Changes in social reward across adolescence in male mice

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Abstract

Background In humans, adolescence is a time of dynamic behavioral and emotional changes, including a transient decrease in affect associated with being among family members. It is not clear if a similar change occurs in rodent species used to model human psychiatric disorders. Here, we investigated in the developmental profile of the reward value of interactions with siblings across adolescence in mice.

Methods Social conditioned place preference test was performed in male mice representing early (around postnatal day 33 [P33]), middle (P38) and late (P43) adolescence stages. Additionally, social interaction in the partition test and cocaine conditioned place preference were measured to assess the specificity of changes observed in social reward.

Results The reward value of interactions with siblings in adolescent male mice followed a similar course to that in humans: high in preadolescence, it decreased in mid-adolescence and returned to the initial level in late adolescence. No age-dependent changes in social interaction or in the preference for cocaine-conditioned context were detected.

Limitations The main limitation of our study is that it does not examine potential developmental changes in the proximate psychological mechanisms underlying social place preference.

Conclusions Taken together, these data show similarities between mice and humans in developmental changes in sensitivity to the rewarding effects of interactions with familiar kin.

Introduction

Adolescence is a time of rapid behavioral and neural changes, as well as the peak onset age for many mental disorders[1,2]. It is postulated that the emergence of psychiatric symptoms during adolescence results from alterations in typical developmental processes[3]. However, causal links between adolescent changes in brain maturation, behavior and pathophysiology have not been firmly established, partly because of the lack of proper animal models. It is thus of great importance to understand to what extent the behavioral development of model animals parallels the features of human adolescence[4].

One of the characteristic features of human adolescence is changes in social preferences. Whereas infants fully depend on parental care and display strong distress following separation from their mothers, mid-adolescents show a decrease in the time spent with their family members along with an increase in the time spent alone or with peers[5,6]. This behavioral shift is accompanied by changes in emotions associated with relatives: early and late adolescents show positive affect in the company of their family members, while mid-adolescents report more negative emotional states[5]. However, it is not known whether affective changes resembling those observed in humans also occur in animals. Therefore, the goal of the present study was to assess changes in the reward value of interactions with familiar kin across adolescence in male mice.

Methods

Animals

Experiments were performed with C57BL/6 male mice bred at the Maj Institute of Pharmacology animal facility. Mice were housed in a 12/12 h light-dark cycle (lights on at 7 AM) under controlled conditions: a temperature of 22 ± 2 °C and a humidity of 40-60%. After weaning, the mice were housed with all littermates of the same sex. Rodent chow and water were available ad libitum. Home and conditioning cages contained aspen nesting material and aspen gnawing blocks. Behavioral tests were conducted during the light phase under dim illumination (5-10 lux). sCPP and social interaction tests were video recorded with additional infrared LED illumination. The age and weight of mice in each experimental group are summarized in **Table S1**.

All behavioral procedures were approved by the II Local Bioethics Committee in Krakow (permit numbers 35/2019, 185/2020, 38/2021, 266/2020, 305/2020) and performed in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The reporting in the manuscript follows the ARRIVE guidelines.

Social conditioned place preference

The test was performed to assess the rewarding effects of housing with siblings and followed the procedure described previously [7]. The test consisted of three phases: pretest, conditioning, and posttest (**Fig. 1A**).

The pretest and posttest phases were performed in a two-compartment cage, as in previously published papers[7–11]. Each cage compartment contained a novel context (context A or context B) defined by type of bedding and gnawing block size and shape. Bedding materials used were beech (context A, P.P.H. "WO-JAR", Poland or PPHU Natur-Drew A. Czaja, Poland or Terrario Peak

79 Wilderness, DMR Group, Poland) and cellulose (context B, Scott Pharma Solutions, cat no. L0107). In
80 the home cages, aspen bedding was used (ABEDD, Latvia or Tapvei GLP, Estonia). Mice were allowed
81 to freely explore the test cage for 30 minutes, and the time spent in each compartment was
82 recorded. Animals that spent more than 70% of the pretest time in one of the contexts were
83 excluded (**Table S2**).

