One defining characteristic of the mammalian brain is its neuronal diversity. For a given region, substructure, layer or even cell type, variability in neuronal morphology and connectivity persists. Although it is well known that such cellular properties vary considerably according to neuronal type, the substantial biophysical diversity of neurons of the same morphological class is typically averaged out and ignored. Here we show that the amplitude of hyperpolarization-evoked sag potential recorded in olfactory bulb mitral cells is an emergent, homotypic property of local networks and sensory information processing. Simultaneous whole-cell recordings from pairs of cells show that the amount of hyperpolarization-evoked sag potential and current (\(I_h\)) is stereotypic for mitral cells belonging to the same glomerular circuit. This is corroborated by a mosaic, glomerulus-based pattern of expression of the HCN2 (hyperpolarization-activated cyclic nucleotide-gated channel 2) subunit of the \(I_h\) channel. Furthermore, inter-glomerular differences in both membrane potential sag and HCN2 protein are diminished when sensory input to glomeruli is genetically and globally altered so that only one type of odorant receptor is universally expressed. Population diversity in this intrinsic property therefore reflects differential expression between local mitral cell networks processing distinct odour-related information.

Neurons show a broad array of biophysical properties that profoundly impact the computations that they perform. Even within cell types, diversity in morphology, and expression of molecular markers and ion channels is well documented, but whether such variation reflects necessary biological noise or perhaps a functional, dynamic system for regulating excitability at the cellular or even network level remains unclear. The hyperpolarization-activated current \(I_h\) has also been observed as a sag potential) is one intrinsic biophysical property that is known to influence the input–output function of principal cells18. In the olfactory bulb, the broad amplitude distribution of \(I_h\)-mediated sag potential recorded across the mitral cell population in mouse is similarly uni-modal (\(P < 0.05\)) and broad (min = \(-10.4\) mV, max = \(30.65\) mV, median = \(3.43 \pm 5.8\) mV, \(n = 105\) cells, \(n = 39\) animals; Fig. 1B and Supplementary Fig. 1). To explore the possibility that such population diversity might reflect differences between local mitral cell networks (Fig. 1A), we performed simultaneous whole-cell recordings of sag from pairs of cells belonging to distinctly different (Fig. 1C, a) or the same glomerular ensemble (Fig. 1C, b). The mean sag potential (SPA) found under these two recording scenarios was not significantly different (inter-glomerular pairs, \(3.3 \pm 6.7\) mV, \(n = 52\) cells versus intra-glomerular pairs, \(2.45 \pm 3.72\) mV, \(n = 28\) cells (\(P = 0.41\); Fig. 1D). For each recorded pair we determined the absolute difference in SPA (Supplementary Fig. 2) and performed a multiple pair-wise comparison, whereby the SPA difference between each cell and all other cells within the same group—excluding its simultaneously recorded ‘partner’—was calculated (‘pseudo pairs’, Fig. 1E). For inter-glomerular pairs of mitral cells, the distribution of SPA difference between recorded and pseudo pairs was similar (recorded: min = \(0.03\) mV, max = \(21.06\) mV, median = \(3.57\) mV, \(Q_1 = 1.87\) mV, \(Q_3 = 5.35\) mV, \(n = 26\) pairs, versus pseudo pairs: min = \(0.01\) mV, max = \(41.05\) mV, median = \(4.435\) mV, \(Q_1 = 1.78\) mV, \(Q_3 = 9.46\) mV, \(n = 1300\) (\(P = 0.16\); Fig. 1E, F). This was also the case when comparing inter-glomerular recorded pairs and pseudo pairs extracted from our entire data set (\(n = 105\) cells, 5,460 pseudo pairs; Supplementary Fig. 2).

In contrast, the sag potential and the \(I_h\)-current amplitude recorded simultaneously from mitral cells belonging to the same glomerular network were virtually indistinguishable (Supplementary Fig. 2). Thus, the difference in the SPA recorded from intra-glomerular pairs was significantly smaller than that determined for inter-glomerular pseudo pairs (recorded SPA difference for intra-glomerular pairs: \(min = 0\) mV, \(max = 3.59\) mV, \(median = 1.22\) mV, \(Q_1 = 0.31\), \(Q_3 = 2.1\) mV, \(n = 14\) pairs, versus intra-glomerular pseudo pairs: \(min = 0\) mV, \(max = 12.75\) mV, \(median = 4.43\) mV, \(Q_1 = 1.99\), \(Q_3 = 6.41\) mV, \(n = 364\) pairs (\(P = 0.0002\); Fig. 1E, F and Supplementary Fig. 2), for inter-glomerular recorded pairs (\(P = 0.024\); Fig. 1F, G) and pseudo pairs extracted from all cells (\(n = 105\) cells; \(P = 0.0001\); Fig. 1F and Supplementary Fig. 2). The broad range of sag and \(I_h\)-current amplitudes recorded across the bulb therefore reflects differences between individual glomerular circuits, in which it is a homotypic feature of the local mitral cell network (Fig. 1G).

