Supplemental Data

Sequential Learning of Pheromonal Cues Modulates Memory Consolidation in Trainer-Specific Associative Courtship Conditioning

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Supplemental References

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Table S1. Cuticular Hydrocarbons of Mature and Immature Virgins

				Immature Virgin (iV)			Mature Virgin (mV)		
Compound	Peak	Source	Time	Mean Amount (Area)	SEM	Percent	Mean Amount (Area)	SEM	Percent
nC19	1	both	17.42	0.278	0.107	0.924	0.362	0.064	0.573
uic	2	both	18.00	0.237	0.088	0.786	0.169	0.030	0.268
nC21	3	both	22.07	0.041	0.016	0.135	0.123	0.014	0.195
uic	4	both	22.31	0.043	0.023	0.143	tr		
Xi-nC20:1	5	both	23.08	tr			tr		
nC22	6	both	24.64	tr			0.076	0.005	0.121
7,11-nC23:2	7	mV	25.87	nd			0.127	0.005	0.202
2-MeC22	8	mV	26.27	nd			0.101	0.006	0.160
9-nC23:1	9	both	26.34	tr			0.105	0.005	0.166
7-nC23:1	10	both	26.54	0.065	0.046	0.214	0.680	0.030	1.077
6-nC23:1	11	mV	26.67	nd			0.076	0.002	0.120
5-nC23:1	12	both	26.82	tr			tr		
nC23	13	both	27.19	0.034	0.022	0.114	2.241	0.100	3.549
9-nC24:1	14	mV	28.85	nd			tr		
7-nC24:1	15	mV	28.94	nd			tr		
5-nC24:1	16	both	29.04	tr			tr		
nC24	17	both	29.66	tr			0.186	0.006	0.295
9,13-nC25:2	18	both	30.67	tr			0.116	0.007	0.184
7,11-nC25:2	19	both	30.86	tr			0.457	0.020	0.724
2-MeC24	20	mV	31.21	nd			0.116	0.116	0.184
9-nC25:1	21	both	31.33	0.060	0.046	0.199	2.334	0.140	3.697
7-nC25:1	22	both	31.52	0.080	0.063	0.267	1.526	0.061	2.417
5-nC25:1	23	both	31.78	tr			0.143	0.016	0.226

(continued)

