Precision and diversity in an odor map on the olfactory bulb

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Supplementary Material

Odor Stimulation

Odorants were diluted in mineral oil (1:100, typically), absorbed onto filter paper and stored in glass vials sealed with a thick rubber septum (Vacutainer™ #366431 tubes), grouped in a rack with 100 tubes. Two different machines were built to deliver odors from this set in arbitrary sequence under computer control. In one device, the rack was moved using two linear translators to position the desired tube under a pair of 20-gauge non-coring needles (Popper and Sons, Inc. #7184). A third translator pushed the needle assembly through the septum. Clean, filtered and humidified air entered through one needle, and the odor stream exited through the other needle at a rate of 1 L/min. In the other device, each tube had a permanent pair of needles through the septum, and the air flow was directed through the tube of choice by a network of solenoid valves and check valves. In either case, Teflon coated tubing carried the odorized air to the animal, through an anesthesia mask surrounding the animal's snout.

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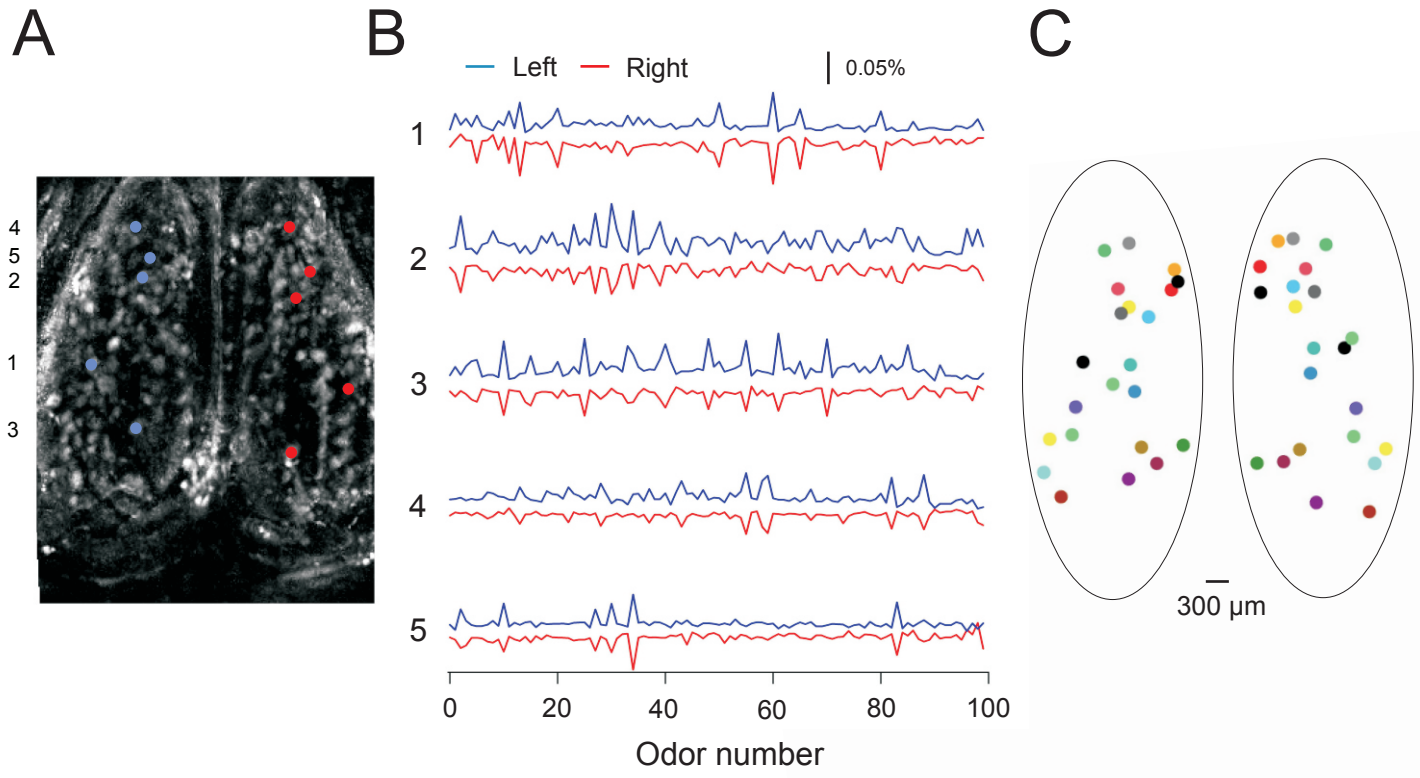
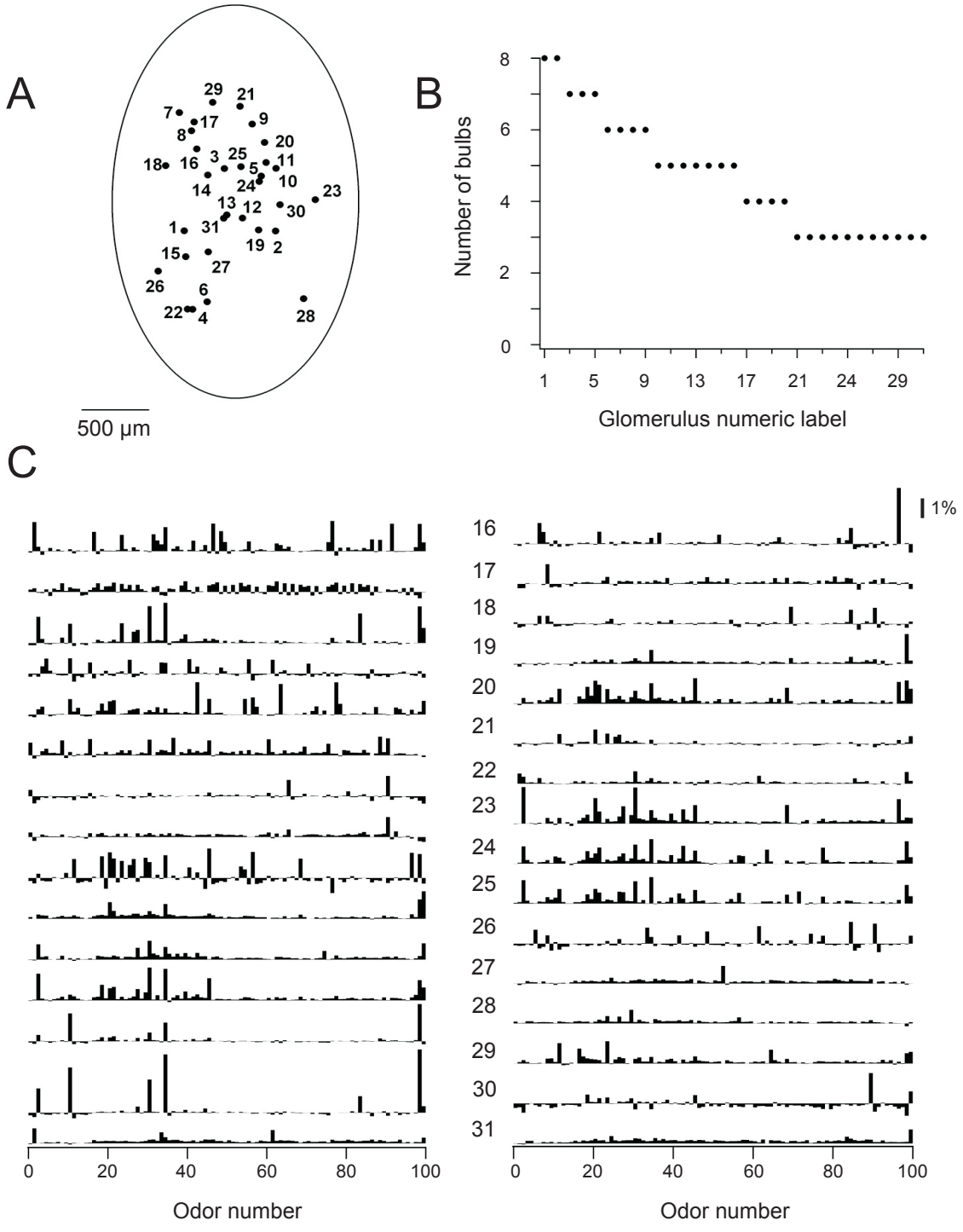
Supplementary Figure 1: Functional identification of glomeruli in rat

