggesting that enhanced responses primarily increase the impact of rewarded stimuli onto appetitive behavior.

A second main effect of associative conditioning was a systematic reorganization of olfactory coding space in dpDp along a dimension closely related to valence. Interindividual variations in the organization of olfactory coding space predicted variations in behavioral odor preference across experimental groups and even among individuals within the same group. Hence dpDp maps odor space onto a low-dimensional representation of valence that is modified by experience and likely to have a direct influence on appetitive behavior. These results are consistent with the notion that the dimensionality of perceptual space in olfaction is low, although the dimensionality of chemical stimulus space may be high46.

The finding that the organization of odor representations predicted appetitive behavior across individuals implies that associative learning modified coding space, whereas the valence axis remained consistent. This indicates that the quality of information transmitted by individual neurons remains stable when odor–value associations change, which is important when neurons have defined effects on behavior. Associative learning by a reorganization of coding space is thus a useful strategy in networks that operate close to outputs controlling behavior. In *Drosophila*, valence is encoded by a dense population code across mushroom body output neurons that project to specific target areas47,48. In this system, valence is represented along a fixed axis, whereas the mapping of odors onto this axis can be modified by experience49, similar to our observations in dpDp. Hence vertebrates and insects may use similar strategies to associate odors with low-dimensional, internal variables.

**Experience-dependent plasticity of inhibition.** To manipulate inhibition, we expressed *enPHR3.0YPF* under the control of the *gad1b* promoter, which targets interneurons broadly. PIN resulted in a pronounced disinhibition in dpDp, but incomplete suppression of action potential firing in subsets of interneurons cannot be excluded. Nonetheless, effects of PIN were highly correlated to interindividual variations in odor-evoked activity and behavior (Fig. 8 and Supplementary Fig. 6), implying that effects of PIN were reliable and effective across individuals.

In PC, photoinhibition of somatostatin-expressing interneurons revealed a form of global subtractive inhibition50, whereas our more broadly targeted approach revealed primarily divisive inhibition in dpDp. Divisive inhibition can efficiently normalize activity in auto-associative networks, which is important to counterbalance recurrent amplification51. Consistent with such a function, PIN unmasked an enhancement of divisive inhibition after associative conditioning that partially balanced the increase in excitatory odor responses.

Inhibition also had nonuniform effects on tuning curves that were systematically modified by associative conditioning, resulting in coordinated changes of inhibitory response components across the population. The underlying synaptic modifications may involve multiple cell types and connections. In principle, specific modifications of inhibition may be indirect consequences of plasticity at synapses between excitatory neurons. Alternatively, modifications of inhibition may involve plasticity of synapses from excitatory neurons to interneurons, from interneurons to excitatory neurons, or both. This hypothesis is consistent with the complex nonuniform effects of PIN and their specific contributions to odor–valence mapping. Moreover, this scenario is consistent with the observation that inhibitory inputs to neurons in posterior Dp exhibit nonrandom tuning52 and with learning-dependent changes in the response selectivity of interneurons in other brain areas53.

Coordinated plasticity of inhibitory response components contributed significantly to experience-dependent plasticity of odor representations in dpDp. PIN reduced interindividual variations in PC scores, even among NAV fish, resulting in the convergence of coding structures toward a common ‘baseline state’. Moreover, effects of PIN mirrored effects of experience on pairwise distances between activity patterns across all odors and fish. Hence a substantial fraction of task-dependent changes and other interindividual variations in odor–value maps can be explained by variations in patterned inhibition. However, PIN did not completely abolish remapping of odor representations, suggesting that plasticity of interactions between excitatory neurons also contributed to the reorganization of odor responses. These results suggest that inhibitory network plasticity enhances excitatory plasticity, particularly during specific memory operations.
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Competing interests
The authors declare no competing interests.

