

Gene-environment interaction influences attachment-like style in mice

G. Lassi and V. Tucci* 

Department of Neuroscience and Brain Technologies (NBT), Genetic and Epigenetics of Behaviour – Istituto Italiano di Tecnologia, Genova, Italy

*Corresponding author: V. Tucci, Department of Neuroscience and Brain Technologies (NBT), Neurobehavioural Genetics Group – Istituto Italiano di Tecnologia, Genova, Italy. E-mail: valter.tucci@iit.it

Attachment styles are established soon after birth and form the basis for a healthy psychological life during adulthood. Here, we investigated whether genetic background (i.e. isogenic strains: C57BL/6N and BALB/c) and parent-of-origin (i.e. reciprocal hybrids) epigenetic effects influence attachment-like styles in mice. We discovered that a specific genetic and epigenetic assortment exerts a role on the development of a secure or insecure attachment-like style. In particular, when biological mothers raise their pups, the attachment-like style is mainly secure, independently of the genetic background. However, when foster mothers raise pups, the attachment-like style can be either secure or insecure, depending on the particular genetic background, and this effect is paternally transmitted. Finally, we observed that secure attachment-like in mice leads to greater sociability during adulthood, while insecure attachment-like leads to reduced sociability. Our study sheds light on gene-environment interactions that shape the attachment-like style early in development and pave the way for a healthy psychological life.

Keywords: Attachment, fostering, mice, parent-of-origin, social behavior

Received 1 September 2016, revised 15 March 2017, 10 April 2017, accepted for publication 10 April 2017

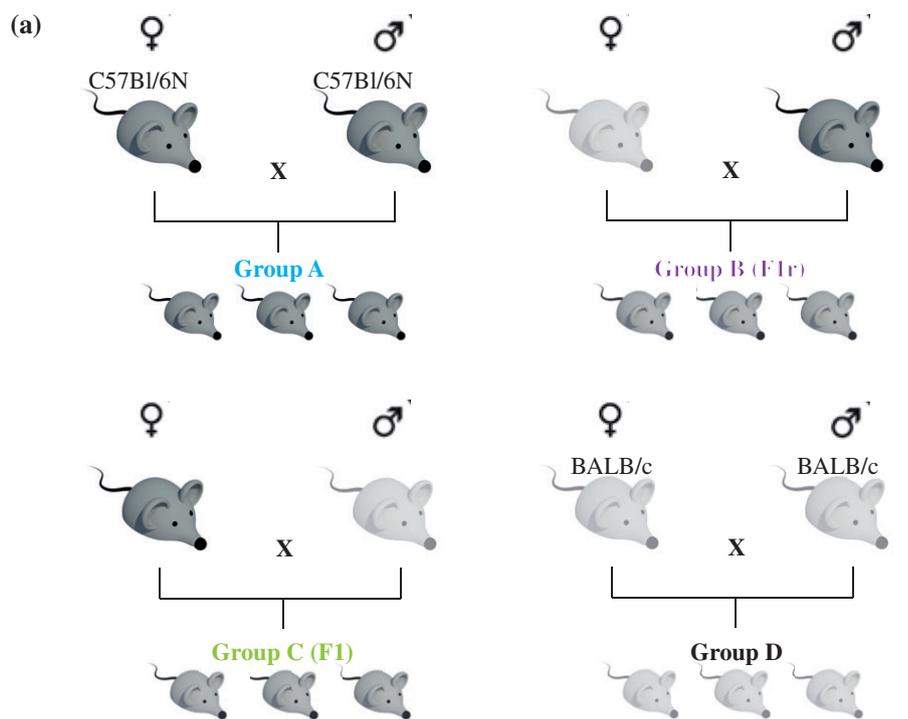
Mammals are mostly altricial animals, which means that they can survive only if adequate parental care is provided after birth. Attachment is the bonding behavior of an infant toward the main caregiver. The type of relationship between immature offspring and caregivers, known as attachment style, paves the way for psychological life in adulthood. Securely attached infants rely on their primary caregiver as a secure base and develop autonomy, a positive self-image and the ability to form emotionally close relationships (Bucci *et al.* 2016). Insecure attachment is developed when the caregiver is inconsistent and does not respond to the infant's needs.

The consequences of dysfunctional attachment styles vary and can be disastrous, adversely affecting adult personality, security, emotionality and sociability (Bremner 2003; Heim & Nemeroff 2001; Landers & Sullivan 2012; Sheinbaum *et al.* 2015; Sullivan 2012). Personality disorders have a profound impact on our society, creating a considerable economic burden for national health services and reducing productivity worldwide (Tyrer *et al.* 2010).

Here, we studied mice as a model for understanding the genetic and epigenetic components that establish attachment styles early in life and the consequences of this attachment in adulthood. In particular, we investigated whether specific parent-of-origin epigenetic mechanisms, e.g. in genomic imprinting, affect attachment behaviors. Genomic imprinting is an epigenetic phenomenon in which maternal and paternal alleles are not functionally equivalent (Bartolomei & Ferguson-Smith 2011). We tested maternal and paternal allelic combinations in setting behavioral responses with a focus on specific genetic effects and environmental factors such as early foster parenthood.

The behavioral premises of our investigation were based on the different mothering styles of two common inbred strains: BALB/c and C57BL/6N. Whilst the latter dams are recognized as 'good mothers', BALB/c dams are reported to spend less time licking their pups and show less arch-backed nursing (Calatayud *et al.* 2004; