

Supplementary material

Chronic within-hive video registrations detect altered nursing behaviour
and retarded larval development of neonicotinoid treated honey bees

Paul Siefert^{1*}, Rudra Hota², Visvanathan Ramesh² and Bernd Grünewald¹

¹Institut für Bienenkunde

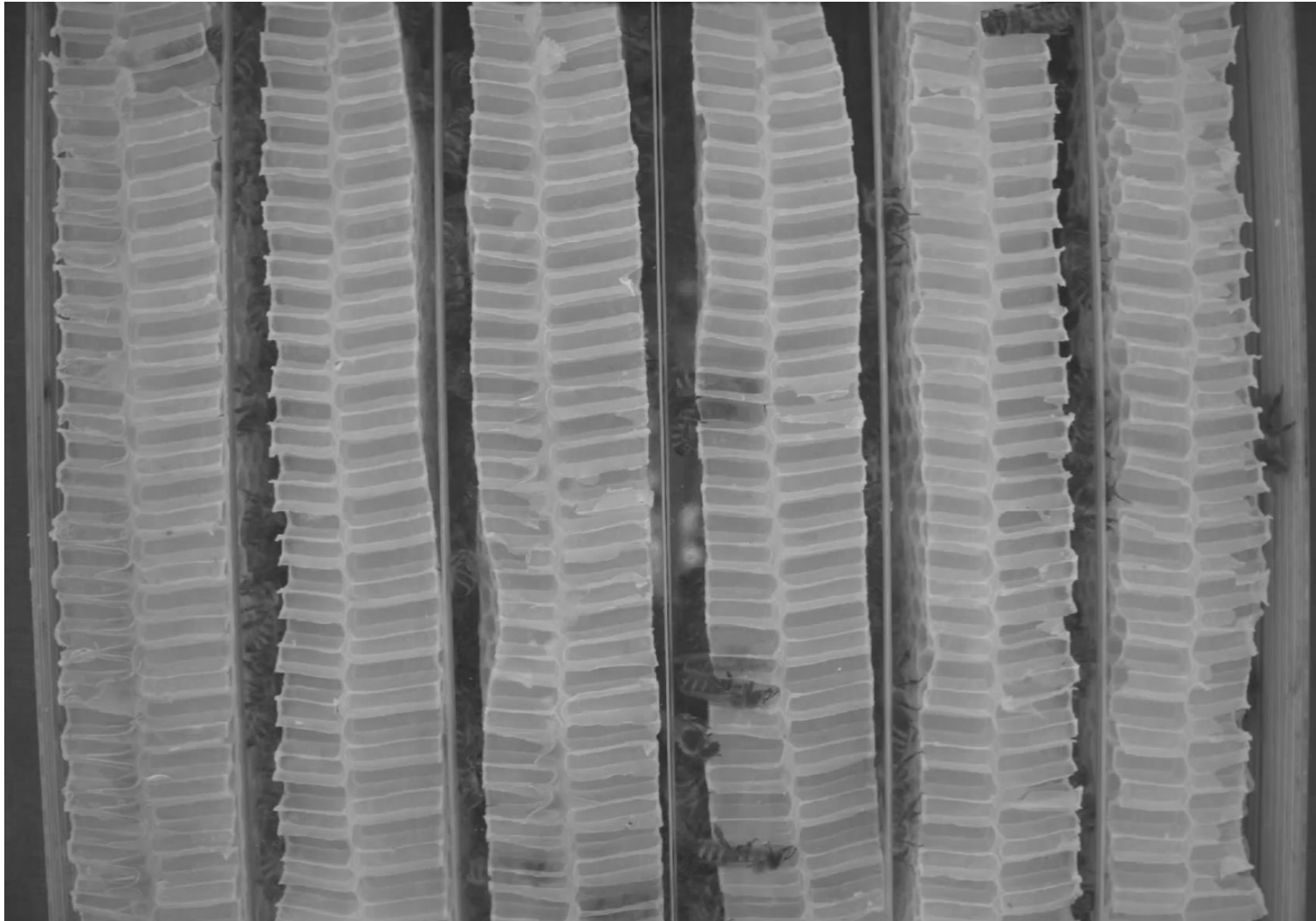
Polytechnische Gesellschaft Frankfurt am Main