Cell-attached experiments in mitral cell apical dendrites indicate that the \(I_h\) current is largest in patches recorded in the very distal region. Thus, the recorded membrane potential sag may reflect activation of HCN channels that are expressed predominantly in the
Mitral cell sag. ORN, olfactory receptor nerve layer. (shown by different coloured circles) may be hetero- (left) or genetically unique input (shown in red, green and blue). Membrane potential population diversity of mitral cell sag. Each olfactory glomerulus receives cell networks. A pairs. a.u., arbitrary units.

Gaussian fits of the recorded data for intra-glomerular and inter-glomerular distribution of SPA recorded across the mitral cell population (individual data points of recorded SPA difference). Recordings, and intra-glomerular pseudo and recorded pairs. Open circles, F D Olfactory bulb A, Box-plot of recorded and pseudo inter-glomerular pairs, pseudo pairs of all recordings, and intra-glomerular pseudo and recorded pairs. Open circles, individual data points of recorded SPA difference. G, Histogram of sag amplitude difference for inter- and intra-glomerular recorded pairs fitted with a half Gaussian (n = 26 and 14 pairs, respectively; bin size, 3 mV). Inset, Gaussian fits of the recorded data for intra-glomerular and inter-glomerular pairs. a.u., arbitrary units.

Figure 1 | Diversity of sag potential amplitude within and between mitral cell networks. A, Schematic of two possible scenarios underlying the population diversity of mitral cell sag. Each olfactory glomerulus receives genetically unique input (shown in red, green and blue). Membrane potential sag (shown by different coloured circles) may be hetero- (left) or homogeneously (right) expressed with the glomerulus. ORN, olfactory receptor nerve layer. B, Histogram (fitted with a Gaussian curve) showing the distribution of SPA recorded across the mitral cell population (n = 105 cells). Traces, three examples recorded from cells belonging to the indicated bins. The sag potential amplitude is determined as the voltage difference between the peak hyperpolarization (shown by an asterisk) and the steady-state membrane potential (shown by two asterisks). C, Example morphologies of two simultaneously recorded mitral cells projecting to either different (Ca) or the same (Cb) glomerular networks. The voltage traces show the sag potential recorded in three examples of inter- and intra-glomerular pairs. D, Histograms of the SPA for all individual cells belonging to either inter- and intra-glomerular pairs. E, Top panel, histograms of recorded SPA differences for inter- (n = 26) and intra-glomerular (n = 14) pairs. Bottom panel, histograms of SPA differences for inter- and intra-glomerular pseudo pairs. F, Box-plot of recorded and pseudo inter-glomerular pairs, pseudo pairs of all recordings, and intra-glomerular pseudo and recorded pairs. Open circles, individual data points of recorded SPA difference. G, Histogram of sag amplitude difference for inter- and intra-glomerular recorded pairs fitted with a half Gaussian (n = 26 and 14 pairs, respectively; bin size, 3 mV). Inset, Gaussian fits of the recorded data for intra-glomerular and inter-glomerular pairs. a.u., arbitrary units.

dendritic tuft, the site of sensory integration within the glomerulus. To explore this possibility, we performed immunohistochemical staining for the HCN2 subunit that can form both homomeric or heteromeric HCN channels known to mediate the slow $I_{h}$ (ref. 23) underlying mitral cell sag. Qualitatively, very little HCN2 protein was seen in the granule cell and mitral cell layers. In contrast to the low but homogeneous HCN2 expression in the external plexiform layer, we found a high-contrast mosaic staining pattern across the glomerular layer (Fig. 2a). To determine whether HCN2 expression within the glomerulus was postsynaptic to olfactory receptor neuron input we next used a transgenic mouse line that expresses the tau-LacZ transgene in the sensory afferents under the olfactory marker protein (OMP) promoter (OMP-IRE-tau-LacZ mice). Double-staining experiments against both LacZ and HCN2 showed that the HCN2 protein is predominantly expressed in dendritic compartments within the glomerulus and downstream of the olfactory receptor input (Fig. 2b–d)25. Irrespective of the potential contribution of other cell types,24,28, this mosaic pattern of HCN2 expression is consistent with the observation of large $I_{h}$ currents in the distal apical dendrite and the broad range of sag amplitudes recorded in mitral cells participating in different glomerular networks.

