Supplementary Information for

Changes in social reward across adolescence in mice

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Supplementary Results

Social Conditioned Place Preference

As a result of trimming procedure (see section 2. Methods) no deviation from the chance value (i.e. 50%) in the time spent in social context during the pre-test was detected in any of the groups, which confirms that the test was fully unbiased **(Table S6).**

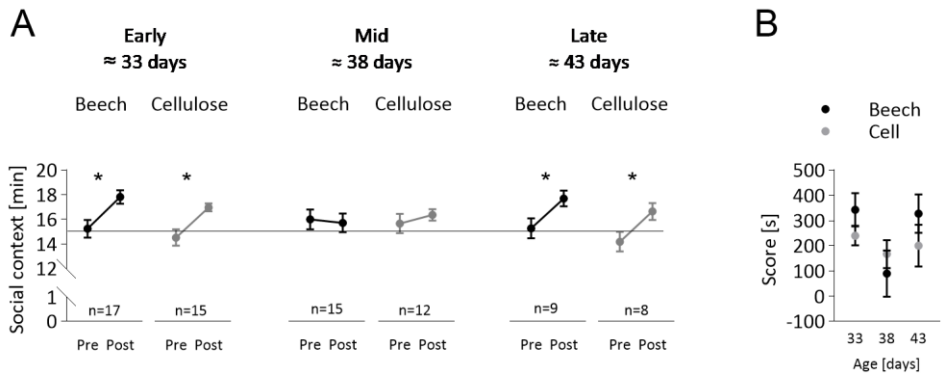

Fig. S1.

Effects of bedding type in sCPP protocol. (**A**) No effect of conditioning context type (or interaction between the context and age) on the time spent in the social context was detected. (**B**) No effect of conditioning context type on social preference score was detected. This figure uses the same dataset that was used on Figure 1 in the Main Text. '*' p <0.05, '**' p <0.01, and '***' p <0.001.

Table S1.

Age and weight of animals used in the study.

Behavioral test	Figure	Age group [days]	n	Age [days]			Weight pre-test [g]			Weight post-test [g]		
				Range	Mean	SEM	Range	Mean	SEM	Range	Mean	SEM
sCPP	1	≈33	30	32-35	33.97	0.2222	7.3-16	10.38	0.3913	12-17.3	14.9	0.3733
		≈38	20	38-39	38.05	0.5000	10.7-17.7	14.68	0.4411	15.7-21.1	18.22	0.3368
		≈43	16	43-44	43.63	0.1250	12.4-21	17.4	0.6862	15.9-21.1	18.69	0.4408
Social interaction in partition cage	2	33	10	33-33	33	0	NA	NA	NA	11.6-18.8	16.09	0.7289
		≈38	18	36-39	37.5	0.3638	NA	NA	NA	13.5-22	17.85	0.6241
		42	10	42-42	42	0	NA	NA	NA	16.6-22	19.67	0.598
Cocaine CPP	3	≈33	12	32-33	32.17	0.1124	8.5-13.1	11.34	0.3963	10.8-16.4	14.13	0.4916
		≈38	20	36-40	37.6	0.2938	13.4-20.5	16.96	0.5141	17-21.2	19.07	0.2865
		≈43	13	41-46	43.23	0.4551	16-21.8	19.42	0.4397	16.4-22.1	19.39	0.4594

Table S2.

Animals excluded from the analysis.

Behavioral test	Figure	n initial	n excluded based on initial context preference (> 70%)	n died	n excluded due to health reasons	n excluded due to technical mistakes	n exluded based on outlier test	n exluded by Python script to equalize the number of animals on the two social contexts	n analyzed
sCPP	**1**	78	2	0	1	0	4	5	66
Social interaction in partition cage	**2**	38	NA	0	0	0	0	NA	38
Cocaine CPP	**3**	47	2	0	0	1	0	NA	44

Table S3.

sCPP, social interaction and cocaine CPP tests: ANOVA results

Behavioral test	Fig	Dependent variable	ANOVA type	Source of variation	df	F	p-value	post-hoc comparison	post hoc p-value
sCPP	1B	time in social context	two-way	age	2,63	0.2182	0.8046	33 pre-post	<0.0001
				pre-post	1,63	21.69	<0.0001	38 pre-post	0.8958
				age x pre-post	2,63	3.253	0.0453	43 pre-post	0.0064
	1C	score	one-way	age	2,63	3.353	0.0420	33 vs 38 33 vs 43 38 vs 43	0.0395 0.9435 0.1617
	S1A	time in social context	three-way	age	2,65	0.1434	0.8667		
				pre-post	1,65	21.55	<0.0001		
				context	1,65	1.738	0.1920		
				age x pre-post	2,65	4.288	0.0178		
				age x context	2,65	0.6916	0.5044		
				pre-post x conext	1,65	0.1533	0.6967		
				age x context x pre-post	2,65	0.2435	0.7846		
	S1B	score	two-way	age	2,65	3.402	0.0394		
				context	1,65	0.8056	0.3727		
				age x context	2,65	1.262	0.2899		
SI	2A	time in the interaction zone	one-way	age	2,35	1.038	0.3648		
	2B	mean distance to the partner	one-way	age	2,35	0.7805	0.4660		
cocaine CPP	3A	time in cocaine context	two-way	age	2,41	0.6877	0.5084	33 pre-post	0.1081
				pre-post	1,41	14.94	0..0004	38 pre-post	0.0071
				age x pre-post	2,41	0.3454	0.7100	43 pre-post	0.3368
	3B	score	one-way	age	2,41	0.9828	0.3829		

Table S4.

Effects of the conditioning context (bedding type) on the sCPP results in different age groups: descriptive statistics and one-sample t-test (refers to Figure S1 in the Supplementary Information). Data not trimmed.

Social context	Age group [days]	df	Pre-test time spent in social context [minutes]				Post-test time spent in social context [minutes]				Score [s]			
			Mean	SEM	One-sample t test (difference from chance value)		Mean	SEM	One-sample t test (difference from chance value)		Mean	SEM	One-sample t test (difference from chance value)	
					t	p-value			t	p-value			t	p-value
Beech	≈33	16	15.24	0.7146	0.3318	0.7443	17.82	0.5528	5.108	0.0001	343	66.28	5.179	<0.0001
	≈38	9	15.98	0.8045	1.212	0.2563	15.69	0.7552	0.9182	0.3825	90	91.65	0.9862	0.3498
	≈43	8	15.24	0.8091	0.3002	0.7717	17.68	0.6359	4.212	0.0029	328	76.40	4..294	0.0026
Cellu-lose	≈33	14	14.53	0.6629	0.7129	0.4876	16.97	0.3259	6.047	<0.0001	241	39.16	6.140	<0.0001
	≈38	11	15.63	0.7829	0.8025	0.4393	16.34	0.4642	2.882	0.0149	167	55.09	3.035	0.0114
	≈43	7	14.17	0.7983	1.051	0.3282	16.62	0.6907	2.346	0.0514	201	82.76	2.427	0.0456

Table S5.

Locomotor activity during the sCPP and cocaine CPP tests: ANOVA (refers to Figure 1 and 3 the Main Text).

Behavioral test	Fig	Dependent variable	ANOVA type	Source of variation	df	F	p-value	post-hoc comparison	post hoc p-value
sCPP	1D	distance moved	two-way	age	2,63	2.279	0.1108	33 pre-post	<0.0001
				pre-post	1,63	85.03	<0.0001	38 pre-post	<0.0001
				age x pre-post	2,63	0.5909	0.5569	43 pre-post	0.0004
cocaine CPP	3C	locomotor activity	two-way	age	2,41	0.8940	0.4168	33 pre-post	
				pre-post	1,41	0.8799	0.7682	38 pre-post	
				age x pre-post	2,41	0.8897	0.4186	43 pre-post	

Table S6.

Social conditioned place preference: descriptive statistics and one-sample t test results (refers to Main Text Figure 1).

Age group [days]	**df**	**Pre-test time spent in social context [minutes]**				**Post-test time spent in social context [minutes]**				**Score [s]**			
		Mean	**SEM**	**One-sample t test (difference from chance value)**		**Mean**	**SEM**	**One-sample t test (difference from chance value)**		**Mean**	**SEM**	**One-sample t test (difference from chance value)**	
				t	**p-value**			**t**	**p-value**			**t**	**p-value**
≈33	29	14.67	0.4882	0.6721	0.5068	17.23	0.3272	6.817	<0.0001	272	39	6.924	<0.0001
≈38	19	15.43	0.5353	0.8093	0.4283	15.87	0.4273	2.027	0.057	111	52	2.157	0.044
≈43	15	14.57	0.5799	0.7369	0.4725	17.02	0.4740	4.271	0.0007	250	57	4.385	0.0005