A. Maximum response projection map of intrinsic signal responses in the rat olfactory bulb: At each pixel the largest odor response is plotted in gray scale. Approximately 110 glomeruli could be stimulated on the dorsal surface of each bulb with a set of 100 odorants.
B. Sample odor response spectra of 5 pairs of glomeruli that were matched between the left and right bulbs of panel A. For odor identities, see Supplementary Table 1, Set A.
C. Locations of the best matches among glomeruli identified in the two bulbs of panel A.



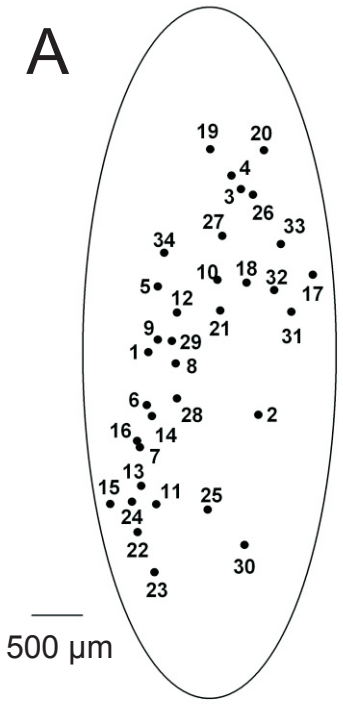
Supplementary Figure 2: Prototype map of the mouse olfactory bulb

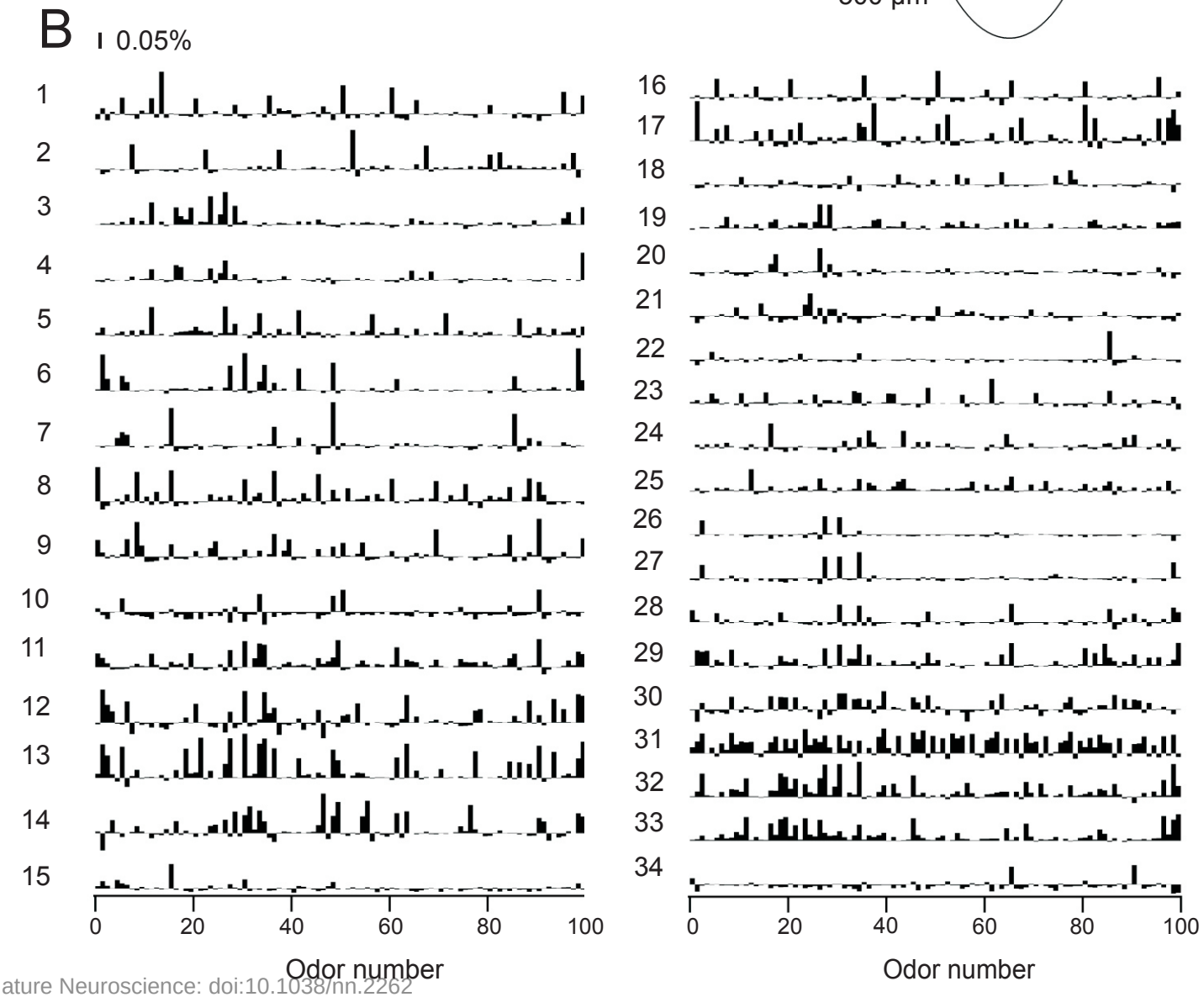
A. Uniquely identifiable glomeruli on the dorsal surface of the right olfactory bulb in mouse. Each glomerulus is plotted at its average location. Glomeruli were included if they could be identified in at least 3 of 8 bulbs inspected.
B. The number of bulbs (of a total of 8) in which each prototype glomerulus was observed. The numerical labels were ordered so that glomeruli with low numbers occurred most frequently.
C. The average odor spectrum for each prototype glomerulus in panel A. For odor identities, see Supplementary Table 1, Set A.



Supplementary Figure 3: Prototype map of the rat olfactory bulb

A. Uniquely identifiable glomeruli on the dorsal surface of the right olfactory bulb in rat, presented as in Supplementary Figure 2A. Glomeruli were included if they were identified in at least 3 of 4 bulbs inspected.
B. The average odor spectrum for each prototype glomerulus in panel A. For odor identities, see Supplementary Table 1, Set A.





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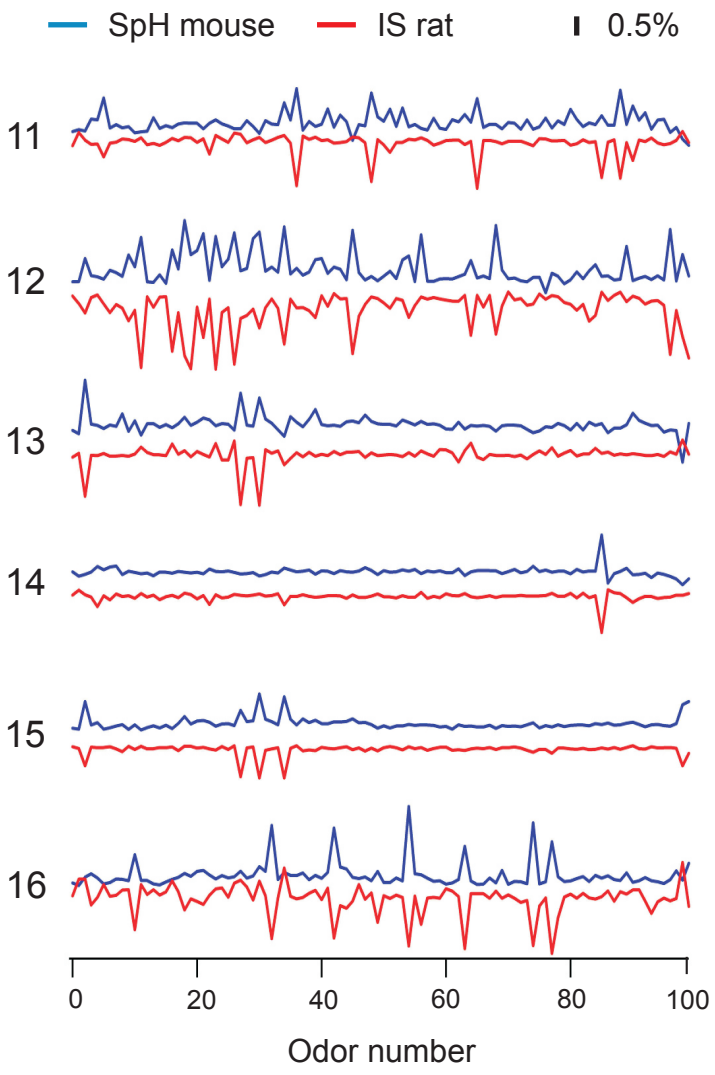
Supplementary Figure 4: Additional matches between glomeruli in mouse and rat

Odor spectra of glomeruli with strong similarity between mouse and rat. This analysis followed an alternate strategy to Fig 5: Glomeruli from individual mouse bulbs were compared directly with those from rat bulbs. All matches with similarity >0.75 were accepted. The resulting set of matching spectra was subjected to a cluster analysis (see Methods), and the average spectrum was computed for each cluster. From the resulting collection of spectra, those already identified in Fig 5 were removed, yielding the 6 additional spectra illustrated here. Scale bar refers to the SpH signal, the IS signal was scaled for easy comparison. For odor identities, see Supplementary Table 1, Set A.



