KU LEUVEN

FACULTY OF PSYCHOLOGY AND
EDUCATIONAL SCIENCES

Laboratory of Biological Psychology

Assessment of parental bonding in single- versus two-parent mouse pups

Master's thesis submitted for the degree of
Master of Science in Master of Psychology:
Theory and Research by
Yang Yang

Supervisor: Prof. Rudi D'Hooge
Co-supervisor: Prof. Guy Bosmans

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Summary

A secure attachment relationship in childhood is closely related to the development of an individual. Attachment to the father especially predicts children's exploratory behavior. It is of importance to gain more insight into the role of father's attachment in the cognitive development of the offspring. Experimental studies with laboratory mice are theoretically and practically feasible alternatives to study the attachment relationship and its outcomes in a more controlled manner. In this study, we aimed to investigate the social preference and exploratory tendency of C57BL/6 laboratory mice, and the effects of cohabitant father, mother sensitivity and age on social preference and exploratory tendency.

Three main research questions were formulated: (1) Do pups show a preference to the mother or the father in case they have a cohabitant father? (2) Do pups show a preference to the mother or to the outside cohabitant father in case they are tested with an outside cohabitant father? (3) Do pups show a preference to their own father or the outside cohabitant father in case they have a non-cohabitant father? In the meantime, the research questions also incorporated the effect of father cohabitation, mother sensitivity and age of the pups on the social preference and exploratory tendency of the pups. Three testing conditions were designed: mother vs. father, mother vs. other father and father vs. other father, to cover all planned comparisons. The social preference data was collected with the assistance of ANY-maze video tracking system.

The statistical results showed that pups preferred mother to father and outside cohabitant father, and pups in general showed equal preference to the father and the comparison father. However, pups with own non-cohabitant father preferred the outside cohabitant father to their own father. Father cohabitation effect was found to be significant in affecting the social preference and the exploratory tendency of the pups in all three conditions. Age was found significant in predicting the exploratory tendency of the pups in all three conditions, and sensitivity was found significantly predicting the exploratory tendency in mother vs. father condition and social preference significant in mother vs. other father condition.

Our results suggest that father cohabitation has a positive effect on the social preference of the pups to the father and the exploratory tendency of the pups. The study can be used as a prototype to study father attachment in laboratory mice.

Acknowledgement

I would like to thank a few people that provided me with great guidance and support during the writing of this thesis. First of all, I would like to thank the supervisors Prof. Dr. Rudi D'Hooge and Prof. Dr. Guy Bosmans for presenting this thesis topic. The subject is very interesting and has great meaning to me. Both Prof. Dr. D'Hooge and Prof. Dr. Bosmans gave me insightful advice on the experimental design, literature, data analysis, writing and the general structure of the thesis. I am very grateful for their valuable feedback. Without their help, I would not have been able to complete the thesis.

My heartfelt gratitude also goes to laboratory technician and administration assistant Leen Van Aerschot. Leen provided me enormous support regarding lab operation, animal handling and care. She helped me resolve technical obstacles and assisted me in experimental tests. The great organization skills and proactive planning of Leen made my time in the lab much easier. I would also like to thank Prof. Dr. Gerrit Storms for tolerating me to carry out experiments and work on the thesis during my internship in the ConCat lab.

Finally, I would like to thank my husband Karel Mycke for taking care of the household and giving me as much time as possible to work on the thesis, and our daughter Lena Mycke, my motivation to choose this subject. I thank her for being a part of my life and letting me be a part of hers.

Explanation of approach and ownership

The subject of this study was chosen from the list of thesis topics provided by the Faculty of Psychology and Educational Sciences. It was presented by the supervisors Prof. Dr. Rudi D'Hooge and Prof. Dr. Guy Bosmans. Before our first discussion, Prof. Dr. D'Hooge gave me an introduction of the background and the general goal of this research. During the first meeting with both supervisors, we brainstormed for the experimental design. Laboratory assistant Leen Van Aerschot was my daily contact concerning lab use and experimental practice. Leen informed me about lab operation and care of the mice.

I enrolled in the course of 'Lab Animal Health' and obtained my certificate of lab animal handling. In the period between 17th December 2014 to 1st October 2015 I kept a total number of 110 laboratory mice. I changed the bedding materials and fed the mice with food and fresh water once a week. After the pups were born, I performed daily checks on the maternity cages. I started experimental tests with the assistance of Leen and completed all tests independently. The data collected from 54 mouse pups were used in this study.

I consulted literature in animal behavior studies, specifically in attachment theory, social preference and exploratory behavior. My supervisors provided me with insightful ideas and interesting reading materials for the theoretical and practical basis of this study. During our meetings, I reported my progress and tried to contribute my ideas for the ongoing and future research.

For the statistical analysis, I used multiple linear regression methods learned in the courses of Statistics IV and Statistics V.

I chose to write this thesis in the article form because it is easier for the readers, and it was planned to be submitted to the *Journal of Experimental Psychology: Animal Learning and Cognition* because of the nature of the study and the possible resources it can provide for future research in animal cognition.

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1. Introduction

Attachment theory was developed by Bowlby (1988) who considered attachment as the emotional bonding between the infant and the mother, in terms that the infant perceives the mother as the base of security, seeks proximity of the mother and experiences distress when separated from the mother. Bowlby (1979) defined attachment behavior as keeping a younger and more vulnerable individual in proximity and taking care of it. Although considerably more attention in the attachment field has been given to mothers, interest in fathers emerged very early in the development of attachment theory (Bretherton, 2010).

Previous researchers have found that the fathers' personality, marital harmony, stress and support in both work and family were associated with infant attachment security to fathers (see Horn, 2000, for a review). However, evidence suggests that the quality of the infant-father attachment relationship may be more closely related to the fathers' motivation and attitudes towards fathering and family than his interactions with the infants during the first year (Crossmann et al, 2002). Lamb (1975) depicted fathers as the link between the children and the outside world. Previous research (Biller, 1993; Lamb, 1997; Booth & Crouter, 1998) also suggested that the accessibility of the father, his closeness and warmth to the children are critical for the father-child relationship.

Crossmann (2002) found that being present at birth was significantly related to how much the fathers valued attachment. Fathers who valued attachment relationships were more sensitive, supportive, and gently challenging during play (Crossmann, 2002). Moreover, secure attachment in childhood was found related to having both a secure attachment to the mother in infancy and a sensitive and gently challenging father (Crossmann, 2002). This indicates that mothers and fathers play different and complementary roles in children's development. Fathers' sensitivity shows to be a significant factor in determining the balance between the infants' attachment and exploratory behavior (Bowlby, 1979). Therefore, it is of importance to gain more insight into the role of father's attachment in the cognitive development of the offspring. However, it is ethically impossible for children development studies to manipulate the cohabitating conditions of the father. Therefore, experimental studies with laboratory mice are theoretically and practically feasible alternatives to study the attachment relationship and its outcomes in a more controlled manner (Andries, 2015).

In mammals, mothers provide nurturance to the offspring and thus have been the focus of parental studies. However, researchers have been trying to find out more about paternal parental behavior in nonhuman mammals. For example, it was discovered that DBA/2J male mice fathers also participate in parental care of the offspring (Lonstein & De Vries, 2000). Lonstein and De Vries (2000) suggested that sex difference in parental behavior in rodents could be related to hormonal, sensory experiential, genetic, social and environmental factors.