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Methods
Animals and Tg lines. All experiments were performed using adult zebrafish (Danio rerio) of both sexes, aged 7 ± 4 months (mean ± s.d.). Fish were raised and kept under standard laboratory conditions (26–27 °C, 13 h/11 h light-dark cycle). Unless otherwise stated, experiments were performed in a double-T g-line that expressed the light-sensitive chloride pump halorhodopsin (eNpHR3.0) (ref. 49) fused to U2AF under the control of the glutamate decarboxylase 1 promoter Tg(gad1b-Ga4, UAS:eNpHR3.0YFP). This fish (gad1b–NpHR) originated from three different crossings (each crossing: females and males). Most fish (9 N; 14 A, 13 TRP and 8 UNC) originated from the first crossing. The remaining fish (4 N; 3 TRP; 15 HS; and 4 UNC) originated from two additional crossings of other individuals. All fish used in the study were assigned randomly to the different training groups. For activity measurements and analysis of innate behavioral odor preference, NAV, were chosen randomly from large populations (>40) of gad1b–NpHR fish. The following non-gad1b–NpHR fish were used in addition: Tg(gad1b–GFP) (ref. 33) (N = 3) and a population of 5 NAV adult zebrafish (Supplementary Fig. 4N; N = 15 animals), and Tg(UAS:eNpHR3.0YFP) fish (Supplementary Fig. 6c; N = 2 animals). Experiments were approved by the Veterinary Department of the Canton Basel-Stadt (Switzerland).

To target optogenetic probes to GABAergic interneurons in Dp, we used the promoter of the gad1b gene (formerly Gad67), which encodes one of the two major isoforms of glutamate decarboxylase in zebrafish28. To enhance the visualization of somata, we also analyzed Tg(gad1b–Ga4) fish49. Throughout Dp; the number and distribution of somata expressing eNpHR3.0YFP in Tg(gad1b-Ga4, UAS:eNpHR3.0YFP) fish and GFP in Tg(gad1b–GFP) fish were compared with the with-o and consistent with published expression patterns28.

A high density of positive somata was found posterior to the prominent mediolateral furrow that runs along the anterior border of the nucleus taeniae. In addition, scattered somata and dense fibers were observed throughout the volume of Dp (Fig. 6u). The number of positive somata was substantially higher than in other Tg lines that target distinct subsets of interneurons in Dp (Tg.EF1α:GFP and Tg.KIF14:GFP). This suggests that the gad1b promoter targeted multiple subtypes of interneurons that account for the majority of, or possibly all, interneurons in Dp.

The UAS:eNpHR3.0YFP expression construct was generated using the To2Kit29, which involved a multisite recombination reaction (invitroGen Multisite Gateway max kit, D.2007) between pSE–UAS (5×UAS and Etf minimal promoter30), pME–eNpHR3.0YFP (third-generation halorhodopsin fused to YFP31) and pSE–polyA as entry vectors, and pDestTol2CG2 as destination vector52. A stack of OGB-1-AM was dissolved in 30 μM stock solutions in deionized water (Fluka), vortexed, sonicated, stored at 20 °C and diluted to a final concentration of 10–5 M in ACSF immediately before the experiment. Oligos were applied in blocks of six trials. In each block, three control and three PIN trials were interleaved. Interstimulus intervals were 2.25 min ± 5% (mean ± s.d.). The sequence of odor blocks was varied across animals to avoid systematic biases, but each experiment was started with one of the neutral odors, followed by one of the familiar odors, the second neutral odor and the second familiar odor. Odors were applied to the nasal epithelium for ~3 s through a controlled stream of ACSF using a syringe pump (Harvard Apparatus) and delivered intranasally to the nostril using a 30° angle needle (Rhodent) as described previously66. No odor stimuli were presented before the first stimulus in an experiment to minimize potential effects of passive odor exposure on odor responses67.

Image acquisition and optical stimulation. Multiphoton calcium imaging in Dp was performed using a custom-built multiphoton microscope38, a 20× objective (NA 1.0; Zeiss) and ScanImage/Ephus software. Fluorescence was excited at 928 nm, and emission was detected by a gated GaAsP photomultiplier tube (PMT; Hamamatsu) through a bandpass emission filter (535/50 nm). In addition, a narrow blocking filter centered on 594 nm was placed in front of the PMT. Laser intensity was adjusted in each focal plane to minimize photobleaching. In each trial, images with 256 lines were acquired at 128 ms per frame, starting approximately 33 s before odor onset in each trial. After each trial, the field of view was re-adjusted to compensate for potential drifts using an automated routine that acquired a small z stack of ≥3 μm (step size: 0.5 μm).