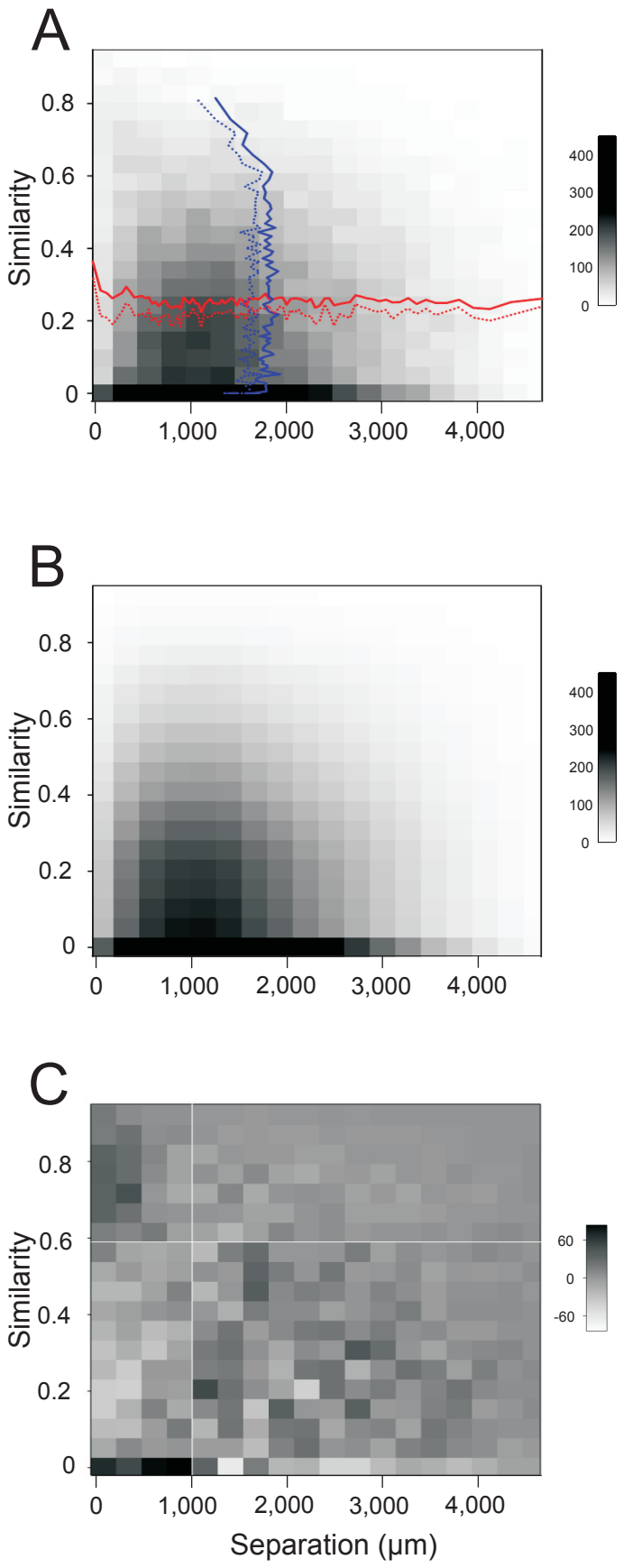
Supplementary Figure 5: Similarity vs distance for pairs of glomeruli on the rat olfactory bulb

We performed a second series of experiments to test for fine-scale chemotopy in the rat. The odor set (Supplementary Table 1, Set A) was different from that in Fig 8A-C, otherwise the analysis proceeded in the same fashion. As before, there is no significant dependence of similarity on inter-glomerular distance (C).

A. Relationship between the odor response similarity (ordinate, Eqn 1) of two glomeruli and their spatial separation (abscissa) in the rat. The analysis extended over 35,991 pairs of glomeruli in 6 olfactory bulbs from 3 rats. Each pair of glomeruli contributes one count in this histogram, and the gray scale reports the number of counts in each bin. Red lines: average (solid) and median (dashed) similarity vs. distance, obtained by binning the distance (150 pairs per bin). Green lines: average (solid) and median (dashed) distance vs. similarity, obtained by binning the similarity. If the response similarity had no dependence on distance, the red lines should be horizontal and the green lines vertical. For odor identities, see Supplementary Table 1, Set A.

B. Under the null hypothesis in which there is no chemotopy whatsoever, the response similarity of a pair of glomeruli should have the same probability distribution at all distances (see Methods). Therefore the joint distribution of similarity and distance should equal the product of the two marginal distributions. This prediction is plotted here; note the close resemblance to the measured distribution (panel A).

C. Difference between the measured distribution (panel A) and the distribution expected in absence of any chemotopy (panel B), plotted on an expanded grayscale. The region with the strongest deviation from the null hypothesis includes the pairs separated by <1 mm (vertical line) with similarity >0.6 (horizontal line): the excess there amounts to 2.4% of glomerular pairs separated by <1 mm or just 0.7% of all pairs.