Unmated male mice are infanticidal, whereas mated male mice exhibit paternal care to their own offspring (Elwood, 1986a). Elwood and Ostermeyer (1984) proved empirically that cohabitation for some time (even one single day) after copulation totally eliminated infanticide in male mice. Bell (1978) suggested that C57BL/10G males cohabitating with their mates were highly parental. The reason might be that the males recognized some features of the pups (Elwood, 1986b). In mice, visual, olfactory input and body contact are important communication and recognition signals (Swaney & Keverne, 2009). Olfactory memory retrieval is an automatic process in animals. Odor recognition is the most common and direct means to measure odor memory (Schab & Crowder, 1995). The maternal olfactory scent, especially the breast odor, becomes associated with food intake, warmth and shelter, enhancing further learning of the cue (Porter & Winberg, 1997), whereas the offspring scent might also trigger the sires' memory about their pups.

Maestripieri and Alleva (1990) suggested that when exposed to unfamiliar lactating female and the pups, recently mated male mice showed parental care towards the pups. It is reasonalbe to infer that pups might exhibit bonding to unfamiliar mice fathers due to their parental behavior. Liu and colleagues (2013) have demonstrated that when male parental mice are continuously housed with their mates and pups for 3- 5 days after parturition, the sires exhibit signs of normal parental care (crouching, licking and pup retrieval).

Although sires do not expereice the same dramatic hormone change as new dams, they do show hormone changes (Wynne-Edwards & Timonin, 2007). Previous researchers studied the hormone change in male rodents before and after birth of their offspring. For example, Brown and colleagues (1995) reported that mated Mongolian gerbils showed a higher prolactin concentration than unmated males. Prolactin has been proven to be related to maternal behavior (Pi & Grattan, 1998) and stimulating female olfactory bulb during pregnancy and lactation (Shingo et al., 2003). The increase in males is synchronous with an increase in female prolactin concentration that is important for normal maternal behavior (Edwards et al., 1995). However, some species (striped mouse) exhibits paternal care but not the same increase in prolactin (Schradin & Pillay, 2004). Increase in estradiol concentration in male rats and hamsters has also been found to be related to paternal behavior (Berg & Wynne-Edwards, 2001; Schum & Wynne-Edwards, 2005), but direct causal relationship cannot be established. Other than hormone changes, evidence has also been found for communication between the dams and sires. Liu and colleagues (2013) found that dams emit auditory signals to sires to induce paternal parental care. Although paternal care cannot simply be attributed to a single cause, it is certain that male rodents especially mice exhibit paternal care.

Despite difference in hormone change, different strains respond variably to novel environments. For instance, C57BL/6 mice perform better in learning and memory tasks but do not respond very well to stress, whereas Balb/c mice show elevated physiological and behavioral response to stress but perform poorly in learning and memory tasks (Blumberg, Freeman, Robinson, Champagne & Curley, 2009). It has also been reported that parental behavior in male mice is dependent on the strain (Wright & Brown, 2000). Liang and colleagues (2014) found that C57BL/6 male mice displayed mate-independent parental behavior.

The mother and infant relationship involves two organisms in different developmental stages: the infant which goes through dramatic changes throughout the developmental process, and the mother who undergoes behavioral changes such as response under stress and other circumstances (Lucion & Bortolini, 2014). Lucion and Bortolini (2014) also argued that the two organisms and the social interaction between them influence each other throughout the dynamic process of development, and therefore missing maternal care can have a big impact on the development of the offspring. Gonzalez and Fleming (2002) found that maternally deprived adult rats were more fearful and less exploratory in an elevated plus maze. However, the effects of the interaction with the father have not been well investigated. It is reasonable to question whether the absence of paternal care causes behavioral changes in the offspring (Lucion & Bortolini, 2014), especially in terms of social preference and exploratory tendency.

Andries (2015) found that that pups showed significant preference to the mother than to the father and other comparison mice. However, Andries (2015) did not study the comparison between father and outside father that has cohabitated with its offspring (referred to as OC father; illustrated in the table below) as comparison, it is a natural extension to test the change on the social preference of the pups once a cohabitant father (referred to as C father) or an OC father is involved. The outside non-cohabitant father is reffered to as ONC father. In this study, three main research questions will be addressed: (1) Do the pups exhibit a social preference to the mother or the father in case they have a C father; (2) Do the pups exhibit a social preference to the mother or the OC father in case they have an OC comparison father; (3) Do the pups exhibit a social preference to the father or OC father in case they have a non-cohabitant father (referred to as NC father). Furthermore, the research questions will also incorporate the effect of father cohabitation, mother sensitivity and age of the pups on the social preference and exploratory tendency of the pups.

Classification of father and social father in the testing conditions

Own father or other father	**Cohabitant**	**Non-cohabitant**
Own father	C father	NC father
Other father	OC father	ONC father

Based on previous findings (Andries, 2015; Liang et al., 2014), it is hypothesized for the first research question that in general pups exhibit strong preference to the mother than to the father, whereas in the cases of C fathers, the pup will show a proximately equal preference to both parents. Regarding the second and third research questions, in case of comparison between the mother and the OC father, it is expected to see a proximately equal preference; in the case of comparison between own C father and OC father, it is hypothesized to see proximately equal preference to both male mice; in the case of own NC father vs. OC father, it is hypothesized that the pups will show a preference to the OC father than to own NC father.

As fathers' sensitivity has been found to be a significant factor in determining the infants' exploratory behavior (Bowlby, 1979), it is of interest to question whether having a C father influence the exploratory tendency of the pups. Furthermore, whether having a C father influences the social preference of the pups is incorporated in the main research question. As Liu and colleagues (2013) argued that preference can be measured by tracing the movements of the pups, distance travelled is used to measure the exploratory tendency. We hypothesize that pups with a C father exhibit stronger exploratory tendency than those without. In other words, pups with a C father will travel longer in the socil preference test.

Previous research (Ainsworth & Wittig, 1969) showed that less sensitivity of mothers predicted abnormal exploratory behavior of the child and its attachment to the mother. We are interested to test whether sensitivity influences the social preference of the pups and their exploratory tendency. Given the unprecedented reference of our experimental design in the literature of attachment behavior, it is hypothesized that sensitivity has an influence on the social preference of the pups and their exploratory tendency.

In the current study, the social preference was tested in a three-chamber box. The mouse pup was initially placed in the middle chamber whereas one parent was placed in one side chamber and the comparison mouse the other side chamber. The test was carried out in two phases: once when the pups were 4 weeks old and another time when they were 6 weeks old. We also intended to test whether the increase in age influenced the pups' social preference and their exploratory tendency. As mice reach sexual maturity after 4 weeks, and based on the reproductive instinct, we hypothesize that as the age increases, the pups show less social preference towards the adult mouse with the same gender and more preference to the opposite gendered mouse. In the meanwhile, gender effects will be investigated. Moreover, as the pups grow older, they become physically and intellectually more mature. Therefore, we hypothesize that the as the age increases, the pups show a stronger exploratory tendency.