For optical stimulation, orange laser light (594 nm) was directed at the posterior Dp through an optical fiber (200-μm diameter; ThorLabs) positioned approximately 100–200 μm from the brain surface. Even though light was directed at the posterior Dp, we cannot rule out that scattered light also had weak effects in adjacent regions. Pulses of light (450 ps) were coupled into the fiber using a digital delay instrument33 and produced by a second pulse of line im injection over the entire range of imaging time. The same procedure was applied to trials without photo-stimulation, resulting in final images with 10 lines and a fill fraction of approximately 40% under all conditions. The intensity of orange light at the tip of the fiber was delivered over the duration of each trial (~6–8 s). In Tg(gad1b-Ga4, UAS:eNpHR3.0YFP) fish that did not carry the gad1b-Ga4 transgene (N = 2), orange laser light had no detectable effect on neuronal activity (Supplementary Fig. 6c).

Regions of interest were drawn manually, using custom-made software (https://github.com/r-namekawa/Pymagen), over all somata in each image plane. The time series of raw fluorescence, averaged over all pixels, was low-pass filtered by a 2-s rolling average, and the baseline fluorescence F0 was defined as the minimum over all time points to calculate the relative change in fluorescence (ΔF/ΔF0) in each region of interest. Time series of Ca2+ signals were aligned to the start of odor-evoked activity to correct for small variations in stimulus onset times across animals. ΔF/ΔF0 was averaged over a 2-s time window starting at response onset. This resulted in a time window that was centered approximately on the peak of the population response (Fig. 1d). Variation of the temporal alignment procedure or of the analysis time window had minimal effects on the results.

Behavioral experiments. Associative conditioning was performed as described previously42. In brief, individual gad1b–NpHR fish were acclimated to the behavioral setup and trained to discriminate between a novel stimulus (CS+) with a food reward, whereas a second odor stimulus (CS−) was not rewarded. Nine trials per odor were delivered each day in odor-alternating sequence (intertrial interval: 20 min). Fish received 27–36 trials of each odor (N = 32 ± 2.5 trials, mean ± s.d.; N = 43 animals; no dependence of discrimination performance on total trial number: Warden corn wall, χ2 = 0.08, df = 1, p = 0.76). Swimming trajectories were monitored three-dimensionally. Appetitive behavioral response components included increased swimming speed, elevated position in water column, increased presence in reward zone, increased surface sampling, decreased distance to odor inflow and decreased rhythmic circular swimming. These behavioral response components were quantified during the 30 s after odor onset and before reward delivery by automated analyses routines, and combined into a composite score of appetitive behavior, ζ (ref. 42). Odor preference was measured as the mean difference between ζ scores in response to the CS+ and CS− over the last nine trials. Alternative metrics for quantification of overall discrimination performance gave similar results.

Uncoupled odor exposure was performed using a similar schedule except that for each block, the remaining 15 min after odor presentation and unstimulated control period were divided by approximately 5 s. The remaining 43 fish (12 ALA, 16 TRP and 15 HHS fish) comprised individuals with a wide range of preference scores (Supplementary Fig. 2c). A total of 12 out of 14 additional gad1b–NpHR fish that received uncoupled odor exposure were used for activity measurements (0.9 ± 0.7 mean ± s.d.) day after last odor exposure; 2 fish
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were excluded upon failed dissection. Exclusion criteria were established before the start of the study. When behavioral data from fish from different experimental groups were combined for analysis (Figs. 3, 5 and 7 and Supplementary Figs. 3–6), the sign of the discrimination score was adjusted to reflect preference for Ala. Image acquisition and primary data analysis of trained fish were performed blind to the odor–reward association of the respective fish for all ALA fish and 13 out of 16 TRP fish. For the remaining fish, the affiliation to the training group could not be concealed from the experimenter.

To analyze innate behavioral responses to odors, we placed individual gad1b–NpHR fish of both sexes (starved for 1 d; N = 71 animals) in a modified setup (no feeding ring, inflow and outflow tubes). Each fish was acclimated for at least 30 min and tested only once on one odor (and fish water facility as control). In each application, 1 ml of facility water or odor solution (His, Ser, Ala or Trp; 10−2 M in facility water) was applied to one corner of the tank during the 20 s after recording baseline swimming activity. We primarily analyzed swimming speed, which has been directly related to appetitive behavior in other tasks14,22. However, differential effects of Ala and the remaining odors were also observed for the distance to the odor application site. Behavioral parameters were normalized to the mean value over 50 s before stimulus onset and quantified over 40 s after stimulus onset.