84 After the pretest, animals were returned to their home cages for approximately 24 h. Then, mice
85 were assigned to undergo social conditioning (housing with cage mates) for 24 h in one of the
86 contexts used in the pretest followed by 24 h of isolate conditioning (single housing) in the other
87 context. Conditioning was performed in cages identical to the home cage, with ad libitum access to
88 food and water. To prevent bias, the social context was randomly assigned such that approximately
89 half of the animals received social conditioning in context A and half in context B. In cases where the
90 final number of animals conditioned in each context was not equal (due to an unequal number of
91 animals passing the 70% criterion or unequal number of animals in the litter), we pseudorandomly
92 trimmed the larger group using a Python script (https://zenodo.org/record/8100281)[12]. The
93 exception from completely random selection was introduced to preserve a mean 50% initial context
94 preference during the pretest, i.e., ascertain that the test was fully unbiased (Table S3). Analysis on
95 the full data set (not trimmed) was also performed, to assess the potential interaction between the
96 age and conditioning context on social reward (**Figure S1, Table S4**). The conditioning phase lasted 6
97 days (3 days in each context, alternating every 24 h), and then the posttest was performed.

98 Two measures of the rewarding effects of social interactions were used: 1) pretest vs. posttest
99 comparison of the time spent in the social context, 2) score: time spent in the social context minus
100 time spent in the isolation context during the posttest.

101 Social interaction in the partitioned cage

102 This test was carried out to assess social contact seeking with a sibling partner after 24 h of isolation.
103 The procedure was performed in a rectangular cage (48 × 12 cm, 25 cm high) divided by a
104 transparent, perforated plastic wall into two compartments: a smaller partner compartment and a
105 larger focal animal compartment (**Fig. 2A**). One day before the test, the animals were weighed, and
106 the heavier animal from each pair was designated as the focal animal. Next, the animals were
107 habituated to their respective cage compartments for 10 minutes. During habituation, only one
108 mouse was present in the test cage. After habituation, mice were placed in separate home cages for
109 approximately 24 h, after which time the focal animals were placed in the test cage for the second
110 adaptation session (5 minutes). After adaptation, the partner was introduced for 10 minutes. Two
111 measures of social contact seeking were used: time spent in close proximity to the partner's
112 compartment and distance to the partner's compartment.

113 Cocaine-induced conditioned place preference

114 For the CPP paradigm, three-compartment cages were used (Med Associates, St. Albans, VT,
115 USAMED-CPP-MSAT); the two peripheral compartments (that contained distinctive visual and tactile
116 cues) were linked to the central compartment by guillotine doors. The test consisted of three phases:
117 pretest, conditioning, and posttest. For the pretest and posttest phases, animals were introduced to
118 the central compartment of the apparatus, and the doors between the compartments were lifted
119 such that the animals could freely explore the apparatus for 20 minutes. Animals that spent more
120 than 70% of the pretest time in one of the contexts were excluded. The less preferred of the two
121 peripheral compartments was designated the cocaine compartment. The next day, the 3-day
122 conditioning phase started. Each day, two 40-minute conditioning sessions were performed,
123 separated by approximately 3 hours, during which animals were kept in their home cages. Before the
124 morning conditioning session, animals received an i.p. saline injection, while before the afternoon
125 session, they were injected with cocaine hydrochloride dissolved in saline (10 mg/kg, 5 µl/g).
126 Immediately after the injection, animals were placed in the respective cage compartment, while the

127 guillotine doors separating the compartments were closed. The posttest was performed on the day
128 after the last conditioning session. The measures used to assess cocaine reward were the same as for
129 the social conditioned place preference.

130 Data analysis

131 The distance traveled and time spent in separate cage compartments in the sCPP and social
132 interaction tests were analyzed automatically using EthoVision XT 15 software (Noldus, The
133 Netherlands). In the social interaction test, the zone close to the partner's compartment was
134 outlined digitally. In the cocaine-induced CPP test, the position of the mouse was registered
135 automatically by the Med Associates system. Comparisons of sample means were performed using
136 analysis of variance (ANOVA) followed by Sidak's post hoc correction or Student's t test for cases
137 with only two samples. The statistical significance threshold was set at $p < 0.05$. Before the analysis,
138 the Grubbs test for outliers was performed. For sCPP results, the outlier test was performed on
139 "score", "index 1", "index 2", and "distance moved during post-test" parameters before the trimming
140 of the data. The test was performed separately for the context A and B data. Four outliers were
141 detected in the sCPP data: one in the P33 group, two in the P38 group, and one in the P44 group. For
142 the social interaction data, the outlier test was performed on the parameter "time in interaction
143 zone". No outliers were detected. For the cocaine experiment, the outlier test was performed on
144 "score", "index 1", "index 2", "exploration during posttest", and "movement during posttest"
145 parameters. No outliers were detected. Data concerning animals excluded from the analysis are
146 presented in **Table S2**. The analysis was performed in GraphPad Prism 9.4.1. For full descriptive
147 statistics and statistical test results, see **Supplementary Materials**.