2. Methods

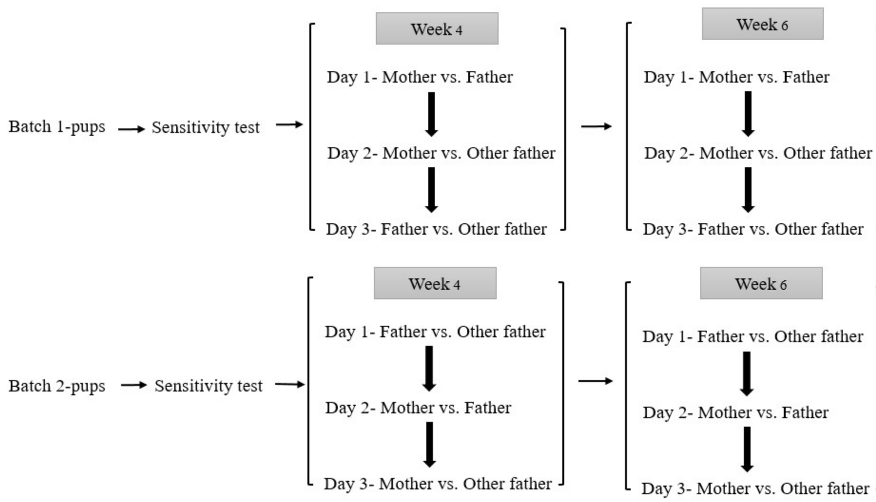
2.1. Participants

Two batches of total 24 nulliparous male and female C57BL/6 inbred mice were paired at 8 weeks. A single male and a single female were continuously housed together in a standard mouse maternity cage from the mating period until the parturition of the pups. In case of C fathers, the sires were kept in the cages until the night before the 4-week social preference test. In case of NC fathers, the sires were removed from the maternity cage upon the birth of the pups and kept in a separate cage. All animals were kept under standard laboratory conditions (between 20 ºC and 22 ºC; 12-h light/dark cycle, lights on at 0800 hours; Liu et al., 2013). A sum of 65 pups were born. Due to the early death of 11 pups and the missing data of 3 pups, the data collected from the remaining 51 pups were validated and used in the further analyses (19 pups from batch 1 and 35 from batch 2). All 51 pups were tested for 3 conditions at 2 phases. In detail, in condition mother vs. other father, 22 pups were tested with an OC father, whereas 29 pups were tested with an ONC father; in condition father vs. other father, 17 pups were tested for NC father vs. OC father, whereas 26 pups were tested for C father vs. OC father.

2.2. Procedure

All the pups underwent two tests: sensitivity test and social preference test (Figure 1). The sensitivity test was done for each litter of the same mother when the pups were 1 week old. The social preference test was done once when the pups were 4 weeks old and again at 6 weeks.

Figure 1. Testing Procedure and social preference testing conditions. All the pups were tested for these 3 conditions. Each condition was tested within 1 day. The complete testing for 1 cage took 3 days.



Sensitivity testing. According to the attachment theory (Bowlby, 1969), maternal sensitivity is the mother's ability to perceive the cues and signals of her infants and respond correctly and promptly. In order to assess the level of sensitivity, we studied the behavior of the dams after removing a pup from the nest. The time taken by the dam to bring the pup back to the nest is used as a measurement for sensitivity.

Two experimenters conducted the testing. One experimenter recorded the timing, whereas the other experimenter mobilized the pups and noted the dams' behavior. In the case of social fathers, the sire was removed before the testing from the maternity cage and kept in a separate clean plastic cage until it was returned to the cage once the testing was finished. In the case of non-social fathers, this step was unnecessary. Pups were taken out of the nest one by one and placed in the far-end corner of the maternity cage. Latency of the dam to begin the retrieval and the total time taken to retrieve the pup was recorded for each pup of the litter. A maximum of 5 minutes was given for a single retrieval. In cases where the dam was feeding in the nest and thus delayed the retrieval, a repeated attempt was added to correct for the prolonged duration. The pups were counted as back in the nest if they were brought back into the nest or left by the dam on the periphery of the nest. If the pups were not picked up by the dam within 5 minutes, they were brought back by the experimenter, and the retrieval time was counted as the maximum. The average retrieval time of each litter was used for further analyses.

Social preference test. The experimental design consists of three testing conditions: mother vs. father, mother vs. other father, and father vs. other father. In order to avoid any influence of fixed condition sequence, the sequence of testing conditions were shuffled for the second batch of mice (Figure 1). The testing apparatus used was a transparent rectangular three-chambered box (94 cm x 28 cm x 30 cm; Figure 2). The chambers are mutually divided with plastic plates. There are small rectangular openings on the plastic walls to allow access to the side chambers. In all the testing conditions, the two comparison adult mice were placed in the side chambers in a counterbalanced order.

The dams were separated from the pups the night before the social preference testing. The pups of each cage were tested one by one. The first phase of the social preference test was acclamation of 5 minutes. A pup was placed in the middle chamber to explore the environment. Afterwards the testing phase started, and the seals on the openings were lifted to allow the test pups to access the side chambers. The pups had 10 minutes to explore the side chambers, and their behavior was recorded.

Mobility was monitored with video cameras and then stored on a computer using specialized software ANY-maze video tracking system (Stoelting Co., Wood Dale, II, USA). The following behavioral variables were measured and used in the analyses: the time the pups spent in each chamber (chamber time), the time the pups sniffed on the two adult mice (sniff time), the distance the pups travelled during the testing period (distance travelled).

Figure 2. The social preference test apparatus (Andries, 2015). The test pup was placed in the middle chamber. The parent mouse and the comparison adult mouse were kept in cylindrical cages. A weight was placed on the cylinders to prevent escape.

2.3. Data analysis

The recordings of the measurements were transferred from ANY-maze to csv. files. The data analyses were done with R v3.1.2. As Liu and colleagues (2013) argued that preference can be measured by tracing the movements of the pups, chamber time as well as sniff time were used to measure the pups' social preference. Student's t tests, repeated measures ANOVA and multiple linear regression models were used for the analyses. In the multiple linear regressions, three dependent variables were defined: distance travelled, chamber time and sniff time. Independent variables included age of the pups (4 weeks and 6 weeks), sensitivity (retrieval time in the sensitivity test), whether the pups had a C father, and whether the comparison father mice in conditions mother vs. other father and father vs. other father were OC fathers. Moreover, the interaction between age, sensitivity and C father were also included in the linear regression models.

3. Results

The study aimed to test the social preference and exploratory tendency of C57BL/6 mouse pups, as well as the effects of C father, sensitivity and age. The following contrasts are of interest: mother and father in case of C father, mother and OC father and NC father vs OC father in case of NC father. The effect of having a C father, sensitivity and age on the exploratory tendency and the social preference of the pups, as well as any possible interaction between these three factors were incorporated in the two main research questions.

We hypothesized that pups with a C father would exhibit approximately equal preference to both father and mother and equal preference to own C father and OC fathers, whereas pups with a NC father would show preference to OC fathers over own NC father. Moreover, we hypothesized that having a C father, high level of sensitivity and increase in age all have an positive effect on the social preference and exploratory tendency of the pups. Three testing conditions were designed, and the social preference data was collected with the assistance of ANY-maze video tracking system. Generally, the pups preferred the mother over father and OC father. No significant difference was found between the preference to the father and the comparison father in condition father vs. other father. However, pups preferred OC father if their own father was a NC father. Father cohabitation effect was found to be significant in affecting the social preference and the exploratory tendency of the pups in all three conditions. Age was found significant in predicting the exploratory tendency of the pups in all three conditions, and sensitivity was found significantly predicting the exploratory tendency in mother vs. father condition and social preference significant in mother vs. other father condition.

3.1. Examination of difference in gender difference and condition sequence

No gender difference was found between male and female pups in parental sensitivity or social preference measures (Table 1a- 1b). Therefore, the gender groups were combined for further analyses. Furthermore, in order to eliminate any effect of the fixed sequence of the experimental conditions, the order of conditions was altered for the second batch of mice. Comparisons were made for condition mother vs. father as an illustration. The comparisons of total sniff time, sniff time and chamber time between the mother and father sides are shown in Figure 3a. The same comparisons between pups with a C father and a NC father are shown in Figure 3b. Student's t test results showed that shuffling the sequence of the conditions did not significantly change the social preference of the pups.

Table 1a.

Comparison of distance travelled between female and male pups. No significant difference was found.

conditions	*mean*		*t-value*	*df*	*p-value*
	Female	Male			
Mother vs. father	47.39	48.26	-0.28	99	0.78
Mother vs. other father	47.65	47.94	1.66	100	0.91
Father vs. other father	49.61	51.81	-0.75	99	0.45

Table 1b.