Statistical analysis. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications15,44,50. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA). N indicates the number of animals (experiments), and unless stated otherwise, n indicates the number of neurons. Unless stated otherwise, sample means are reported as ± s.e.m.

We tested pooled samples for normality using the Jarque–Bera test. We used a repeated-measures t test for all paired samples with n > 250. For smaller sample sizes or if the null hypothesis of normality was rejected, we used nonparametric tests: nonparametric Kruskal–Wallis test followed by a Dunn–Hollander–Wolfe test for nonparametric multiple comparisons with one control group (reported P values are adjusted for multiple comparisons) or Wilcoxon signed rank test for paired samples.

To compare two samples of categorical data (for example, correctly identified trials in template-matching classification analysis), we used a Pearson χ2 test for unpaired samples (reported P values are not adjusted for multiple comparisons) or a McNemar test with Edward’s correction for paired samples. The correlation between two variables was quantified using either Pearson correlation (for linear relationships) or Spearman correlation (for nonparametric data).

To test whether the regression slope of linear fits was significantly larger than zero, the correlation coefficient was compared with a standard normal distribution and a P value was calculated.

Further analyses. Defining responses. For the analysis of signal correlation and SBR (see below), only neurons that responded to at least one odor were considered (criterion: mean Ca2+ signal during odor responses > 3 × s.d., of Ca2+ signal during prestimulus phase). Modifying this criterion had no major effect on results. SBR. To quantify discriminability of odor responses (signal) from the background (spontaneous) activity, we calculated the SBR for odor responses in individual trials (Supplementary Fig. 3b).

\[
\text{SBR} = \frac{(\Delta F/F)_{\text{odor}} - (\Delta F/F)_{\text{spont}}}{\sqrt{0.5 \times (\sigma_{\text{odor}}^2 + \sigma_{\text{spont}}^2)}}
\]

where \((\Delta F/F)_{\text{odor}}\) and \(\sigma_{\text{odor}}^2\) represent mean and variance of Ca2+ signal during odor response (3 s), and \((\Delta F/F)_{\text{spont}}\) and \(\sigma_{\text{spont}}^2\) represent mean and variance of Ca2+ signal before odor response (25.6 s). For odor-specific SBR comparisons (Supplementary Fig. 3c), we considered only neurons that responded to both odors. To compare PIN and control trials (Supplementary Fig. 5d), we determined background activity in separate trials. Neuron–odor pair trials with a Ca2+ signal z-score <2 were excluded from all SBR analyses; lifting this criterion had no effect on odor-specific SBR comparisons.

Sparseness measures: lifetime and population sparseness. Lifetime and population sparseness were calculated using the metric11:

\[
\text{Sparseness} = \frac{1 - \frac{\sum \sigma^2}{N}}{1 - \frac{1}{N}}
\]

This normalized metric describes the ‘peakness’ of a distribution and ranges between 0 (all responses are equal) and 1 (all responses are zero except one).

Lifetime sparseness is the distribution of response amplitudes across odors in single neurons. Population sparseness is the distribution of response amplitudes to a single odor across neurons.

Cosine distance. The cosine distance of two population vectors, \(\mathbf{x}\) and \(\mathbf{y}\), was defined as:

\[
\text{Cosine distance} = 1 - \cos(\theta) = 1 - \frac{\sum x_i y_i}{\sqrt{\sum x_i^2} \sqrt{\sum y_i^2}}
\]

Mean cosine distance matrices were calculated by averaging matrices of individual animals. To assess whether the mean cosine distance, across all six pairwise distances, differed from NAV fish (pattern separation; Fig. 6d and Supplementary Fig. 6e), we subtracted the mean cosine distance matrix of NAV fish from the cosine distance matrices in each individual fish (\(\Delta\) Cosine dist.). To assess differences in the structure of cosine distance matrices in each group (pattern reorganization; Fig. 6c and Supplementary Fig. 6f), we (1) centered cosine distance matrices of each fish by subtracting the mean distance of the corresponding experimental group, (2) subtracted the centered mean NAV matrix from the centered matrix of each fish, and (3) subsequently calculated the mean of the absolute values in the difference matrices (\(\langle|\Delta\text{Cosine dist.}|\rangle\)). Large values therefore indicate changes in specific distances that cannot be accounted for by a change in the mean distance.