Goethe-Universität, Frankfurt am Main, Germany

²Center for Cognition and Computation

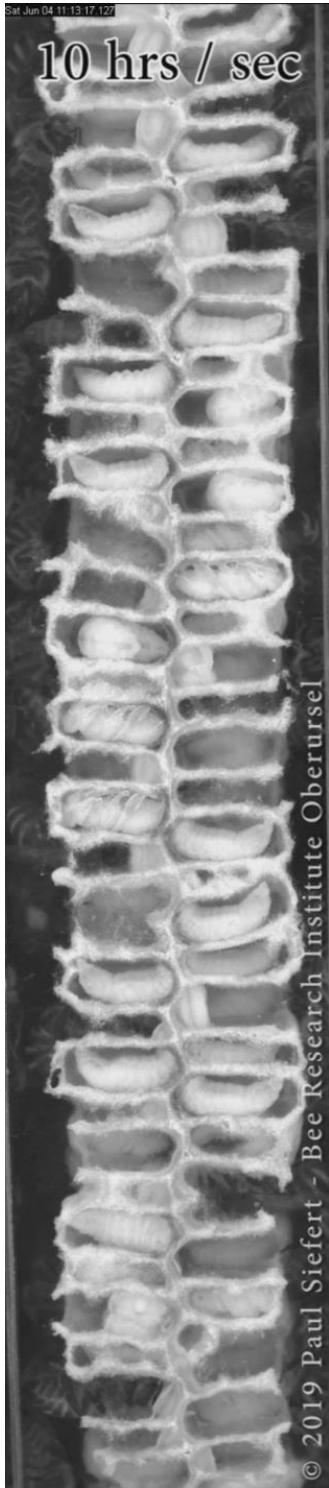
Institut für Informatik

Goethe-Universität, Frankfurt am Main, Germany



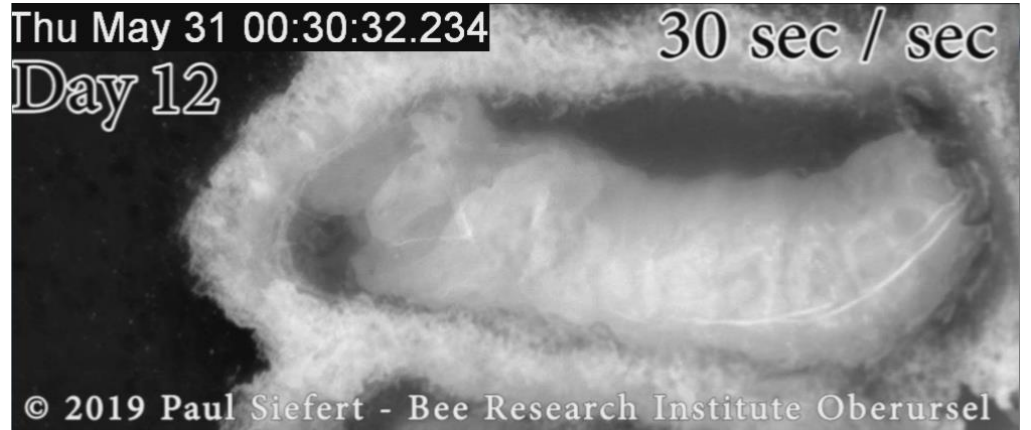
Supplementary Video S1. Time-lapse of initial building activity after setup of a hive.

This recording of a full-sized camera view in a pre-experiment in 2015 started right after setup of the hive. The wax of the cells adjacent to the glass (first cell layer) of the provided well-constructed combs was remodelled to fixate the combs to the glass. Therefore, the cells of the first cell layer initially appear to shrink in size and are extended again afterwards. In later experiments, the full-sized image was divided into six images as shown in Supplementary Video S2 to increase performance of the space-time image generation script. Furthermore, this initial building phase was not recorded to save hard-disc space since no feeding behaviour was present. Over the course of the video, adjustments were being made to the camera hardware to optimise image brightness, contrast and hard-disk requirements (capturing frequency). This material is protected by copyright. All rights reserved.



Supplementary Video S2. Comb stipe in a control colony, demonstrating the development in the breeding area within the observation hives.

This video footage was used for analysis. However, quality has been reduced to enable online publication. The colony was setup on May 17th 2016. Recording started on May 22nd and ended on June 5th, as displayed in the upper left corner. We recorded with 1 frame per second throughout the experiment, as shown in the initial 14 seconds of the video (running speed displayed at the top). Afterwards, the video is in time-lapse to demonstrate colony development. Eggs were visible shortly after colony setup and appeared descending only when workers pushed them during a deep visit in the cell, or when the larva hatched. As seen in numerous cells, young larvae of the initial eggs were cannibalized (though initially fed) until development in the invisible inner cells progressed to a certain degree (almost all inner cells were capped when colonies were disassembled at the end of the experiment). After observable development in the second layer of cells, nurses started to rear brood in the first layer shortly after, displaying that inner cells were prioritised.



Supplementary Video S3. Complete worker ontogenesis within a truncated cell.

This video was recorded from May – June 2018 for illustrational purposes in a setup specially designed for macro recordings. On the top right is the playback speed. On the top left is the date and below it the day of worker ontogenesis. Initially, the queen inspects the cell with frequent antennal movements, which are also characteristic for inspections by workers. Hereafter, the queen places an egg at the bottom of the cell. The video now focuses a different cell in which oviposition was not recorded. We approximated the larval hatch after 72 hours of development in this cell. Workers showed release of a clear substance on the glass that was taken up shortly afterwards. We assume cooling purposes since the colony stood in a very well-heated location and this behaviour was not observed in experiment colonies. On the end of day eight, after five days of larval development, the cell is capped and cocoon spinning starts for about 36 hours. The prepupa state lasts approximately 48 hours showing some movement during the first of the two days. On day twelve pupal metamorphoses takes place: The larval skin is ripped open on the side of the individual from caput to abdomen, opening zipper-like within 20 minutes. Approximately an hour later, the pupa remains motionless until day 19. The last pupal ecdysis is observable at noon on June 7th and shortly after, the imago outstretches its third pair of legs to prepare for wing development. Within 10 minutes the wings are extended and the imago does some longitudinal movements for another 15 hours before perforating the cell capping during day 20 of development. The estimated total development time of about 19.5 days is below the mean duration described in literature, possibly due to setup circumstances.

Supplementary Table S1. (continued)

June 2016

July 2016

	LDD	ANOVA		C n = 6		C1 n = 5				C10 n = 14				T200 n = 4				ANOVA		C n = 5		C1 n = 5				C10 n = 5				T200 n = 5			
		F	p	Mean	SEM	Mean	SEM	t	Sig.	Mean	SEM	t	Sig.	Mean	SEM	t	Sig.	F	p	Mean	SEM	Mean	SEM	t	Sig.	Mean	SEM	t	Sig.	Mean	SEM	t	Sig.
Feeding visits	6	5.19	0.0063	23.17	8.44	28.60	9.02	-0.64	0.5366	51.64	3.55	-2.87	0.0800	35.50	2.96	-2.03	0.0918	2.25	0.1220	6.20	2.62	14.20	3.99			7.60	2.32			3.80	2.62		
	5	8.14	0.0006	49.67	7.95	59.60	6.75	-1.06	0.3149	32.29	2.38	2.95	0.0255	35.50	2.53	1.41	0.1973	1.52	0.2478	49.40	3.08	46.60	3.91			51.20	2.29			56.60	3.97		
	4	4.52	0.0115	24.50	4.58	22.20	2.85	0.11	0.9137	13.57	1.73	2.80	0.0353	15.25	1.03	1.37	0.2093	3.00	0.0615	25.20	2.35	20.60	2.69			25.40	1.99			29.40	1.29		
	3	2.98	0.0505	11.50	1.88	7.60	0.93			7.43	0.75			7.25	0.75			2.02	0.1511	12.40	0.68	9.80	1.28			11.40	0.24			12.20	0.97		
	2	1.72	0.1887	6.33	2.17	3.00	0.55			3.93	0.46			3.50	0.50			7.08	0.0030	8.80	1.16	4.20	0.20	5.17	0.0026	5.60	0.98	2.23	0.0567	7.00	0.55	1.43	0.1904
	1	1.23	0.3186	3.67	0.67	3.20	0.58			3.29	0.45			2.00	0.41			0.09	0.9643	5.20	0.97	4.40	0.68			5.00	1.10			4.80	0.58		
Cumulative feeding visits	6	2.31	0.1012	118.83	9.98	124.20	9.29			112.14	2.49			99.00	3.76			2.69	0.0813	107.20	3.48	99.80	3.72			106.20	3.92			113.80	2.67		
	5	6.72	0.0018	95.67	14.79	95.60	8.42	-0.23	0.8240	60.50	5.06	3.11	0.0180	63.50	1.89	2.22	0.0741	4.22	0.0223	101.00	2.45	85.60	6.59	2.09	0.0939	98.60	4.88	1.09	0.6349	110.00	4.09	-1.87	0.0981
	4	3.81	0.0224	46.00	8.66	36.00	3.51	0.86	0.4122	28.21	2.80	2.83	0.0333	28.00	0.91	1.89	0.0959	4.64	0.0162	51.60	3.71	39.00	3.61	2.44	0.1214	47.40	2.93	0.40	0.4153	53.40	1.50	-0.56	0.5937
	3	2.60	0.0748	21.50	4.48	13.80	1.77			14.64	1.28			12.75	0.75			3.48	0.0406	26.40	2.42	18.40	1.44	2.68	0.0838	22.00	1.79	0.57	0.2181	24.00	1.00	0.73	0.4840
	2	1.59	0.2179	10.00	2.73	6.20	1.07			7.21	0.69			5.50	0.87			2.23	0.1245	14.00	1.90	8.60	0.81			10.60	1.81			11.80	0.58		
	1	1.31	0.2925	3.67	0.67	3.20	0.58			3.29	0.45			2.00	0.41			0.08	0.9697	5.20	0.97	4.40	0.68			5.00	1.10			4.80	0.58		
Feeding duration	6	5.84	0.0036	29.57	12.32	38.32	10.06	-1.03	0.3291	67.16	4.64	-2.74	0.1066	55.33	5.49	-2.43	0.0549	2.37	0.1092	7.59	3.27	20.01	5.97			12.34	3.49			5.58	3.39		
	5	5.76	0.0039	72.36	6.68	86.45	6.52	-1.60	0.1444	60.21	2.88	2.01	0.0602	66.38	4.13	0.61	0.5583	0.39	0.7628	70.45	3.45	74.71	5.79			75.12	3.38			80.80	9.69		
	4	3.34	0.0354	52.13	8.93	45.53	4.92	0.31	0.7667	32.58	2.94	2.54	0.0611	35.94	3.25	1.18	0.2723	3.14	0.0545	48.68	3.14	44.69	4.81			50.85	2.85			59.80	1.96		
	3	4.18	0.0158	26.28	3.68	16.57	1.52	2.63	0.0547	16.97	1.55	3.04	0.0213	15.68	1.94	2.69	0.0273	3.53	0.0391	29.90	1.03	22.64	2.58	2.31	0.0500	26.05	0.81	2.89	0.0609	28.73	1.42	0.71	0.4966
	2	1.35	0.2794	12.97	4.04	6.56	1.19			8.60	1.09			7.19	0.85			8.79	0.0011	18.13	2.08	8.65	0.45	5.65	0.0014	10.45	1.63	2.83	0.0440	14.89	1.15	1.29	0.2347
	1	1.94	0.1488	9.15	1.89	5.93	1.38			7.32	0.89			4.47	1.01			0.58	0.6342	10.53	1.66	7.45	0.73			9.74	2.38			9.93	1.27		
Cumulative feeding duration	6	0.63	0.6006	202.45	13.52	199.36	5.74			192.83	4.83			184.98	5.41			2.46	0.1000	185.29	4.31	178.14	5.65			184.54	4.51			199.74	8.11		
	5	5.06	0.0071	172.88	19.92	161.04	9.96	0.35	0.7316	125.67	7.30	3.01	0.0228	129.65	2.35	2.30	0.0669	4.68	0.0157	177.70	3.55	158.13	8.26	2.12	0.0672	172.20	5.29	0.87	0.4078	194.15	8.92	-1.77	0.1150
	4	4.05	0.0177	100.52	16.68	74.59	5.29	1.43	0.1863	65.46	5.16	2.95	0.0256	63.28	2.47	2.19	0.0602	8.27	0.0015	107.25	5.10	83.42	6.21	2.93	0.0568	97.08	3.46	1.65	0.1366	113.35	1.97	-1.17	0.2738
	3	3.85	0.0215	48.39	9.05	29.06	3.32	2.31	0.0463	32.88	2.59	2.41	0.0810	27.34	2.06	2.46	0.0783	7.01	0.0032	58.56	4.02	38.73	2.83	3.79	0.0160	46.23	3.45	2.23	0.0565	53.55	1.81	1.02	0.3390
	2	2.07	0.1299	22.12	5.77	12.49	2.48			15.92	1.56			11.66	1.40			4.06	0.0254	28.67	3.54	16.10	1.08	3.76	0.0167	20.18	3.66	1.63	0.1418	24.82	0.50	0.84	0.4450
	1	1.94	0.1488	9.15	1.89	5.93	1.38			7.32	0.89			4.47	1.01			0.58	0.6342	10.53	1.66	7.45	0.73			9.74	2.38			9.93	1.27		

Supplementary Table S1. (continued)

		Mean										
		ANOVA		C		C1		C10		T200		
		LDD	F	p	n = 4		n = 4		n = 4		n = 4	
					Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Feeding visits	6	0.18	0.9088	19.28	4.65	22.40	3.65	30.88	9.01	33.03	10.31	
	5	1.16	0.3665	47.45	1.74	48.58	4.58	40.18	3.97	42.03	5.03	
	4	0.91	0.4649	23.13	1.27	20.50	0.74	19.65	2.41	18.83	3.53	
	3	1.2	0.3509	11.23	0.56	9.53	1.00	9.48	0.94	8.93	1.13	
	2	1.97	0.1729	7.05	0.60	4.88	1.00	4.80	0.79	4.40	0.90	
	1	1.18	0.3598	4.43	0.32	4.48	0.72	4.00	0.62	3.25	0.60	
Cumulative feeding visits	6	0.13	0.9426	112.50	2.39	110.35	5.61	108.98	2.00	110.38	5.44	
	5	1.43	0.2815	93.25	3.47	87.95	2.57	78.08	7.99	77.35	11.06	
	4	1.42	0.2843	45.80	2.09	39.38	2.66	37.90	4.31	35.33	6.07	
	3	1.42	0.2852	22.68	1.35	18.88	2.68	18.25	2.29	16.55	2.54	
	2	1.46	0.2745	11.45	0.90	9.35	1.71	8.78	1.43	7.65	1.41	
	1	1.18	0.3598	4.43	0.32	4.48	0.72	4.00	0.62	3.25	0.60	
Feeding duration	6	0.30	0.8239	26.03	6.49	31.03	4.90	43.55	11.43	49.88	15.30	
	5	0.78	0.5270	71.38	2.40	76.38	6.47	66.40	3.38	69.70	4.33	
	4	0.82	0.5051	47.75	2.31	43.45	1.19	42.70	3.89	40.20	6.62	
	3	1.00	0.4257	24.73	2.12	20.45	1.69	21.00	1.98	19.80	3.13	
	2	2.02	0.1656	13.80	1.45	9.65	1.49	9.25	1.06	8.78	2.16	
	1	1.23	0.3401	8.90	0.60	8.18	1.29	7.48	1.11	6.35	1.21	
Cumulative feeding duration	6	0.19	0.9016	192.55	4.01	189.15	6.63	190.38	1.99	194.73	7.68	
	5	1.15	0.3704	166.55	6.35	158.10	2.27	146.85	9.57	144.90	17.08	
	4	1.36	0.3007	95.18	5.36	81.73	4.23	80.45	6.62	75.18	12.97	
	3	1.44	0.2808	47.45	4.07	38.28	4.25	37.70	3.77	34.93	6.43	
	2	1.7	0.2197	22.73	2.05	17.83	2.75	16.75	2.12	15.13	3.31	
	1	1.23	0.3401	8.90	0.60	8.18	1.29	7.48	1.11	6.35	1.21	