Comparison of sniff time in all three testing conditions between female and male pups, as an illustration for social preference. No significant difference was found.

conditions	*mean*		*t-value*	*df*	*p-value*
	Female	Male			
Mother vs. father	275.93	252.88	1.50	99	0.14
Mother vs. other father	284.37	283.85	0.04	100	0.97
Father vs. other father	181.65	173.90	0.54	99	0.59

Figure 3a. Mother vs Father condition tested on various days

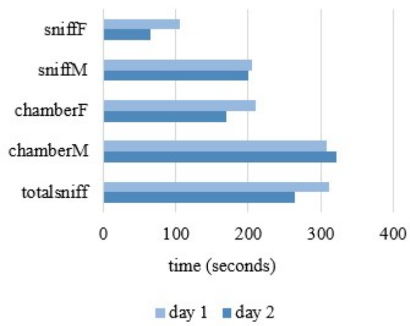
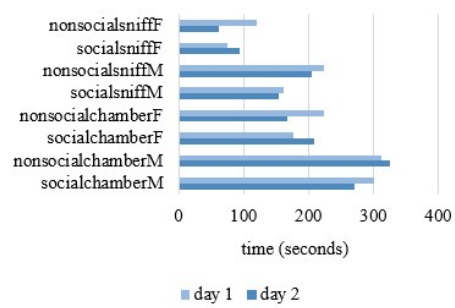


Figure 3b. Contrast between pups with and without C father in Mother vs Father condition tested on various days



3.2. Preference to the mother

Preference to the mother was addressed in condition mother vs. father and mother vs. other father. The pups showed in general preference to the mother whenever she was present.

3.2.1. Condition mother vs. father

Regardless of having a C father or a NC father, student's t tests showed that pups strongly preferred the mother to the father ($p_{\text{sniff time}} < 2.2e\text{-}16$, $p_{\text{chamber time}} < 2.2e\text{-}16$, Figure 4a). In the same testing condition, pups with a C father still sniffed significantly longer on the mother side and stayed significantly longer in the mother chamber than the father chamber ($p_{\text{chamber time}} = 6.12e\text{-}12$, $p_{\text{sniff time}} = 1.18e\text{-}10$, Figure 4b).

Figure 4a. Comparison between preference to the mother and the father of all pups, desregarding C father or NC faher

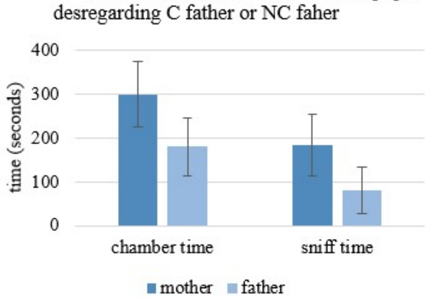
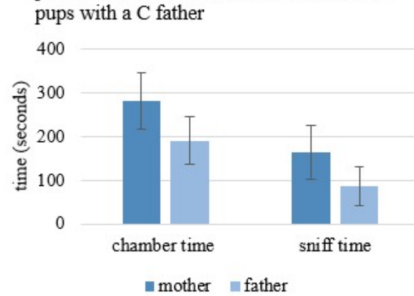


Figure 4b. Comparison between preference to the mother and C father of pups with a C father



Multiple linear regressions revealed only main effect of C father in predicing the sniff time and chamber time on the both the mother side and the father side (Table 2a - 2d; Figure 5a - 5b).

Table 2a.

Predictors for sniff time on the mother side in mother vs. father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	207.30	14.88	13.93	< 2.2e-16 ***
sensitivity	-0.17	0.14	-1.22	0.22
gender	20.54	13.83	1.49	0.14
C father	-47.10	13.93	-3.38	0.001 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2b.

Predictors for chamber time on the mother side in mother vs. father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	331.14	14.87	22.27	< 2.2e-16 ***
sensitivity	-0.19	0.14	-1.40	0.17
gender	12.85	13.84	0.93	0.36
C father	-47.10	13.98	-3.37	0.001 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table2c.

Predictors for sniff time on the father side in mother vs. father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	74.38	9.97	7.46	3.81e-11 ***
sensitivity	-0.09	0.09	-0.93	0.35
gender	-2.05	9.25	-0.22	0.83
C father	17.88	9.35	1.92	0.059 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2d.

Predictors for chamber time on the father side in mother vs. father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	163.60	13.19	12.41	< 2.2e-16 ***
sensitivity	-0.04	0.12	-0.35	0.73
gender	7.82	12.24	0.64	0.52
C father	24.47	12.38	1.98	0.05 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure 5a. Sniff time and chamber time (seconds) on the mother side of pups with a C father and a NC father in condition mother vs. father

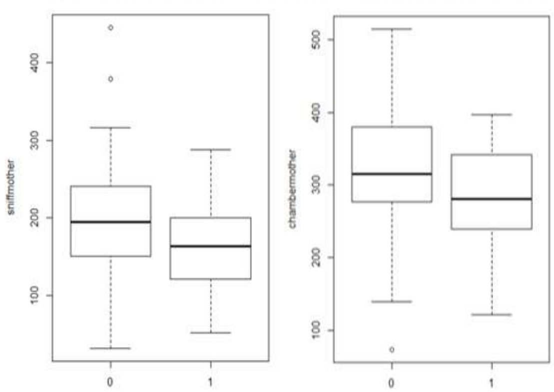
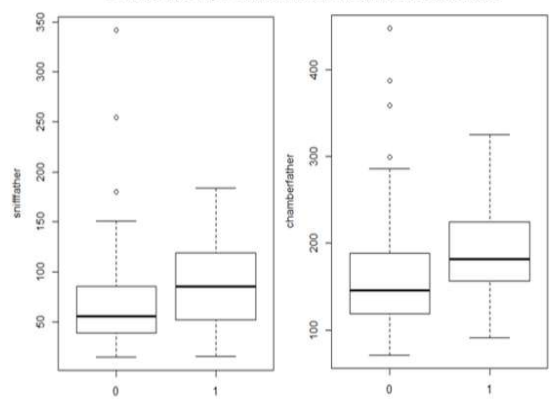


Figure 5b. Sniff time and chamber time on the father side of pups with a C father and a NC father in condition mother vs. father



* 0 for NC father, 1 for C father

3.2.2. Condition mother vs. other father

Regardless of having a C father or a NC father, student's t tests revealed that pups in general showed clear preference to the mother than to the comparison father ($p_{\text{sniff time}} < 2.2e\text{-}16$, $p_{\text{chamber time}} = 9.174e\text{-}15$; Figure 6a). If we focus on the social preference of the pups with a NC father for their mother or the OC comparison father, they still showed significant preference to their mother than to the OC father ($p_{\text{sniff time}} = 9.63e\text{-}12$; $p_{\text{chamber chamber}} = 8.5e\text{-}13$; Figure 6b). The difference between mother chamber time and other father chamber time as well as the difference between mother sniff time and other father sniff time of all the pups and only pups with an OC comparison father were also compared with a t test, and no significant difference was found ($p_{\text{chamber time difference}} = 0.60$, $p_{\text{sniff time difference}} = 0.56$, Figure 6c).

Figure 6a. Chamber time and sniff time of all pups on the side of the mother and other father in condition mother vs. other father

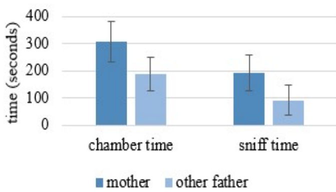


Figure 6b. Chamber time and sniff time of pups with a NC father on the side of the mother and OC father in condition mother vs. other father

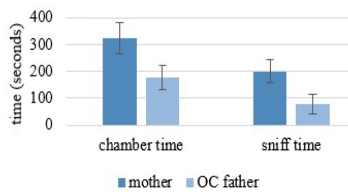
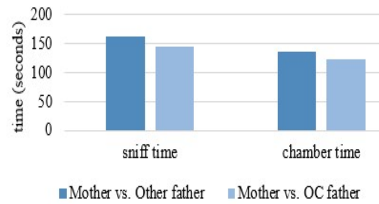


Figure 6c. Chamber time and sniff time difference on the side of the mother and other father regardless of OC father, and beween mother and OC father



Multiple linear regression revealed that the preference to the mother in terms of chamber time and sniff time was influenced by sensitivity, own C father and the interaction between them. With the same level of sensitivity, pups with a C father stayed about 153 seconds shorter and sniffed about 93 seconds less on the mother side than those without a C father. However, whether the comparison father was a C father or not did not significantly affect the preference to the mother ($p_{sniff} = 0.07$, $p_{chamber} = 0.15$, Table 3a - 3b).