148

149

Results

Rewarding effects of interactions with siblings across adolescence

To investigate the possible changes in the rewarding effects of social interactions with siblings during adolescence in mice, we used the social conditioned place preference (sCPP) test (**Fig. 1A**[7]) with male mice representing early (around postnatal day 33 [P33]), middle (P38) and late (P43) adolescence stages (for information about experimental groups, see **Tables S1** and **S2**). Animals were conditioned to associate one environmental context with group housing and another with social isolation, and then were tested to determine context preference. Mice aged 33, and 43 (but not 38) days at posttest showed a significant increase in the time spent in the social context from pretest to posttest, as revealed by significant interaction between age and conditioning (**Fig. 1B, Table S5,** Age $F_{2,63}$ = 0.2182, p = 0.8046; Pre-Post $F_{1,63}$ = 21.69, p < 0.0001; Age x Pre-Post $F_{2,63}$ = 3.253, p = 0.0453; post hoc P33 p < 0.0001, P38 p = 0.8958, P43 p = 0.0064). There was no effect of context (i.e., the type of bedding) on preference (**Fig. S1, Table S4**). It had no significant effect as main factor or in interactions with age or conditioning, while interaction between age and conditioning remains significant when context is added as variable (Context $F_{1,65}$ = 1.738, p = 0.1920; Age x Context $F_{2,65}$ = 0.6916, p = 0.5044, Pre-Post x Context $F_{1,65}$ = 0.1533, p = 0.6967; Age x Pre-Post x Context $F_{2,65}$ = 0.2435, p = 0.7846; Age x Pre-Post $F_{2,65}$ = 4.288 , p = 0.0178). Thus, interactions with siblings had lower reward value for mid-adolescent mice. The decrease in preference for the compartment associated with social contact in mid-adolescent mice was also clearly apparent in the social preference score (**Fig. 1C**). The score showed that the rewarding effects of social interactions were more than two times lower in mid-adolescent (P38) than in early-adolescent (P33) mice and returned to the early adolescent level in late-adolescent (P43) mice (Age $F_{2,63}$ = 3.353, p = 0.0420). Importantly, motor activity was not significantly affected by age or interaction between age and conditioning; thus, it was not a confounding factor (**Fig. 1D, Table S6,** Age $F_{2,63}$ = 2.279, p = 0.1108; Pre-Post $F_{1,63}$ = 85.03, p

174 < 0.0001; Age x Pre-Post $F_{2,63}$ = 0.5909, p = 0.5569). Taken together, the results reveal a transient

175 decrease in the rewarding effects of interactions with related individuals in mid-adolescent mice.