Template matching. Odor classification by template matching21 was performed by choosing the population response vector of one trial as a test vector and comparing it with reference vectors for each odor (similar to leave-one-out cross-validation). Reference vectors were constructed by averaging over all trials except for the test trial. The test trial was then assigned to the odor that was represented by the reference vector with the lowest cosine distance entry. Similar results were obtained when Pearson correlation distance or Euclidean distance was used (Supplementary Fig. 1). To account for differences in the size of the population vectors between fish, we randomly selected n = 95 neurons in each classification (minimum population size across all animals: n = 98). This procedure was repeated 400 times in each fish, using sampling without replacement.

Spontaneous correlation. Spontaneous correlations of Ca2+ signals between pairs of neurons from the same animal were calculated over the last 20 s of the prestimulus phase of 28 trials (560 s total). Time traces were binned to 1-s bins.

Signal correlation. The analysis of signal correlation was restricted to neuron pairs in which both neurons responded to at least one odor. After determining signal correlations for all pairs in one animal, signal correlations were averaged over all pairs involving each neuron, resulting in a mean signal correlation for each neuron. Further averaging was then performed over neurons. Direct averaging of signal correlations over all pairs gave similar results. Signal correlations across animals were calculated accordingly.

Analysis of coding structures. The organization of odor representations in dPdP was defined in each fish by the six pairwise cosine distances between all four odor-evoked activity patterns (His, Ser, Ala and Trp). Vectors representing these ‘coding structures’ were pooled across all fish (NAV, ALA, TRP HIS and UNC; N = 68 animals) and optical stimulation conditions (control and PIN). The resulting 6 × 136 matrix (or its transform), representing a total of 136 coding structures, was the input for principal component analysis (Fig. 5 and Supplementary Fig. 4). In each of the six coding structure dimensions, the 136 individual values were centered by subtracting their mean. We did not scale each dimension to unit variance to retain information that may be contained in variance differences between dimensions.

Chl and analysis of nonuniform inhibition. Odor-specific effects of inhibition on individual cells were quantified by the Chl:

\[
\text{Chl} = \frac{(\Delta F/F)_{\text{PIN}} - (\Delta F/F)_{\text{control}}}{(\Delta F/F)_{\text{PIN}} + (\Delta F/F)_{\text{control}}}
\]

Mean strength of inhibition is given by the mean Chl over odors. Nonuniformity of inhibition is the s.d. of Chls across odors. Only neurons that were modulated by PIN were considered (criterion: P < 0.1 for a pairwise comparison between control and PIN trials; Wilcoxon signed rank test, two-sided). Note that with this threshold a selective modulation of one out of the four odor responses (that is, 3 out of 12 trials) does not suffice to classify a neuron as PIN modulated. In NAV fish, 87% of responsive neurons were classified as PIN modulated using this criterion.

Linear regression. To describe the effects of inhibition on odor responses, we fitted lines to different combinations of parameters derived from control and PIN responses, respectively (Figs. 6g and 8c,e and Supplementary Fig. 6h). Fitting was performed using a total least squares procedure, which minimizes the orthogonal distance of the regression line from the data points. Thus, this method does not
distinguish between dependent and independent variables, and accounts for
the equality of measurement errors in the two variables. In the remaining linear
regression analyses (Figs. 3c,d, 5e,f and 7bc and Supplementary Figs. 3–6) we used
a standard least squares procedure.

**Reporting Summary.** Further information on research design is available in the
Nature Research Reporting Summary linked to this article.

**Data availability**
The data that support the findings of this study are available from the
the corresponding authors upon reasonable request.

**Code availability**
All codes used in this study are available from the corresponding authors upon
reasonable request.

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐  The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

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☐  For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

Give P values as exact values whenever suitable.

☐  For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐  For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐  Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

We used Scanimage (v. 3.8.1; Pologruto et al., 2003) and custom code written in MATLAB 2013a and 2017a (The MathWorks) for data collection.