Table 3a.

Predictors for sniff time on the mother side in mother vs. other father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	239.73	13.44	17.84	< 2.2e-16 ***
sensitivity	-0.31	0.13	-2.34	0.02 *
gender	17.22	12.07	1.43	0.16
OC father	-25.24	13.74	-1.84	0.07
C father	-94.88	25.03	-3.79	0.0003 ***
sensitivity : C father	1.01	0.40	2.55	0.01 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3b.

Predictors for chamber time on the mother side in mother vs. other father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	368.11	13.22	27.85	< 2.2e-16 ***
sensitivity	-0.52	0.13	-3.99	0.0001 ***
gender	20.66	11.88	1.74	0.09
OC father	-19.73	13.51	-1.46	0.15
C father	-154.79	24.62	-6.29	9.462-09 ***
sensitivity : C father	1.95	0.39	4.99	2.73e-06 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.3. Effect of father cohabitation on social preference

In condition father vs. other father, the cohabitation effect of both own father and comparison father was addressed. The comparisons of chamber time and sniff time were made for the following three pairs: own father vs. other father (without distinction beween C father, NC father, OC father or ONC father), NC father vs. other father regardless of OC or ONC, and NC father vs. OC father. The general comparison between father and other father showed no significant difference ($p_{chamber} = 0.42$, $p_{sniff} = 0.18$; Figure 8a).

However, pups with a NC father showed significant preference to the comparison father mouse than to their own father (t test; $p_{chamber\ time} = 0.02$; $p_{sniff\ time} = 0.005$; Figure 8b), disregarding the comparison mouse being an OC father or ONC father. More specifically, if the comparison father was an OC father, the pups with a NC father also sniffed and stayed significantly longer on the OC father side than their own father (t test; $p_{chamber} = 0.0006$, $p_{sniff} = 0.0003$; Figure 8c). On the other hand, the other father being an OC father or an ONC father did not have any significant influence in predicting the chamber time and sniff time on their own father side (t test; $p_{sniff} = 0.97$, $p_{chamber} = 0.67$).

Figure 8a. Chamber time and sniff time in father vs. other father condition regardless of status of own father and other father

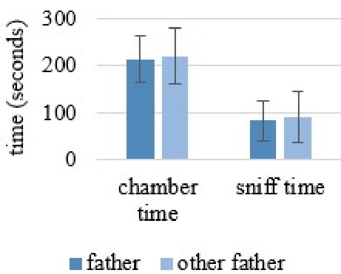


Figure 8b. Comparison of chamber time and sniff time of pups with a NC father on the side of the NC father and other father

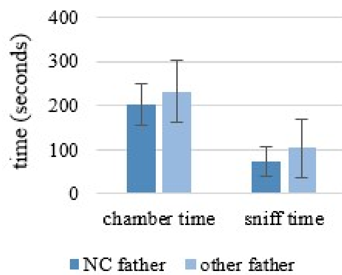
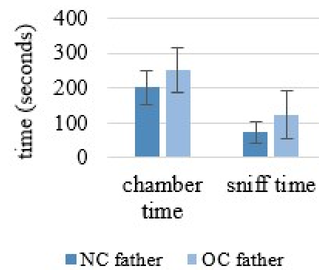
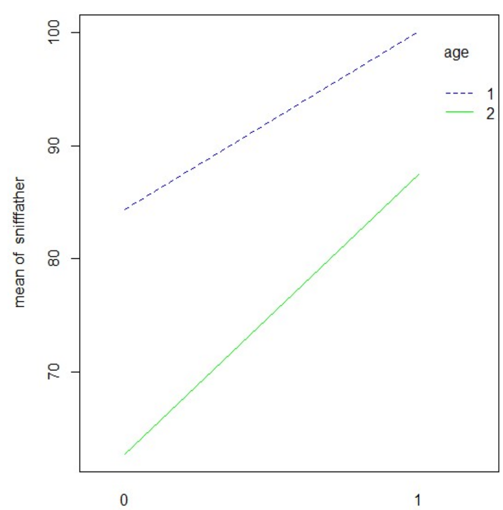


Figure 8c. Comparison of chamber time and sniff time of pups with a NC father and an OC comparison father



Regarding sniff time on the father side, multiple linear regressions showed that age and C father were significant ($p_{age} = 0.007$, $p_{C\ father} = 0.05$). At the same age, pups with a C father sniffed about 17.2 seconds longer on the father side than those with a NC father. As the pups grew older, they sniffed about 21 seconds shorter on the father side. The cohabitation effect on father sniff time is shown in Figure 9.

Figure 9. Sniff time on the father side in condition father vs. other father



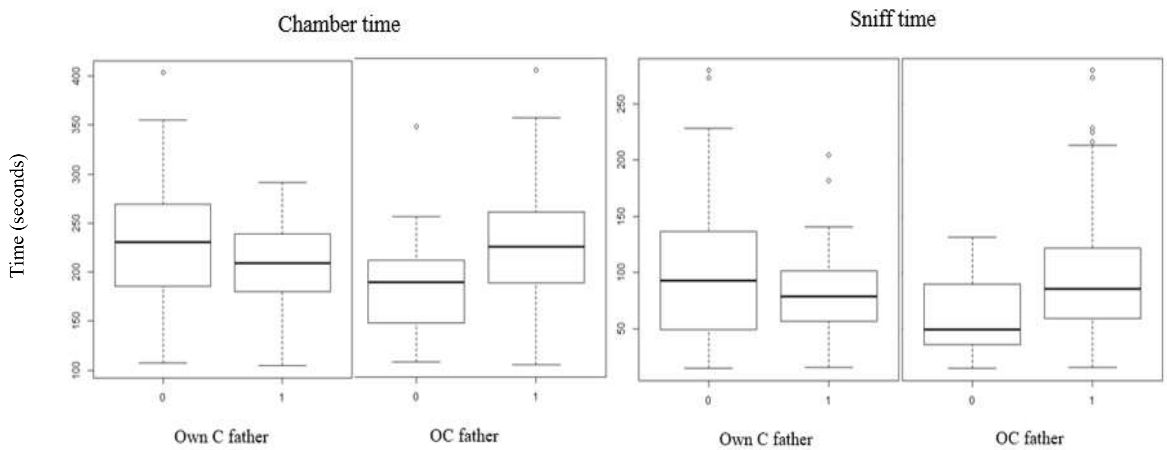
Age
1 - 4 weeks
2 - 6 weeks

Status father
0 – NC fahter
1 – C father

Regarding sniff time and chamber time on the other father side, multiple linear regressions showed that having a C father and an OC comparison father were found significant. In terms of sniff time, if the comparison mouse was an OC father, pups in general sniffed about 62 seconds longer than when it was an ONC father, and pups with an own NC father sniffed about 40 seconds longer on the other father side than those with a C father ($p_{C\ father} = 0.0009$, $p_{OC\ father} = 0.0002$; Figure 10).

The same predictors were found significant for chamber time ($p_{C\ father} = 0.0005$, $p_{OC\ father} = 0.0001$; Figure 10). In particular, if the comparison father was an OC father, the pups stayed about 69 seconds longer in its chamber than when it was an ONC father. Pups with own NC father stayed about 45 seconds longer in the comparison father chamber than those with own C father.