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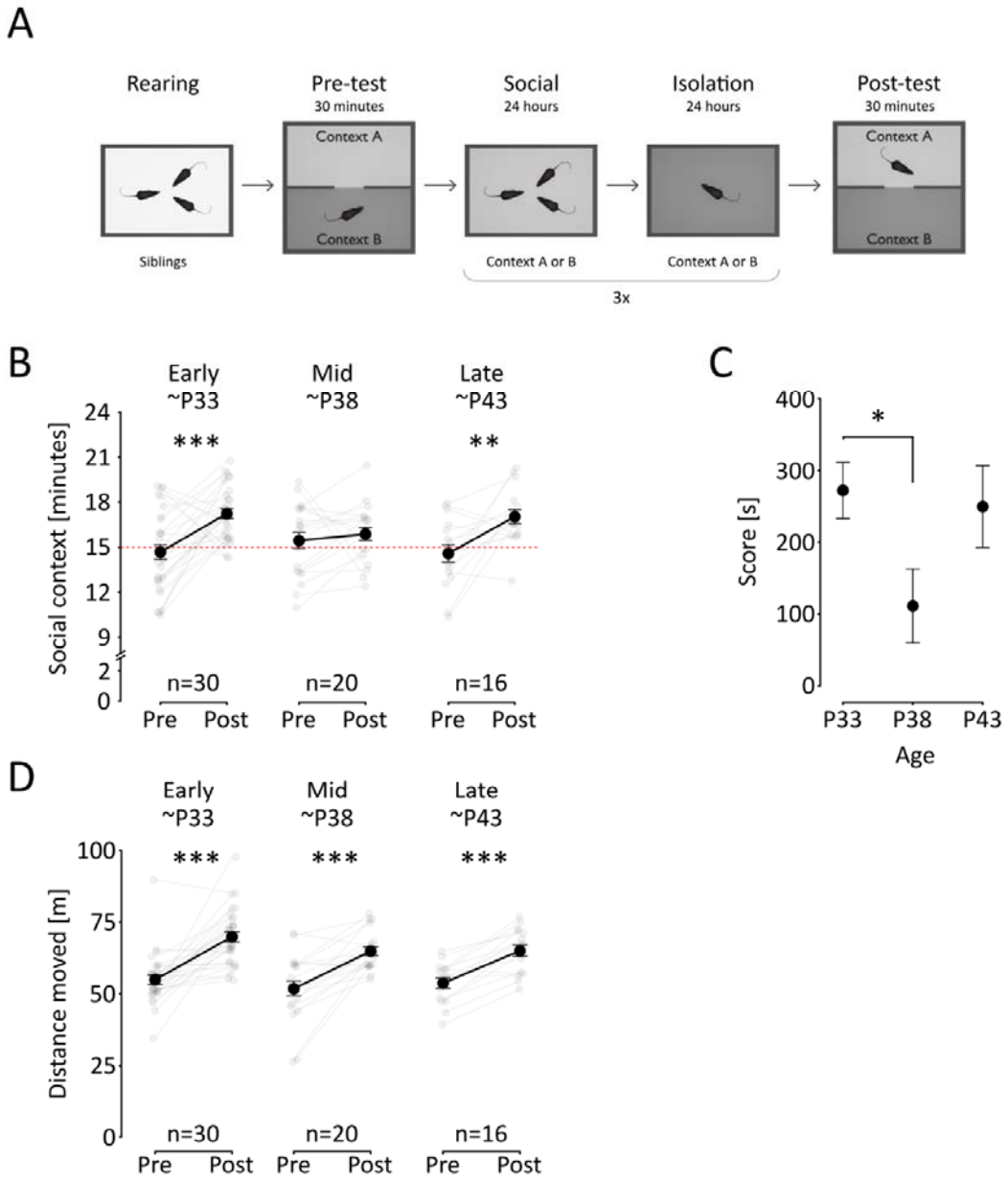

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178 **Fig. 1. Social conditioned place preference during adolescence.** (**A**) Schematic representation of the

179 experimental schedule. (**B**) Time spent in the social context. The graphs show the mean time spent

180 on the social-conditioned context during the pretest and posttest (black dots and line, error values

are s.e.m.). The gray points and lines show all individual mice in the corresponding age bracket as indicated above. (**C**) Preference of the social context. The points show mean values of the preference score, i.e., the difference between the time spent in social context and isolation context during posttest of mice at different adolescence stages as indicated below. (**D**) Distance traveled during the tests. Points represent mean distance travelled during pretest and posttest, error bars are s.e.m., gray points and lines represent individual animals. Significant post-hoc (ANOVA with Šidák correction) differences between mean values are shown as: '**' $p < 0.01$, and '***' $p < 0.001$.

Social contact

Next, we investigated if the change in social behavior in mid-adolescent mice was specific to the rewarding effects of social interactions or if contact seeking was also altered. To explore this possibility, we administered a test in which contact with another mouse is enabled through a transparent, perforated plexiglass wall (**Fig. 2A**)[13]. The interaction partners were siblings reared in the same cage but isolated for one day before the test to match the conditions of the sCPP posttest. We observed no age-related changes in the time spent in the proximity to the partner's compartment (**Fig. 2B, Table S5,** Age $F_{2,65} = 1.038$, $p = 0.3648$) or the distance between the focal mouse and the partner's compartment (**Fig. 2C,** Age $F_{1,65} = 0.7805$, $p = 0.4660$). These results might indicate that the decrease in the rewarding effects of interactions with related individuals in mid-adolescent mice was not accompanied by a general decrease in social contact seeking.