Data analysis

We analysed the imaging data with Pymagor 2.6 (https://github.com/i-namekawa/Pymagor) and custom code written in Igor Pro 8 (Wavemetrics) and MATLAB 2016a (The MathWorks). We analysed the behavioral data with TopSideMonitor 1.0 (https://github.com/i-namekawa/TopSideMonitor) and custom code written in Python 2.7, MATLAB 2013a and 2017a, and Igor Pro 8. Details of the different analyses are specified in the text and Online Methods.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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The data and code that support the findings of this study are available from the corresponding authors upon reasonable request.
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications (Chapuis & Wilson, 2012; Poort et al., 2015; Khan et al., 2018) |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | After termination of associative odor discrimination training, fish were ranked according to an initial assessment of their discrimination performance. Individuals were then successively selected for measurements of odor-evoked activity patterns following this rank order. In total, 49 out of 94 trained fish were used for activity measurements. Five of these fish had to be eliminated from the dataset later, due to technical problems during the behavioral training that became evident only after the fish already had been imaged, and one fish had to be discarded due to problems during the dissection procedure. Out of 14 fish that received uncoupled odor exposure, twelve were used for activity measurements and two fish were discarded due to problems during the dissection procedure. Exclusion criteria were established prior to the start of the study. |
| Replication | In both associative odor discrimination training and uncoupled odor exposure groups, fish from several different training batches (13 for discrimination training, five for uncoupled odor exposure) were imaged. From each training batch, at least two fish were imaged (except for one uncoupled odor exposure training batch, in which only one fish was imaged). Random selection of 50% of all fish in each group from the full dataset and subsequent analysis revealed that a major reduction in the number of datapoints had no obvious effect on key findings related to neuronal population activity analyses reported in the manuscript, indicating that results were not limited by the number of datapoints. Furthermore, for other analyses, we pooled neurons across fish as the pooled sample was not dominated by a small number of fish with unusual properties (“outliers”). In our datasets, the number of neurons did not vary much between fish, implying that no individual fish was strongly overrepresented by a high number of neurons. Moreover, for all measures analyzed in the manuscript, we calculated mean values for each fish and examined their distribution across fish. This analysis did not reveal any obvious outliers except in one case (mean lifetime sparseness of one UNC fish). To confirm these observations we tested for the deviation between the observed distributions from a normal distribution using the Jarque-Bera test. This test identified no deviations from a normal distribution except for the one case mentioned above. Eliminating this fish from the analysis had no major consequences. We therefore conclude that pooling of neurons across fish is justified and prudent because there is no evidence that the pooled sample is dominated by “outliers”. Thus, in summary, all attempts at replication were successful. |
| Randomization | All fish used in the study were assigned randomly to the different training groups. For activity measurements and analysis of innate behavioral odor preference, naive fish were chosen randomly from large populations (> 40) of gad1b-NpHR fish. |
| Blinding | Image acquisition and primary data analysis of trained fish were performed blind to the odor-reward association of the respective fish for all ALA fish and 13 out of 16 TRP fish. For the remaining fish, the affiliation to the training group could not be concealed from the experimenter. |

Reporting for specific materials, systems and methods

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| Materials & experimental systems | n/a |
|---------------------------------|-----|
| Involved in the study | Antibodies |
| Eukaryotic cell lines | Palaeontology |
| Palaeontology | Animals and other organisms |
| Animals and other organisms | Human research participants |
| Human research participants | Clinical data |

| Methods | n/a |
|---------|-----|
| Involved in the study | ChIP-seq |
| Flow cytometry | MRI-based neuroimaging |

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | All fish used in this study were adult zebrasfish (Danio rerio) of both sexes with an age of 7 ± 4 months (mean ± SD). With few exceptions (below), experiments were performed in a double-transgenic line that expressed the light-sensitive chloride pump halorhodopsin (eNpHR3.0) fused to yellow fluorescent protein (YFP) under the control of the glutamate decarboxylase 1b |
promoter Tg(gad1b:Gal4, UAS:eNpHR3.0YFP): ‘gad1b-NpHR’ fish. Exceptions (non gad1b-NpHR fish): Fig. 6a (Tg(gad1b:GFP)); Supplementary Fig. 4f (mixed population of naïve adult zebrafish); Supplementary Fig. 6c (Tg(UAS:eNpHR3.0YFP)). See Online Methods for more details.

Wild animals
The study did not involve wild animals.

Field-collected samples
The study did not involve samples collected from the field.

Ethics oversight
All experiments were approved by the Veterinary Department of the Canton Basel-Stadt (Switzerland).

Note that full information on the approval of the study protocol must also be provided in the manuscript.