Figure 10. Chamber time and sniff time on the other father side in condition father vs other father.



3.4. Effect of mother sensitivity on social preference

Multiple linear regressions were built for chamber time and sniff time in all 3 testing conditions as a measurement for social preference. Sensitivity was only found significant in predicting the sniff time and chamber time on both the mother side and the other father side in condition mother vs. other father. An interaction between sensitivity and C father was also found in predicting the chamber time on the father side in condition father vs. other father.

In condition mother vs. other father, generally, pups with a NC father stayed and sniffed shorter on the mother side as the sensitivity decreased, whereas pups with a C father stayed and sniffed longer on the mother side as the sensitivity decreased. With the same type of father, the less sensitive the pups were, the shorter they sniffed and stayed on the mother side ($p_{sniff} = 0.02$, $p_{chamber} = 0.0001$, Figure 12a).

In condition father vs. other father, as the level of sensitivity decreased, the pups with a C father stayed longer in the father chamber whereas those with a NC father stayed slightly shorter ($P_{sensitivity*C\ father} = 0.006$, Figure 12b).

Figure 12a. Chamber time and sniff time (seconds) on the mother side influenced by sensitivity mother and C father in condition mother vs. other father.

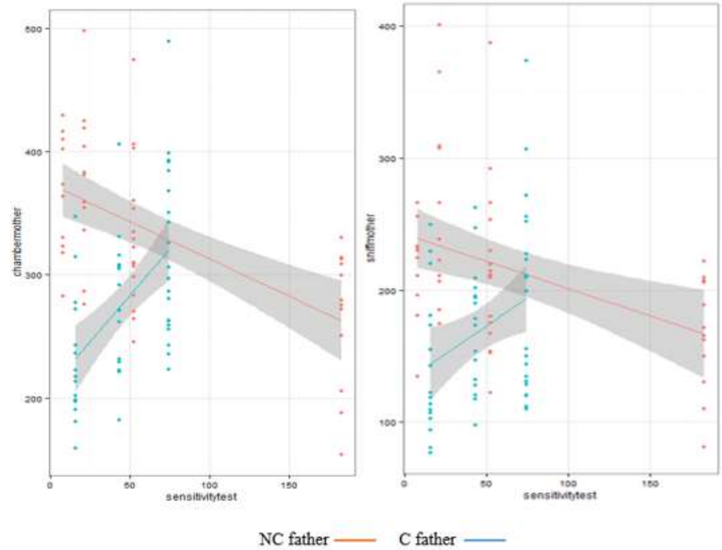
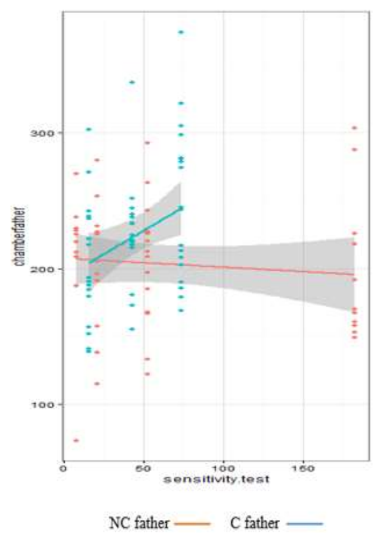


Figure 12b. Chamber time (seconds) on the father side influenced by sensitivity mother and C father in condition father vs. other father.



* Eight cages of pups were tested, and two of them had the same sensitivity time, so there are 7 values for sensitivity.

3.5. Effect of age on social preference

Multiple linear regressions were built for distance travelled in all three testing conditions, and age was only found significant in predicting the sniff time and chamber time on the father side in condition father vs. other father. In general, pups sniffed and stayed about 36 seconds shorter on the father side when they were 6 weeks old than when they were 4 weeks old (Table 4a - 4b; Figure 11a - 11b).

Table 4a.

Predictors for sniff time on the father side in father vs. other father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	102.35	15.99	6.40	6.06e-09 ***
age	-20.88	7.58	-2.76	0.007 **
sensitivity	0.04	0.07	0.47	0.64
gender	0.69	7.85	0.09	0.93
OC father	-0.41	12.00	-0.03	0.97
C father	17.22	8.66	1.99	0.05 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4b.

Predictors for chamber time on the father side in father vs. other father condition

Coefficients	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	261.45	18.99	13.77	< 2.2e-16 ***
age	-35.76	8.98	-3.98	0.0001 ***
sensitivity	-0.05	0.10	-0.54	0.59
gender	8.30	9.35	0.89	0.38
OC father	-6.12	14.30	-0.43	0.67
C father	-15.07	17.42	-0.87	0.39
sensitivity: C father	0.78	0.28	2.81	0.006 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure 11a. Sniff time (seconds) on the father side as age of pups increase in condition father vs. other father. Age is coded with 1 standing for 4 weeks and 2 for 6 weeks.

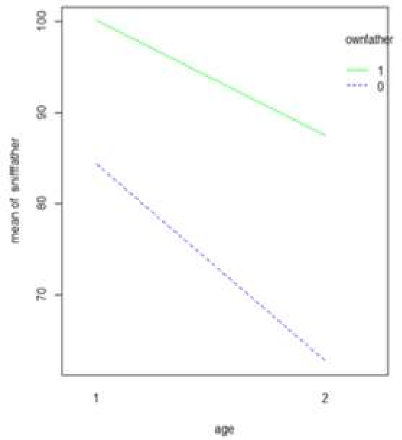
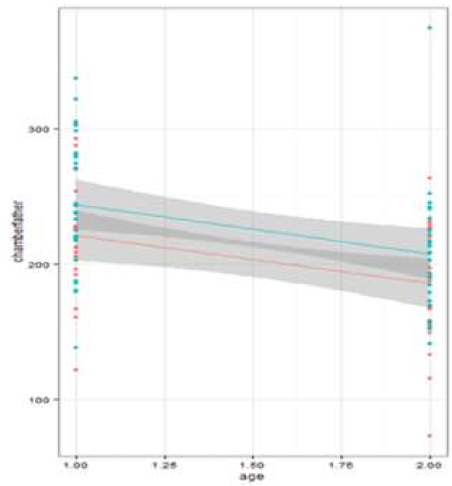


Figure 11b. Chamber time (seconds) on the father side as age of pups increase in condition father vs. other father. Age is coded with 1 standing for 4 weeks and 2 for 6 weeks.



3.6. Exploratory tendency

Multiple linear regressions were built to investigate what affected the exploratory tendency of the pups. In all three testing conditions, pups' age and having a C father were found to be significantly affecting their exploratory tendency. Specifically, in condition mother vs. father, predicting variables included C father, sensitivity, age and their interactions. Main effects were found for sensitivity, C father and age, and interaction effects were also found between sensitivity and age as well as between C father and age (Table 5a, Figure 13a). In condition mother vs. other father, predicting variables included C father, sensitivity, age, OC father and the interactions between C father, sensitivity and age. Only age and OC father were found significant (Table 5b). In condition father vs. other father, besides age and own C father, whether the comparison father was an OC father was also found significant (Table 5c).

Table 5a

Distance travelled in condition mother vs. father. Pups that have the same level of mother sensitivity and type of father, travelled about 24.65 meters longer when they were 6 weeks than when they were 4 weeks. For pups at the same age and have the same level of mother sensitivity, the ones with a C father travelled about 8.85 meters longer than those with a NC father. Although the positive effect of sensitivity is significant, pups who had lower mother sensitivity by 1 second travelled only 6 centimeters longer.