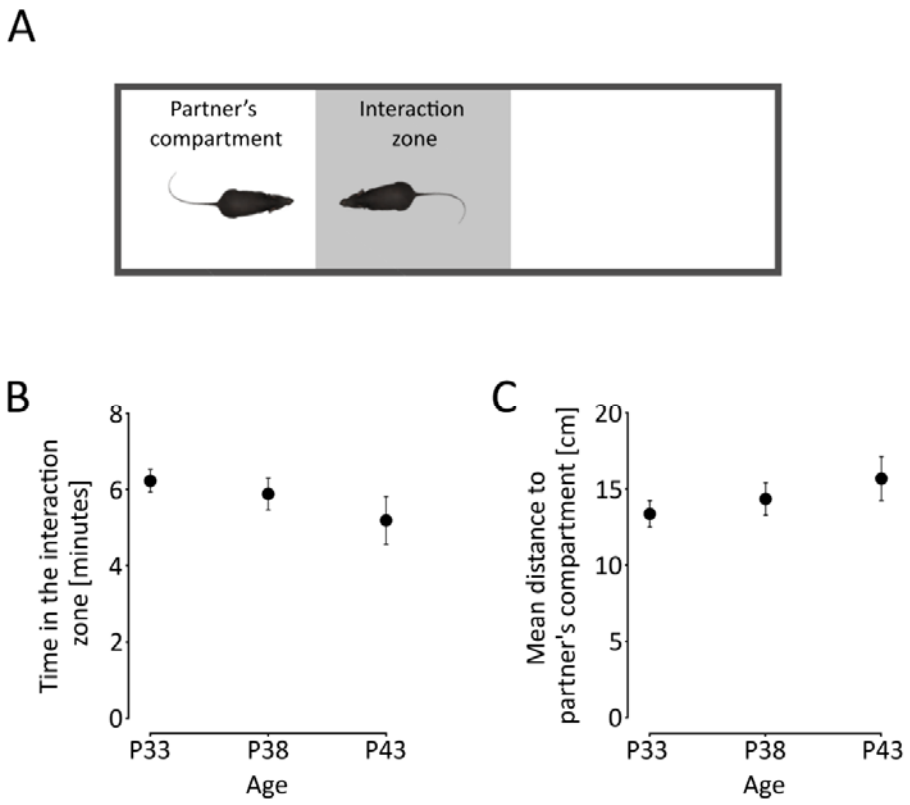
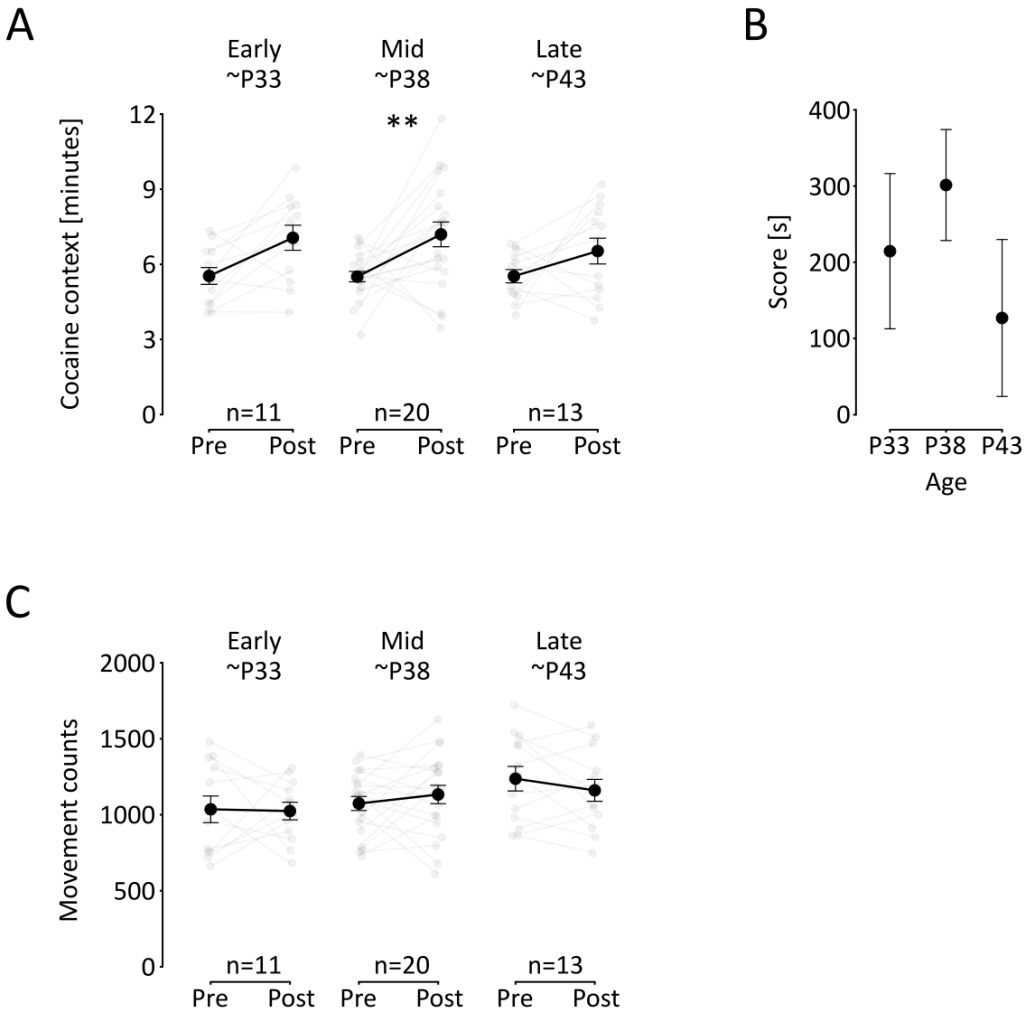