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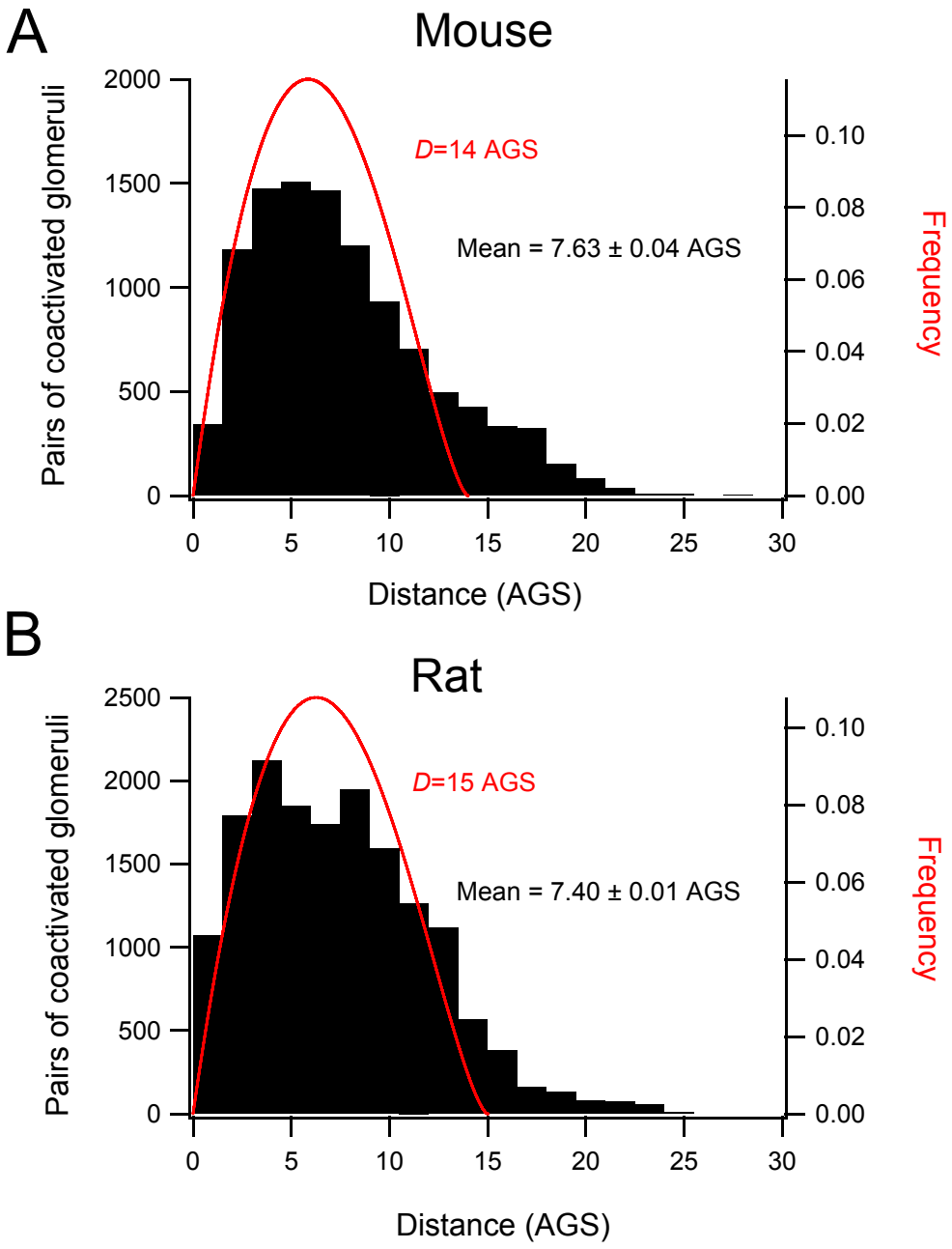
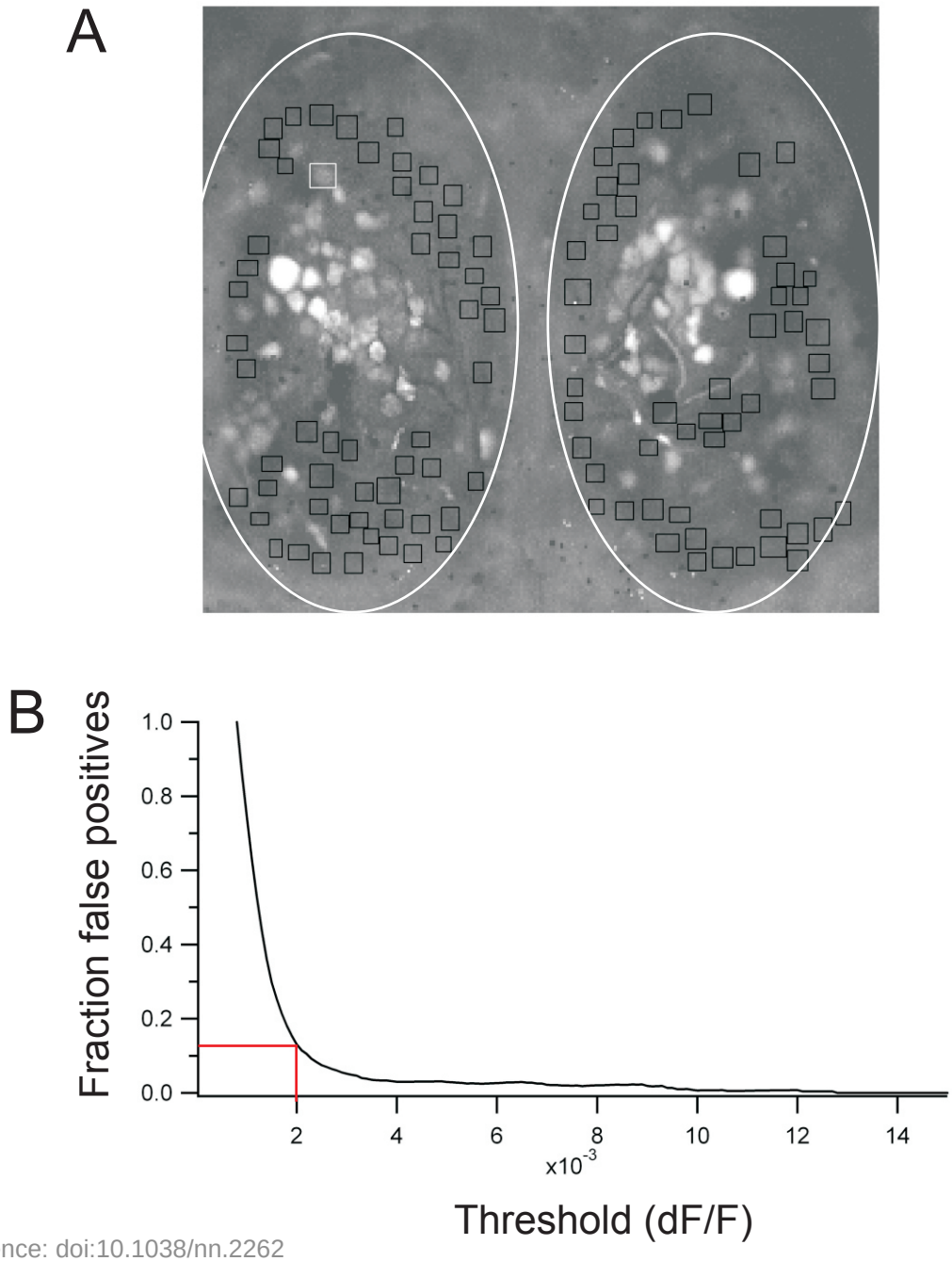
Supplementary Figure 6: The size of single-odor activation patterns

In general, a single odor activates a set of glomeruli sparsely scattered on the bulb. Here we measure the spatial extent of these activation patterns in mouse (A) and rat (B). For each odor, we identified the glomeruli activated above threshold (see Methods). Within that set, we computed the distribution of pairwise distances and averaged that distribution over all odors (solid bars). For reference, we draw the distribution expected if the activated glomeruli were distributed randomly inside a circle of diameter *D* (line; see Garwood, 1947, Biometrika 34, 1-17). By adjusting *D* to approximate the observed distribution, we estimate the size of the activated pattern. In the rat, the typical pattern involved 17 glomeruli in an area with a diameter of ~15 spacings; in the mouse 11 glomeruli in an area of diameter ~14 spacings. The analysis covered 8 olfactory bulbs in (A) and 6 in (B). For odor identities, see Supplementary Table 1, Set A.



Supplementary Figure 7: Receiver Operating Characteristic (ROC) analysis determines a signal threshold

A. Maximum intensity projection map of SpH responses from olfactory bulbs in the mouse (see Figure 1B). Overt spots were taken to be glomeruli, and each of these was enclosed by a rectangular region of interest (ROI, e.g. sample white rectangle) used to measure its response amplitude with a Gaussian fit of the profile (see Methods). In addition, control ROIs were drawn in non-responsive regions of the dorsal bulb (black rectangles). The control ROIs were processed in the same way to yield response amplitudes caused by imaging noise.
B. For any given threshold value we counted the number of control regions whose amplitude exceeded threshold (false positives) and compared it to the number of bona fide glomeruli exceeding threshold (hits). We adopted a threshold of 0.002 which yielded a ratio of false positives to hits of ~10%. Signals below this threshold were set to zero. A similar analysis was applied to the IS responses.



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Supplementary Table 1: Reverse lookup list of all odors used

For each odor set, the odor number refers to the order of presentation during the experiment, and the value along the abscissa in the respective odor spectra. The odor index can be used to look up the corresponding odor name in Supplementary Table 2. For example, in Set A the substance presented at position 46 has odor index 11 and Supplementary Table 2 indicates this is 1-pentanol. In Set B, on the other hand, position 46 is a compound with odor index 206, which Supplementary Table 2 reveals to be methyl 2-pyrollyl ketone.

Odor number	Odor index					
	Set A	Set B	Set C	Set D	Set E	Set F
0	216	216	216	216	216	235
1	235	147	147	83	35	252
2	48	139	139	232	208	149
3	275	67	67	179	158	195
4	270	276	276	175	65	13
5	30	214	66	227	257	179
6	44	38	38	223	50	245
7	145	71	71	84	128	143
8	3	135	135	233	129	191
9	19	253	93	182	107	177
10	14	143	143	177	209	71
11	43	231	231	229	255	189
12	205	149	149	225	67	227
13	91	72	249	90	190	61
14	256	140	140	276	221	223
15	255	44	44	180	93	94
16	181	137	137	176	172	138
17	176	148	188	228	203	280
18	58	207	207	224	215	136
19	117	35	35	204	200	91
20	56	213	213	211	52	213
21	63	136	136	20	119	144
22	17	180	180	234	73	38
23	179	146	146	192	4	188
24	245	192	192	157	245	110
25	143	141	141	159	251	59
26	175	226	226	266	274	81
27	71	59	59	146	138	147
28	227	255	255	141	98	67
29	189	245	245	149	160	93
30	147	234	234	144	142	11
31	81	273	273	143	123	141
32	188	228	228	145	256	31
33	38	189	189	162	243	270

34	213	227	227	163	88	66
35	280	40	40	170	193	146
36	138	176	176	166	140	64
37	94	179	179	165	97	269
38	223	190	190	167	110	172
39	61	52	52	207	198	115
40	171	256	256	250	118	192
41	64	250	250	195	279	248
42	146	69	69	183	136	98
43	66	166	166	69	217	249
44	1	34	34	78	269	140
45	141	163	163	213	62	156
46	11	206	206	252	231	10
47	93	168	168	196	86	62
48	67	105	105	184	219	231
49	110	169	169	71	273	276
50	62	29	29	80	45	91
51	210	21	21	29	26	43
52	156	39	39	28	137	139
53	140	92	92	27	7	122
54	249	275	275	270	85	223
55	98	191	191	212	139	228
56	0	9	9	60	70	126
57	192	89	89	273	218	124
58	115	87	87	41	236	180
59	172	125	125	34	122	255
60	95	193	193	46	134	190
61	40	57	57	18	76	164
62	164	133	133	16	220	95
63	190	272	272	36	58	40
64	180	54	54	17	194	58
65	5	53	53	55	31	88
66	228	79	79	44	47	70
67	122	82	82	42	2	87
68	276	111	111	12	8	274
69	231	127	127	104	186	86
70	132	112	112	202	59	234
71	116	178	178	230	91	97
72	158	242	242	226	185	158
73	97	187	187	96	77	116
74	234	238	238	244	222	132
75	86	267	267	173	199	137
76	274	240	240	154	271	52
77	87	281	281	235	246	130
78	70	241	241	94	51	33

79	88	282	282	74	22	273
80	226	237	237	75	120	74
81	173	283	283	105	68	244
82	202	247	247	201	121	202
83	244	284	284	258	24	173
84	74	268	268	277	278	226
85	273	285	285	206	108	230
86	49		270	37	109	201
87	131		161	25	155	207
88	52			23	153	
89	137			15	152	
90	6			261	101	
91	68			260	99	
92	186			263	100	
93	254			262	103	
94	207			259	102	
95	201			265	114	
96	230			264	113	
97	174				106	
98	197				286	
99	150					

Supplementary Table 2: Alphabetical list of all odors used

The odor index listed here is used to reference odors in Supplementary Table 1. The odor number refers to the position on the abscissa in the response spectra illustrated in various figures. It also reflects the order of odor presentations during the experiment. Six different odor sets (A-F) were used in various parts of the study, as identified in the respective figure legends. For example, 1-pentanol has odor index 11 in this table and was presented at position 46 in Set A, at position 30 in Set F but was not used in Sets B, C, D, or E.