Such glomerular-based SPA and HCN2 expression might reflect network-related homeostatic regulation27,28 of excitability, in which glomerular differences arise from the processing of functionally and
we next performed experiments in a transgenic ‘monoclonal nose’ mouse that expresses the M71 odorant receptor in more than 95% of receptor neurons (M71tg mice, Fig. 3A). We calculated mean pixel intensities of glomeruli in HCN2–DAB-stained M71tg and control animals that revealed significantly different variances (P = 0.001) whereby the mean pixel intensity for glomeruli varied less in the M71tg mice than in the control mice (P = 0.0025; Fig. 3A, Supplementary Fig. 3). Simultaneous recordings from inter-glomerular pairs of mitral cells in M71tg mice (min = 0.08 mV, max = 6.3 mV, median = 3.3 mV, Q1 = 1.32 mV, Q3 = 3.78 mV; n = 24; Fig. 3B, a) also revealed a significantly narrower distribution of SPA difference compared to wild-type and control mice (min = 0.23 mV, max = 21.04 mV, median = 3.4 mV, Q1 = 1.78 mV, Q3 = 10.78 mV, n = 24 (P = 0.004); Fig. 3B, a and b, C). This reduction in sag amplitude diversity was also notable for pseudo-pair comparisons for all recorded pairs (Fig. 3D, a) and the overall population data set (M71tg mice: min = 0 mV, max = 36.01 mV, median = 3.4 mV, Q1 = 1.6, Q3 = 6.1 mV, n = 91 cells, 4,095 comparisons, versus M71 control mice: min = 0 mV, max = 51.4 mV, median = 4.6 mV, Q1 = 2, Q3 = 10.19 mV, n = 81 cells, 3,321 comparisons (P < 4 × 10^{-4}); Fig. 3D, a and c). Thus, mitral cells and glomeruli in M71tg mice are more homogeneous in their sag and HCN2 expression profile than those in wild-type and M71 control mice receiving the normal, genetically diverse, array of olfactory receptor neuron input.

Despite the overall reduction in SPA variance in the M71tg mice, the SPA in the intra-glomerular pairs remained more similar (M71tg intra-glomerular pairs: min = 0.2 mV, max = 6.5 mV, median = 0.8 mV, Q1 = 0.3 mV, Q3 = 1.26 mV; n = 9, versus M71tg inter-glomerular pairs (P = 0.04); Figs 3C and 4a). Indeed, we observed no effect of wholesale expression of the M71 receptor on intra-glomerular sag diversity (M71tg versus control and wild-type mice; P = 0.61 and 0.46, respectively; Fig. 4a). Thus, sensory afferent input seems unlikely to be the sole driver of inter-glomerular diversity (Fig. 4b).

Using the hyperpolarization-evoked sag potential as a general proxy, we have identified several organizing principles regarding the population diversity of h channel expression in mitral cells\textsuperscript{20–22}. First, this intrinsic property is a biophysical fingerprint of local constellations of mitral cells forming a functionally discrete olfactory network (Fig. 4c). As mitral cells are electrically and exclusively coupled to their intra-glomerular counterparts, co-regulation of the h channel and current via gap junctions may contribute to their biophysical similarity\textsuperscript{9}. Second, analysis of the M71tg mouse shows directly that this network-affiliation-based signature depends on sensory information processing. The fact that in the M71tg mouse intra-glomerular sag diversity remained more homogeneous than in the overall population also suggests that other factors such as feed-forward and lateral inhibition may contribute to sag regulation at the level of the glomerulus. From a functional perspective, mitral cell F–I curves (frequency of spiking output of neurons (F) in response to varying current injection...
5. Brown, S. P. & Hestrin, S. Intracortical circuits of pyramidal neurons reflect their all recorded pairs.

2. Brochtrup, A. & Hummel, T. Olfactory map formation in the mice.

30. Irrespective of the cellular mechanisms mechanism and contribute to correlated output patterning at the level this delineation may therefore reflect a network-based gain control.

1. Gupta, A., Wang, Y. & Markram, H. Organizing principles for a diversity of types C57Bl/6J and M71 transgenic or control littermate mice aged 4–6 weeks. The Whole-cell recordings using standard intracellular and extracellular solution were for mitral cells6, and the details of the experimental and analytical procedures are for intra-glomerular

Figure 4 | Population diversity reflects local network membership and sensory processing. a. Left, example membrane-voltage traces showing the sag potential recorded in three different intra-glomerular pairs from the M71tg mouse. Right, histogram of the SPA difference recorded for intra-glomerular pairs in M71tg mice (n = 9 pairs) versus M71 control and wild-type (WT) mice (pooled: n = 17 pairs). Inset, box-plot of SPA difference for intra-glomerular pairs recorded in wild-type (n = 14), M71 control (n = 3) and M71tg (n = 9) mice. b. Summary data plotted as a cumulative histogram of SPA difference of all recorded pairs. c. Schematic highlighting the relationship between glomerulus affiliation, sensory input and mitral cell sag diversity.

amplitudes (I)) are known to shift left, from a sigmoid towards a linear operation with increasing sag2. We suggest that the glomerular basis of this delineation may therefore reflect a network-based gain control mechanism and contribute to correlated output patterning at the level of mitral cell networks26. Irrespective of the cellular mechanisms underlying this glomerular phenomenon, the network-based regulation of this mitral cell property appears fundamental to the organizational and function of olfactory bulb circuits.

METHODS SUMMARY
Whole-cell recordings using standard intracellular and extracellular solution were carried out in horizontal olfactory bulb slices (300-μm thick) prepared from wild-type C57Bl/6J and M71 transgenic or control littermate mice aged 4–6 weeks. The Ih current and Ih-mediated sag potential has recently been extensively characterized for mitral cells26, and the details of the experimental and analytical procedures are provided in the Supplementary Information.

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