Fig. 2. Social contact seeking during adolescence. (**A**) A schematic representation of the task. Mice were tested for seeking social contact with a sibling after 24 h of isolation. (**B**) Time spent in the interaction zone. The points show mean time spent in the zone, error bars are the s.e.m. (**C**) Mean distance from the partner's compartment. Group sizes: P33 n = 10, P38 n = 18, P43 n = 10.

Rewarding effects of cocaine

An alternative explanation for the observed decrease in the rewarding effects of social interactions could be a general anhedonia or impairment in associative learning. To test whether the decrease in the rewarding effects of social interaction reflected a stimulus-independent reduction in the expression of conditioned behaviors, the cocaine-induced CPP was assessed. As expected, a significant increase in time spent in cocaine-paired compartment was observed but without the effect of age or interaction between age and conditioning (**Fig. 3A, Table S5,** Age $F_{2,41}$ = 0.6877, p =

215 0.5084; Pre-Post $F_{1,41}$ = 14.94, p = 0.004; Age x Pre-Post $F_{2,41}$ = 0.3454, p = 0.7100). In line with this
216 interpretation, no significant effect of age on the place preference score was detected (**Fig. 3B,** Age
217 $F_{1,41}$ = 0.9828, p = 0.3829). No significant effect of age or conditioning on locomotor activity was
218 observed (**Fig. 3C,** Age $F_{2,41}$ = 0.8940, p = 0.4168; Pre-Post $F_{1,41}$ = 0.8799, p = 0.7682; Age x Pre-Post
219 $F_{2,41}$ = 0.8897, p = 0.4186). These data show that no general impairment of reward-conditioned
220 preference occurred during mid-adolescence.



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222 **Fig. 3. Cocaine-induced conditioned place preference during adolescence.** (**A**) Time spent in the
223 cocaine-conditioned context. The points show mean time spent in the cocaine-paired context during
224 the pretest and posttest, error values are s.e.m.. The gray points and lines show individual mice in

225 age brackets indicated above. (**B**) Preference of the cocaine-conditioned context. The points show
226 mean values of the preference score, i.e. the difference between the time spent in cocaine-
227 conditioned context and saline-paired context during posttest of animals at different adolescence
228 stages as indicated below. (**C**) Distance traveled during the tests. Points represent mean distance
229 travelled during pretest and posttest, error bars are s.e.m., gray points represent individual animals.
230 A significant post-hoc (ANOVA with Šidák correction) difference between mean values is shown as:
231 '**' $p < 0.01$.

232

233

234 # Discussion

235 We show that the rewarding effects of interactions with familiar kin in male mice exhibit a
236 transient decrease during mid-adolescence (around P36-40). This decrease was specific to social
237 reward, as no change in social contact seeking, and the reward value of cocaine was observed during
238 this period. Our results resemble human data reported by Larson and Richards (1991), who observed
239 that the affect associated with time spent with family members was more positive in 10-year-old and
240 16-year-old boys than in 12- to 14-year-olds[5]. The age range of 11 to 16 years in male humans
241 corresponds to P30-40 in male mice, and this period is considered "peripubertal"[14]. This indicates
242 that the phenomenon observed in our study, i.e., the temporary decrease in the reward value of
243 interactions with familiar kin, is evolutionarily conserved. This finding may facilitate future research
244 on the neuronal and physiological underpinnings of rebellious behaviors in adolescence.