Table S1. Continued

Compound	Peak	Source	Time	Immature Virgin (iV) Mean Amount (Area)	SEM	Percent	Mature Virgin (mV) Mean Amount (Area)	SEM	Percent
nC25	24	both	32.12	0.034	0.024	0.112	2.888	0.104	4.573
uic	25	both	32.90	0.286	0.193	0.948	tr		
7,11-nC26:2	26	both	33.27	0.294	0.197	0.975	0.116	0.004	0.184
2-MeC25	27	both	33.60	tr			0.131	0.003	0.207
9-nC26:1	28	mV	33.70	nd			tr		
7-nC26:1	29	both	33.79	tr			0.069	0.003	0.109
5-nC26:1	30	both	33.89	tr			0.085	0.003	0.134
nC26	31	STD	34.44	1.000	0.000	3.320	1.000	0.000	1.580
9,13-nC27:2	32	both	35.40	tr			0.341	0.015	0.540
7,11-nC27:2	33	both	35.69	tr			8.628	0.274	13.664
2-MeC26	34	both	35.91	0.392	0.042	1.304	7.883	0.210	12.484
9-nC27:1	35	both	36.09	0.044	0.030	0.146	2.310	0.091	3.658
7-nC27:1	36	both	36.21	0.124	0.069	0.412	3.998	0.147	6.332
5-nC27:1	37	both	36.48	tr			0.283	0.007	0.448
nC27	38	both	36.72	0.069	0.021	0.229	3.119	0.036	4.940
8,12-nC28:2	39	both	37.78	tr			0.077	0.007	0.123
7,11-nC28:2	40	both	37.90	tr			0.408	0.013	0.646
2-MeC27	41	mV	38.01	nd			0.060	0.002	0.100
6,10-nC28:2	42	both	38.15	0.084	0.011	0.277	0.116	0.003	0.184
uic	43	both	38.58	0.052	0.035	0.173	tr		
nC28	44	both	38.94	0.033	0.002	0.111	0.093	0.005	0.148
9,13-nC29:2	45	mV	39.90	nd			0.328	0.042	0.519
7,11-nC29:2	46	both	40.23	tr			13.987	0.326	22.151
2-MeC28	47	both	40.37	5.073	0.484	16.846	4.839	0.121	7.663
9-nC29:1	48	both	40.48	0.072	0.022	0.238	0.108	0.013	0.172
7-nC29:1	49	both	40.66	0.868	0.142	2.881	0.483	0.036	0.765
5-nC29:1	50	both	40.88	tr			tr		
nC29	51	both	41.10	0.168	0.008	0.558	0.477	0.011	0.756
uic	52	both	41.78	0.126	0.008	0.419	0.019	0.005	0.031
uic	53	iV	41.89	0.040	0.016	0.132	nd		
uic	54	both	41.99	0.158	0.026	0.526	0.071	0.004	0.113
uic	55	both	42.44	0.160	0.012	0.532	tr		
uic	56	both	42.67	0.312	0.064	1.037	tr		
uic	57	iV	42.87	0.029	0.005	0.100	nd		
nC30	58	both	43.18	tr			tr		
uic	59	both	43.87	0.042	0.042	0.138	tr		
9,13-nC31:2	60	both	44.00	0.699	0.064	2.321	0.110	0.020	0.174
uic	61	both	44.16	0.125	0.011	0.414	0.066	0.008	0.105
7,11-nC31:2	62	mV	44.33	nd			0.673	0.023	1.065
2-MeC30	63	both	44.52	4.905	0.275	16.287	1.044	0.067	1.653
9-nC31:1	64	both		tr			tr		
7-nC31:1	65	both	44.67	1.365	0.253	4.534	0.097	0.016	0.153
5-nC31:1	66	both	44.82	0.337	0.052	1.120	0.053	0.013	0.083
nC31	67	both	45.21	0.219	0.010	0.728	0.068	0.008	0.108
uic	68	both	45.84	0.187	0.015	0.622	tr		
uic	69	both	45.96	0.247	0.020	0.820	tr		
uic	70	iV	46.42	0.399	0.138	1.326	nd		
uic	71	iV	47.53	0.035	0.035	0.115	nd		
11,17-nC33:2	72	both	47.53	0.104	0.054	0.345	tr		
Xi-nC33:2	73	both	47.91	4.496	0.585	14.928	0.204	0.069	0.323
uic	74	iV	48.16	0.091	0.020	0.304	nd		
Xi-nC33:1	75	both	48.38	2.716	0.338	9.018	0.146	0.040	0.232
uic	76	iV	48.50	0.060	0.037	0.198	nd		
uic	77	both	49.09	0.141	0.068	0.469	tr		
uic	78	iV	49.73	0.464	0.082	1.542	nd		
uic	79	iV	50.22	0.343	0.198	1.137	nd		
uic	80	iV	50.36	0.148	0.099	0.491	nd		
uic	81	iV	51.18	0.307	0.117	1.020	nd		
Xi-nC35:2	82	iV	51.31	tr			nd		
Xi-nC35:2	83	both	51.58	2.256	0.152	7.492	tr		
Xi-nC35:1	84	both	52.14	0.577	0.062	1.917	tr		
uic	85	iV	53.64	0.158	0.030	0.524	nd		
uic	86	both	54.19	0.068	0.023	0.226	tr		

(continued)

Table S1. Continued

Compound Xi: unknown position of double bond(s); uic = unidentified compound (absent from vial control). The peak numbers refer to peaks that showed up in all samples. Other peaks were not included in this analysis. Source indicates which samples included the designated compound: both = present in both; iV = present in immature virgins; mV = present in mature virgins. Mean amount is expressed as peak area counts normalized to the area of the standard (peak 36; nC26 [10 ng]). n = 5 independent samples. nd = not detected (limit of detection 10 pg on-column) tr= trace $< 0.1\%$. Percent refers to the proportion that each compound comprises of the total amount of hydrocarbons extracted.