Odor index	Odor name	Odor number					
		Set A	Set B	Set C	Set D	Set E	Set F
0	1-propanethiol	56					
1	2-butenol	44					
2	2-pentanol					67	
3	4-allyl anisole	8					
4	1,1-diethoxyethane					23	
5	1,3- dimethoxy-benzene	65					
6	1,4 dimethoxy benzene	90					
7	1-butanethiol					53	
8	1-decanol					68	
9	1-heptanol		56	56			
10	1-methyl pyrrole						46
11	1-pentanol	46					30
12	2 isobutyl 3 methyl pyrazine				68		
13	2,3 ethyl pyrazine						4
14	2,3 pentane dione	10					
15	2,3,5,6 tetramethyl pyrazine				89		
16	2,3,5-trimethyl pyrazine				62		
17	2,3-diethyl pyrazine	22			64		
18	2,3-dimethyl pyrazine				61		
19	2,4 decadienal	9					
20	2,5- dimethyl pyrazine				21		
21	2,5-dimethyl thiazole		51	51			
22	2,5-dimethyl phenol					79	
23	2,6- dimethyl pyrazine				88		
24	2,6-dimethyl phenol					83	
25	2-acetyl pyrazine				87		
26	2-acetyl furan					51	
27	2-acetyl pyridine				53		
28	2-acetyl thiazole				52		

29	2-acetyl thiophene		50	50	51		
30	2-acetyl thiophenone	5					
31	2-butanol					65	32
32	2-butenol						
33	2-butyl cyclohexanone						78
34	2-ethoxy thiazole		44	44	59		
35	2-ethyl butyric acid		19	19		1	
36	2-ethyl pyrazine				63		
37	2-furyl methyl ketone				86		
38	2-heptanone	33	6	6			22
39	2-hexanal, trans		52	52			
40	2-hexanone	61	35	35			63
41	2-isobutyl thiazole				58		
42	2-methoxy 3-methyl pyrazine				67		
43	2-methoxy naftalene	11					51
44	2-methoxy pyrazine	6	15	15	66		
45	2-methoxy phenol					50	
46	2-methyl pyrazine				60		
47	2-octenal (E)					66	
48	2-propyl tiglate	2					
49	2-secbutyl cyclohexanone	86					
50	2-undecanone					6	
51	3,4-dimethoxy acetophenone					78	
52	3-acetyl 2,5-dimethyl furan	88	39	39		20	76
53	3-acetyl furan (solid)		65	65			
54	3-ethoxy, 4-hydroxy benzaldehyde		64	64			
55	3-ethyl 2-methyl pyrazine				65		
56	3-ethyl valerate	20					
57	3-hexanone		61	61			
58	3-methyl 2-buten 1-ol	18				63	64
59	4'-methoxy acetophenone (solid)		27	27		70	25
60	4,5-dimethyl thiazole				56		
61	4-heptanone	39					13
62	4-isopropyl benzaldehyde	50				45	47
63	4-propyl butyrate	21					
64	5 hydroxyethyl 4-methyl thiazole	41					36

65	5,6,7,8-tetrahydroquinoxaline					4	
66	acetal	43		5			34
67	acetophenone	48	3	3		12	28
68	acetovanillone	91				81	
69	allyl butyrate		42	42	43		
70	allyl cyclohexyl propionate	78				56	66
71	allyl tiglate	27	7	7	49		10
72	allyl tiglate (12%)		13				
73	ammonium sulfide					22	
74	anise oil (10%)	84			79		80
75	apple flavor				80		
76	benzaldehyde					61	
77	benzoic acid					73	
78	benzyl butyrate				44		
79	benzyl propionate		66	66			
80	benzyl tiglate				50		
81	benzyl trans 2-methyl 2-butenoate	31					26
82	bicyclononanal lactone		67	67			
83	butanal				1		
84	butanol				7		
85	butyl acetate					54	
86	butyl formate	75				47	69
87	butyl propionate	77	58	58			67
88	butyl sulfide	79				34	65
89	butylamine		57	57			
90	butyrate				13		
91	camphor	13				71	19
92	carvone		53	53			
93	carvyl acetate	47		9		15	29
94	cedarwood oil (10%)	37			78		15
95	cineole	60					62
96	cinnamon oil (10%)				73		
97	citral cis + trans	73				37	71
98	citronellal	55				28	42
99	citrus arantium					91	
100	citrus arantium v.amara					92	
101	citrus arantium v. bergamia					90	

102	citrus limon					94	
103	citrus reticulata v. mandarin					93	
104	clove oil (10%)				69		
105	coffee (10%)		48	48	81		
106	cupressus sempervirens					97	
107	cyclohexanone					9	
108	cyclohexanone / butylacetate					85	
109	cyclohexanone / butyrolactone					86	
110	cyclohexyl acetate	49				38	24
111	cyclohexyl ethyl acetate		68	68			
112	cyclohexyl ethyl alcohol		70	70			
113	cymbogom martini					96	
114	cymbogom nardus					95	
115	DBE-dibasic ester	58					39
116	decanolactone	71					73
117	decyl alcohol	19					
118	delta-decalactone					40	
119	delta-dodecalactone					21	
120	dextro-camphene					80	
121	dibenzyl disulfide					82	
122	diethyl maleate	67				59	53
123	difurfuryl disulfide					31	
124	dimethoxy acetophenone						57
125	dimethoxy benzaldehyde (solid)		59	59			
126	dimethoxy benzene						56
127	dimethyl benzyl carbonyl acetate		69	69			
128	dimethyl succinate					7	
129	dipropyl ketone					8	
130	dodecen acetate						77
131	dodecyl acetate	87					
132	dyhidrocarvone	70					74
133	dymethyl phenol (solid)		62	62			
134	estragole					60	
135	ethyl 2-mercaptopropionate		8	8			
136	ethyl 2-methyl butyrate		21	21		42	18
137	ethyl 3-hydroxy butyrate	89	16	16		52	75