245 Our results complement those of two previous studies that assessed the rewarding effects of
246 social interactions using the sCPP paradigm at selected time points in adolescent mice[11,15]. In contrast
247 to the methodology presented here, both previous studies used animals that were familiar but were
248 not specifically kept in sibling groups. As we have shown previously, regarding the reward value of
249 social interactions, eight weeks of familiarization with nonrelated mice is not equivalent to being
250 reared in the same cage before weaning, at least in females. Mice do not form an sCPP when
251 conditioned as adults in groups coming from different litters and housed together since weaning[7].
252 Moreover, both previous studies used a paradigm with only two days of conditioning, which
253 produces different results than the paradigm with six conditioning sessions used in this study[7,10].
254 Bearing these differences in mind, we note that Cann and collaborators (2020) reported that social
255 contact is rewarding in mice tested on postnatal day 29 but not on postnatal day 38, which aligns
256 with our results. Conversely, in the study by Nardou and collaborators (2019), a decrease in the
257 rewarding effects of social interactions during adolescence was not observed in male mice (although
258 it was observed in females). However, the previous results were not interpreted in terms of the

259 possible decrease in the reward value of social interactions during adolescence. Instead, Cann and
260 collaborators conclude that sCPP, under the applied conditions, can be induced in early adolescent,
261 but not adult mice, implying that the observed lack of conditioning effects at the age of 38 days is
262 considered permanent. Results presented in this work, together with our previous paper where adult
263 mice were studied[10], suggest that sCPP can be induced in late adolescent and adult male mice,
264 provided that the 6-day protocol is used.

265 Recent study by Murray and collaborators (2024) investigated developmental changes in the
266 motivation to obtain social reward in rats[16], a species in which the equivalent of P30-40 in mice is
267 P42-55[14]. In this study, the operant conditioning protocol was employed and an unfamiliar
268 conspecific was used as a stimulus animal. The authors found that social motivation is higher in pre-
269 adolescent (P30) and mid-adolescent (P50) rats than in early adolescent animals (P40). This might
270 indicate that the rewarding effects of and motivation to obtain social reward differ depending on the
271 familiarity of the stimulus animal, with mid-adolescence being a period of decreased familiar kin
272 contact seeking, and higher motivation to interact with unfamiliar individuals, which could serve as
273 one of the proximate mechanisms of dispersal from natal environment[17–19]. A study using the 6-day
274 sCPP protocol on mice housed with familiar and unfamiliar conspecifics in different adolescence
275 timepoints should be performed to test this hypothesis. However, it must be noted that Murray and
276 coworkers use different labels for the developmental periods, and describe P40 as mid-adolescence
277 and P50 as late adolescence[16].

278 In contrast to social reward, there were no apparent changes in social contact seeking
279 (assessed with the partition test) during adolescence. This finding indicates that the changes in social
280 interactions in adolescent mice are qualitative rather than quantitative. We speculate that the
281 amount of time spent with siblings may not change, but aggressive encounters may replace affiliative
282 interactions. This interpretation is supported by earlier studies showing a profound decrease in
283 passive social contact[20] and play behavior[21], along with an increase in fighting[20,22], in the second

284 postnatal month of mice. Strikingly, the cocaine-induced CPP did not change during the adolescent
285 period. These results indicate that the decrease in social CPP in mid-adolescent mice cannot be
286 explained by a general decline in learning abilities and confirm that different neuronal processes
287 underlie the rewarding effects of cocaine and social contact[9]. Developmental changes in cocaine-
288 induced CPP were previously studied in rats, and their results are inconsistent. For example,
289 Brenhouse and coworkers reported a greater cocaine (10 mg/kg)-induced CPP in adolescents (P44)
290 than in adults (P105)[23]. Notably, preadolescent rats (P27-37) showed similar levels of cocaine-
291 induced CPP as adult animals[23,24], which suggests an inverted U-shaped relationship between cocaine
292 reward and age. These results are consistent with previous reports of heightened sensitivity to other
293 drugs of abuse in adolescent animals[25]. Conversely, the results by Badanich and collaborators
294 indicated the highest sensitivity to cocaine reward in pre-adolescent rats, because a low cocaine dose
295 (5 mg/kg) induced CPP only in pre-adolescents (P35), but not in adolescents (P45) or young adults
296 (P60 animals)[26]. This suggests that some factors unidentified yet, for example the level of stress
297 imposed by the experimental protocol, might differentially influence the rewarding effects of cocaine
298 in different sub-periods of adolescence. Obviously, more studies are needed to resolve this issue.
299 Irrespective of these inconsistencies, our results indicate that social and drug reward follow different
300 developmental trajectories in the peri-adolescent period.