Coefficients	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*
Intercept	-6.45162	5.97709	-1.079	0.28317
sensitivity	0.19228	0.06169	3.117	0.00242 **
C father	18.68161	6.20165	3.012	0.00333 **
age	34.60996	3.79414	9.122	1.35e-14 ***
sensitivity : age	-0.12545	0.03983	-3.149	0.00219 **
C father : age	-9.83317	3.93606	-2.498	0.01422 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure 13a. Distance travelled (meter) in condition mother vs. father. The interaction between age and C father as well as between sensitivity and age are demonstrated.

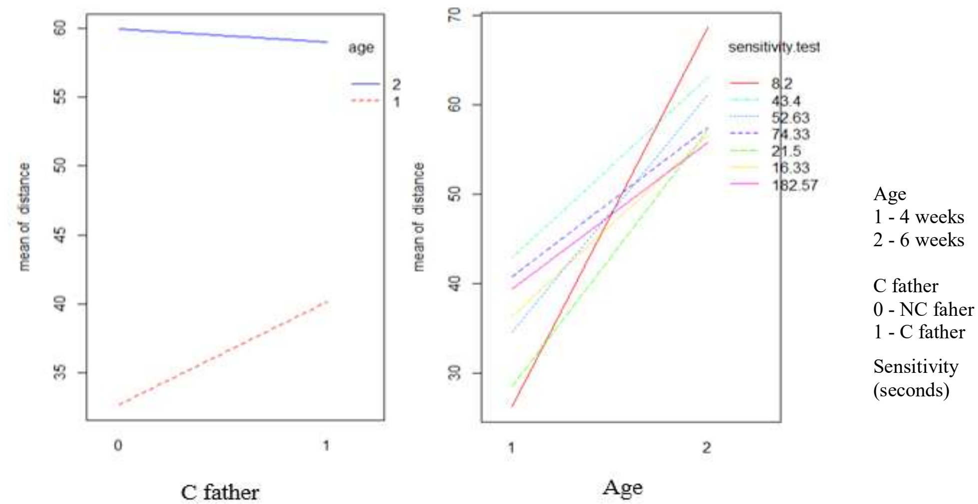


Table 5b

Distance travelled in condition mother vs. other father. Pups tested with an OC father travelled about 4.23 meters longer than those tested with an ONC father. Pups travelled about 18.31 meter longer when they were 6 weeks than when they were 4 weeks. Own C father was not found significant, and no interaction effect was found significant.

Coefficients	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*
Intercept	16.62113	3.10662	5.35	5.86e-07 ***
sensitivity	0.01597	0.01754	0.910	0.3650
OC father	4.23242	1.82501	2.319	0.0225 *
age	18.3111	1.70513	10.739	< 2e-16 ***
C father	0.76823	1.82287	0.421	0.6744

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 5c

Distance travelled in condition father vs. other father. Pups with a C father travelled about 7.27 meters longer than those with a NC father. Pups that had an OC comparison father travelled about 5.86 meters shorter than those that had a ONC comparison father. Moreover, pups travelled about 22.86 meters longer when they were 6 weeks than when they were 4 weeks. No interaction effect was found significant.

Coefficients	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*
Intercept	17.16881	3.6536	4.699	8.82e-06 ***
sensitivity	0.0111	0.01766	0.629	0.530974
C father	7.27089	1.99934	3.637	0.00045 ***
OC father	-5.8555	2.81911	-2.077	0.040493 *
age	22.86384	1.75887	12.999	< 2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

4. Discussion

A secure attachment relationship in childhood is closely related to the development of an individual (Crossmann, 2002). Attachment to the father especially predicts children's exploratory behavior (Bowlby, 1979). Cognitive, affective and neurobiological processes all contribute to the attachment relationship (Ainsworth, 1989). In this study, we aimed to investigate what affects the social preference and exploratory tendency of C57BL/6 laboratory mice.

Three major research questions were formulated: (1) Do pups exhibit a preference to the mother or the father in case they have a cohabitant father; (2) Do pups exhibit a preference to the mother or the OC father in case they have an outside cohabitant father; (3) Do pups exhibit a preference to their own father or OC father in case they have a non-cohabitant father. The tests were carried out in three conditions: mother vs. father, mother vs. other father and father vs. other father, to cover all planned comparisons. It was hypothesized for the first question that pups would in general exhibit preference to the mother than to the father, whereas pups with a C father would exhibit approximately equal preference to the mother and the father. Conforming with previous research (Pearson, Defensor, Blanchard & Blacnchard, 2010; Andries, 2015), pups generally preferred the mother to the father in terms of chamber time and sniff time, regardless of having a C father or a NC father.

However, contrary to our hypothesis, pups with an C father also preferred the mother significantly more than the C father. As Crossmann (2002) suggested that mothers and fathers play different and complementary roles in the development of children. Although cohabitant fathers participate in the raising activities of the offspring, mothers are still the main source of nurture and care. We may therefore conclude that when both parents are present, although pups are familiar with the fathers, they still prefer the mothers. It is consequently more interesting to reveal what are the factors that predicted this preference.

Although pups showed significant preference to the mother than to the father regardless of having a C father or not, the pups with a C father showed shorter sniff time and chamber time on the mother side than those without. Corresponding to the hypothesis, pups with a C father showed more preference to the father than those without. Multiple linear regressions revealed that having a C father is the sole main predictor for preference to the mother or the father.

Secondly, it was hypothesized that the comparisons between the mother and the OC father as well as between own C father and OC father would lead to a approximately equal preference, whereas the comparison between NC father and OC father would show preference to the OC father. In the comparison between mother and other father mouse, regardless of having a C father or a NC father, pups showed significant preference to the mother than to the comparison mouse. In the comparison between father and other father mouse, regardless of having a C or a NC father, pups showed equal preference to both male mice. However, under the condition mother vs. other father, pups with an own

NC father and an OC comparison father showed significant preference to the mother than to the OC father. Therefore, we could conclude that pups prefer the mother whenever she is present.

The preference to the mother can be linked to sensitivity, C father and the interaction between them. The lack of high-level maternal sensitivity leads to insecure and anxious attachment relationships between the mother and the child (Ainsworth & Wittig, 1969). Conformingly, our study demonstrates that pups with low sensitivity sniffed and stayed shorter on the mother side than those with higher sensitivity. As the pups were familiar with the C father, and the C fathers might have exhibited parental behavior during the social preference testing phase, the pups also spent relatively longer time on the C father side. In other words, having a C father substantially changes the preference of the pups to the mother. Therefore, we could conclude that the more sensitive, the more the pups preferred the mother, and having a C father reduced the preference of the pups to the mother. However, less preference to the mother under the testing condition of mother vs. other father could also be interpreted as less dependence on the mother. As Ainsworth and Wittig (1969) suggested, high maternal sensitivity lead to secure attachment relationships that fostered exploratory behavior and independence. Low sensitivity, on the other hand, was demonstrated by the decrease of preference to the mother.

Corresponding to the hypothesis, regardless of the status of the own father and the comparison father in terms of C, NC, OC or ONC, pups in general showed equal preference in terms of chamber time and sniff time towards both own father and the comparison father. However, pups with NC father showed significant preference to the comparison father mouse than to their own father. Particularly, if the comparison mouse was an OC father, pups with an NC father stayed and sniffed longer on the comparison father side. As mentioned in the introduction, when recently mated male mice are exposed to unfamiliar pups, they exhibit parental care towards the pups (Maestripieri & Alleva, 1990). Our results show that pups preferred an OC father in the condition of own NC father vs. other OC father. The OC fathers had been cohabitating with the mother and the new born pups until the night before the social preference testing. Therefore, our results also correspond with the conclusion of Liu and colleagues (2013) that co-housing of the sires with their mates and pups stimulated paternal parental care.