Results: We and others have suggested that although *n*-alkanes and externally branched monomethylalkanes perform a structural function, alkenes act as signals (e.g., [S1]). Consistent with this hypothesis, the proportion of alkanes (externally branched monomethylalkanes + *n*-alkanes) to total hydrocarbons did not significantly differ between immature and mature virgins. No internally branched alkanes were detected in our samples. The proportion of alkenes (monoenes + dienes) did significantly increase with age (immature virgins: $47.8 \pm 1.7\%$. Mature virgins: $61.2 \pm 0.4\%$, $p < 0.01$). Although no difference was observed in the proportion of "total" alkanes, the proportion of monomethylalkanes was increased in the immature virgins ($34.7 \pm 1.3\%$) compared to the mature virgins ($22.5 \pm 0.3\%$; $p < 0.01$). The proportion of *n*-alkanes significantly increased in the mature virgins (immature virgins: $3.0 \pm 0.2\%$. Mature virgins: $15.3 \pm 0.3\%$, $p < 0.01$). With respect to the alkenes, the proportion of dienes significantly increased in the mature virgins ($26.5 \pm 1.0\%$ and $40.8 \pm 0.2\%$, respectively; $p < 0.01$). The proportion of monoenes did not differ between immature and mature virgins.

Methods: Hexane extracts, prepared as described, were reconstituted in 60 μl of hexane containing 10 ng/μl hexacosane (nC26) as an injection standard. A 1 μl sample of the extract was then injected on a Varian CP3800 gas chromatograph with a flame ionization detector and PTV injector (cool-on-column mode), fitted with 0.25 mm × 15 m Varian CP8510 fused silica capillary column with a 0.25 μm film thickness and a 2.5 m deactivated silica retention gap (Varian, Mississauga, Ontario, Canada). Carrier gas was Helium at a flow rate of 1 ml/min.

Analysis of the extract was carried out with a column temperature profile that began at 50°C (held for 1 min) and was ramped at 15°C/min to 150°C and then at 3°C/min to 280°C, where it was held for 5 min. The injector oven was programmed at 50°C for 0.1 min and then ramped to 280°C at 200°C/min. Varian Star Integrator software was used to calculate the retention time and total area of each peak for subsequent analysis.

Compound identification was conducted on a Shimadzu GC-17A gas chromatograph fitted with a HP-5MS fused silica capillary column (0.25 mm × 30 m, 0.25 μm film thickness) linked to a mass analyzer (Shimadzu QP5050A mass spectrometer). The injector was used in splitless mode with a splitless time of 0.5 min, and the carrier gas was helium at 1 ml/min. Injector temperature was held constant at 280°C. An oven program that began at 60°C (1 min) and was ramped at 6°C/min to 225°C and then 3°C/min to 310°C(10 min) and a pressure program of 57 kPa (1 min) to 185 kPa (1.83 min) at 2 kPa/min were employed. Electron impact positive ions at 70 eV were recorded in the scanning mode (mass range scanned 45–550 amu). The mass spectra were interpreted by fragmentation analysis and comparison to published criteria [S2, S3–S8]. Retention indices, based on a series of n-alkane standards (C10–C32; extrapolation to C36), were used to match GC-FID and GC-MS data and to obtain approximate comparisons to published data.

Statistical analysis of the proportion of hydrocarbon types (i.e., straight and branched alkanes and mono- and dienes) was conducted by Steel's 2-tailed Rank Test ($p \leq 0.01$) following Bartlett's F-test for equality of variance with ToxStat v3.4 software (Western Ecosystems Technology Inc., Cheyenne, WY). All calculations were made after removal of the standard area from the data matrix.