301

Limitations

The main limitation of our study is that it does not examine potential developmental changes in the proximate psychological mechanisms underlying social place preference. Generally, grouping may be driven by both negative and positive reinforcement[27]. Specifically, it may be reinforced either by the alleviation of negative affect caused by social isolation or by the rewarding nature of interactions with conspecifics. Panksepp and collaborators demonstrated that in early adolescent mice (postnatal days 31–32), social place preference is primarily driven by aversion to isolation. Mice subjected to a 'reversed' social conditioned place preference protocol (where the post-test immediately follows the group conditioning session instead of the isolation session) did not display a preference for the conditioning context[28]. In contrast, a study by Wei and colleagues found that adult mice did develop a context preference under the reversed sCPP protocol[29]. This suggests that the motivation underlying social place preference may shift during adolescence from being driven by the aversiveness of isolation to being driven by the rewarding nature of social interactions, that is, from negative to positive reinforcement. Accordingly, the mid-adolescent decline in preference for the social context observed in the present study may reflect a transitional period in which social isolation no longer acts as a negative reinforcer, while the mechanisms of positive social reinforcement are not yet fully developed. Future research should test this hypothesis.

A second limitation of our study is that it was conducted exclusively on male mice. Female mice may exhibit a different developmental trajectory in the valuation of social rewards.

Conclusion

Taken together, our data show similarities between mice and humans in the pattern of social reward development and suggest that a decrease in rewarding effects of interactions with familiar kin occurring in mid-adolescence might serve as a proximate affective mechanism of dispersal from the natal environment.

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Data availability:

All data are available at https://zenodo.org/records/15284680.

Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

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Supporting Information for

Changes in social reward across adolescence in male mice

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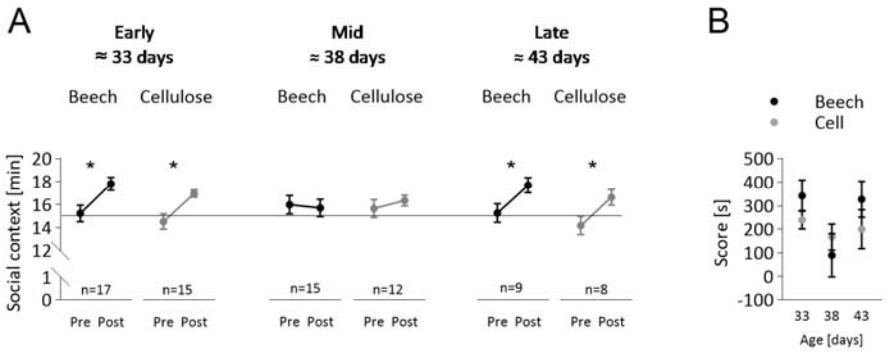
This PDF file includes:

Fig. S1

Tables S1 to S8



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433 **Fig. S1.**

434 **Effects of bedding type in sCPP protocol.** (**A**) No effect of conditioning context type (or interaction

435 between the context and age) on the time spent in the social context was detected. (**B**) No effect of

436 conditioning context type on social preference score was detected. This figure uses the same dataset

437 that was used on Figure 1 in the Main Text. '*' $p < 0.05$, '**' $p < 0.01$, and '***' $p < 0.001$.

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441 **Table S1.**

442 **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

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445 **Table S2.**

446 **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

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449 **Table S3.**

450 **Social conditioned place preference: descriptive statistics and one-sample t test results (refers to**

451 **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

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453

454 **Table S4.**

455 **Effects of the conditioning context (bedding type) on the sCPP results in different age groups:**

456 **descriptive statistics and one-sample t-test (refers to Figure S1 in the Supplementary Information)**

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

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Table S5.

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	0.0395
								33 vs 43	0.9435
								38 vs 43	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		

461 **Table S6.**

462 **Locomotor activity during the sCPP and cocaine CPP tests: ANOVA (refers to Figure 1 and 3 the**

463 **Main Text).**

464

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	

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