138	ethyl 3-mercaptopropionate	36				27	16
139	ethyl acrylate		2	2		55	52
140	ethyl benzoylacetate	53	14	14		36	44
141	ethyl butyrate	45	25	25	28		31
142	ethyl formate					30	
143	ethyl heptanoate	25	10	10	31		7
144	ethyl hexanoate				30		21
145	ethyl octanoate	7			32		
146	ethyl propionate	42	23	23	27		35
147	ethyl tiglate	30	1	1			27
148	ethyl tiglate (1.6%)		17				
149	ethyl valerate		12	12	29		2
150	ethyl valerate 12%	99					
151	ethyl-benzyl-acetate						
152	eucalyptus citriodora					89	
153	eucalyptus globulus					88	
154	eucalyptus oil (10%)				76		
155	eucalyptus staigerana (natural oil)					87	
156	eugenol	52					45
157	farnesene				24		
158	fenchone (-)	72				3	72
159	filter paper (no mineral oil control)				25		
160	formic acid					29	
161	fox anal gland extract			87			
162	furfuryl proprionate				33		
163	furfuryl butyrate		45	45	34		
164	furfuryl disulfide	62					61
165	furfuryl heptanoate				37		
166	furfuryl hexanoate		43	43	36		
167	furfuryl octanoate				38		
168	furfuryl pentanoate		47	47			
169	furfuryl propionate		49	49			
170	furfuryl valerate				35		
171	gamma terpinene	40					
172	geraniol	59				16	38
173	ginger oil (10%)	81			75		83
174	hanoki oil (10%)	97					

175	heptanal	26			4		
176	heptanoic acid	17	36	36	16		
177	heptanol				10		9
178	hexalon		71	71			
179	hexanal	23	37	37	3		5
180	hexanoate	64	22	22	15		58
181	hexanoate (1/200)	16					
182	hexanol				9		
183	hexyl butyrate				42		
184	hexyl tiglate				48		
185	hydroquinone dimethyl ether					72	
186	indole	92				69	
187	ionone beta		73	73			
188	isoamyl acetate	32		17			23
189	isoamylamine	29	33	33			11
190	isobutyl propionate	63	38	38		13	60
191	isobutyl thiazole		55	55			8
192	isobutylamine	57	24	24	23		40
193	isoeugenol		60	60		35	
194	isoheptanol					64	
195	isopropyl butyrate				41		3
196	isopropyl tiglate				47		
197	isopropyl tiglate (12%)	98					
198	L-(-)-carvone					39	
199	L-menthol					75	
200	L-verbenone					19	
201	lavender oil (10%)	95			82		86
202	lemon oil (10%)	82			70		82
203	m-dimethoxy benzene					17	
204	meister bräu beer				19		
205	methoxy acetophenone	12					
206	methyl 2-pyrrollyl ketone solid		46	46	85		
207	methyl butyrate	94	18	18	39		87
208	methyl n-amyl ketone					2	
209	methyl propyl disulfide					10	
210	methyl pyruvate	51					
211	methyl sulfoxide				20		

212	methyl thiazole				55		
213	methyl tiglate	34	20	20	45		20
214	methyl tiglate (12%)		5				
215	methyl pyrrole					18	
216	mineral oil	0	0	0	0	0	
217	n-butyl propionate					43	
218	n-butylamine					57	
219	n-butyrophenone					48	
220	n-hexanoic acid					62	
221	n-propyl acetate					14	
222	naphthalene					74	
223	nonanal	38			6		14
224	nonanoic acid				18		
225	nonanol				12		
226	nutmeg oil (10%)	80	26	26	72		84
227	octanal	28	34	34	5		12
228	octanoic acid	66	32	32	17		55
229	octanol				11		
230	orange oil (10%)	96			71		85
231	p-anis aldehyde	69	11	11		46	48
232	pentanal				2		
233	pentanol				8		
234	pentyl acetate	74	30	30	22		70
235	peppermint oil (10%)	1			77		0
236	phenethylamine					58	
237	phenoxy ethyl isobutyrate		80	80			
238	phenoxy ethyl propionate		74	74			
239	phenyl acetate						
240	phenyl ethyl acetate		76	76			
241	phenyl ethyl alcohol		78	78			
242	phenyl ethyl isobutyrate		72	72			
243	phenyl mercaptan					33	
244	pine oil (10%)	83			74		81
245	piperidine	24	29	29		24	6
246	piperine					77	
247	prenyl acetate		82	82			
248	propane thiol						41
249	propyl acetate	54		13			43

250	propyl butyrate		41	41	40		
251	propyl mercaptan					25	
252	propyl tiglate				46		1
253	propyl tiglate (12%)		9				
254	pyrazine	93					
255	pyridine	15	28	28		11	59
256	pyrrolidine	14	40	40		32	
257	quinoline					5	
258	rose oil (10%)				83		
259	sigma cocaine scent				94		
260	sigma corpse 2				91		
261	sigma corpse 1				90		
262	sigma explosive scent				93		
263	sigma heroin scent				92		
264	sigma LSD scent				96		
265	sigma marijuana scent				95		
266	soiled bedding				26		
267	strawberriff		75	75			
268	styralyl propionate		84	84			
269	terpinene					44	37
270	thiazole	4		86	54		33
271	thymol					76	
272	tislic acid - isobutyl ester		63	63			
273	trimethyl thiazole	85	31	31	57	49	79
274	undecane	76				26	68
275	valeraldehyde	3	54	54			
276	valeric acid	68	4	4	14		49
277	vanilla butternut flavor				84		
278	veratraldehyde					84	
279	veratrole					41	
280	verbenone	35					17
281	verdox HC		77	77			
282	verdural B extra		79	79			
283	verdural extra		81	81			
284	vertenex		83	83			
285	vertenex HC		85	85			
286	vetiveria zizanioides					98	