Linear regressions revealed that the preference to the own father is affected by age of the pups and C father. The same group of pups sniffed about 21 seconds shorter on their father side when they were 6 weeks old than when they were 4 weeks old. As the pups grew more mature, their dependence on the father also decreased. At the same age, pups with a C father sniffed about 17 seconds longer on the father side than those with a NC father. Moreover, whether the comparison mouse was an OC father or ONC father did not have any significant effect on the preference to the own father.

On the contrary, having an OC father significantly influenced the preference to the comparison father mouse. In fact, having a C father and an OC father are both found to be significantly affecting the preference to the comparison father in terms of chamber time and sniff time, but no interaction effect was discovered. In general, with the same type of own father, pups that had an OC father as the comparison mouse stayed and sniffed longer on the other father side than those with a ONC father as the comparison mouse. Corresponding to the results of Liu and colleagues (2013), cohabitant father mice received more preference than the non-cohabitant ones.

Moreover, the comparison between pups with an own C father and own NC father showed that pups with a C father sniffed significantly longer on the own father than those with a NC father ($p_{sniff\ father} = 0.017$), indicating their preference to the own C father; however, the pups with a NC father sniffted significantly longer on the comparison father mouse side than those with an own C fahter ($p_{sniff\ other\ father} = 0.037$), which indicated their lack of preference to their own fathers.

Furthermore, we were interested in what affects the exploratory tendency of the pups. Three hypotheses were proposed: (1) pups with a C father exhibit stronger exploratory tendency than those without; (2) sensitivity has an influence on the exploratory tendency of the pups; (3) pups show a stronger exploratory tendency as their age increases. The results confirm all the hypotheses. More specifically, for condition mother vs. father, interaction effects were found between sensitivity and age as well as between C father and age.

Moreover, in the mother vs. other father condition, only main effects were found for age and C father. At 4 weeks, pups with an own C father showed significantly stronger exploratory tendency than those with a NC father ($p_{C\ father} = 0.012$). As predicted, when the pups became more mature, they also became significantly more exploratory ($p_{age} < 2e\text{-}16$). This finding corresponds to the findings of Bowlby (1979) and Crossmann (2002) concerning the relationship between father's attachment and the exploratory behavior in early childhood. However, in condition father vs. other father, whether the comparison mouse was an OC father was also found significant. If the pups were from same age group and had the same type of own fathers, the ones tested with an OC father travelled about 5.9 meters shorter than those with an ONC father (p= 0.04). As discussed in the section about social preference in the father vs. other father condition, cohabitant father mice might have displayed parental care and prolonged the time the pups spent on their side, thus affecting the distance travelled by the pups. In other words, the pups might have spent more time on the OC father side and consequently reduced the time on travelling between the chambers.

5. Conclusion

This study is aimed to test the factors that influence the social preference and exploratory tendency of C57BL/6 mouse pups. We found that having a C father, high level of mother sensitivity and increase in age all increase the exploration of the pups. Furthermore, having a C father was found to be a significant predictor for the social preference of the pups in all three testing conditions. We can confidently conclude that having a C father has a positive effect on the social preference of the pups to the father and the exploration of the pups. In order to avoid any effect of the fixed sequence of testing conditions, the testing conditions were randomized for the second batch of mice. The comparison for the same testing condition on different days showed no significant difference in the performance of the pups and thus proved randomizing conditions to be an efficient method. However, due to the restriction of time and limited number of mouse pups, some limitations were not able be overcome. As the study focused on the cohabitation effect of the father, it would be of interest to test the father sensitivity as well. The retrieval behavior of the father may have effects on the attachment between the pups and the father. Combining with our results, this can be a new direction for future researchers to study paternal bonding.

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Appendix

Table 1.

Comparison of condition mother vs. father on two different days

measures	***mean***		***t-value***	***df***	***p-value***
	Day 1	Day 2			
Total sniff time	311.26	261.53	1.59	18.95	0.13
Mother chamber time	308.61	300.46	0.26	18.46	0.80
Father chamber time	209.7	188.07	0.70	17.47	0.49
Mother chamber time with C father	300.84	284.17	0.60	6.32	0.57
Mother chamber time without C father	312.14	324.89	-0.29	12.10	0.78
Father chamber time with C father	177.28	202.26	-0.69	4.87	0.52
Father chamber time without C father	224.44	166.79	1.38	11.49	0.19
Mother sniff time	205.39	181.66	0.79	19.71	0.44
Father sniff time	105.87	79.87	1.13	17.36	0.27
Mother sniff time with C father	162.66	165.94	-0.11	6.61	0.92
Mother sniff time without C father	224.81	205.24	0.48	12.69	0.64
Father sniff time with C father	74.54	91.68	-0.68	5.03	0.53
Father sniff time without C father	120.11	62.15	1.89	11.00	0.09

Table 2.

Comparison of chamber time and sniff time between mother and father in mother vs. father condition

measures	***mean***		***t-value***	***df***	***p-value***
	Mother	Father			
Chamber time	305.22	180.06	12.69	198.62	< 2.2e-16
Sniff time	192.09	80.19	13.23	190.82	< 2.2e-16

Table 3.

Comparison of chamber time and sniff time between mother and C father in mother vs. father condition

measures	***mean***		***t-value***	***df***	***p-value***
	Mother	C father			
Chamber time	282.38	190.51	7.80	100	6.12e-12
Sniff time	163.94	87.05	7.19	100	1.18e-10

Table 4.

Comparison of chamber time and sniff time between mother and other father in mother vs. other father condition

measures	***mean***		***t-value***	***df***	***p-value***
	Mother	Other father			
Chamber time	305.22	188.94	12.19	194.84	< 2.2e-16 ***
Sniff time	192.09	92.01	11.6	195.14	< 2.2e-16 ***

Table 5.

Comparison of chamber time and sniff time between mother and OC father in mother vs. other father condition

measures	***mean***		***t-value***	***df***	***p-value***
	Mother	OC father			
Chamber time	322.60	177.1	9.29	42	9.63e-12 ***
Sniff time	200.38	76.84	10.09	42	8.51e-13 ***

Table 6.

Comparison of chamber time and sniff time between father and other father in father vs. other father condition

measures	***mean***		***t-value***	***df***	***p-value***
	Father	Other father			
Chamber time	214.71	220.9	-0.80	195.17	0.42
Sniff time	83.43	92.55	-1.34	195.3	0.18

Table 7.

Comparison of chamber time and sniff time between NC father and other father in father vs. other father condition

measures	***mean***		***t-value***	***df***	***p-value***
	NC father	Other father			
Chamber time	203.02	232.06	-2.38	96	0.02 *
Sniff time	73.29	103.63	-2.86	96	0.005 **

Table 8.

Comparison of chamber time and sniff time between NC father and OC father in father vs. other father condition

measures	***mean***		***t-value***	***df***	***p-value***
	Father	Other father			
Chamber time	201.68	252.14	-3.59	66	0.0006 ***
Sniff time	73.67	122.22	-3.75	66	0.0003 ***

Table 9.

Predictors for sniff time on the other father side in father vs. other father condition

Coefficients	***Estimate***	***Std. Error***	***t value***	***Pr(>|t|)***
Intercept	63.51	14.55	4.37	3.19e-05 ***
sensitivity	-0.02	0.10	-0.21	0.84
gender	-3.39	10.42	-0.33	0.75
OC father	61.88	16.00	3.87	0.0002 ***
C father	-39.60	11.50	-3.44	0.0009 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 10.

Predictors for chamber time on the other father side in father vs. other father condition

Coefficients	***Estimate***	***Std. Error***	***t value***	***Pr(>|t|)***
Intercept	193.93	15.77	12.29	< 2e-16 ***
sensitivity	-0.13	0.00	-1.14	0.26
gender	-2.62	11.30	-0.23	0.82
OC father	68.91	17.35	3.97	0.0001 ***
C father	-44.96	12.47	-3.61	0.0005 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1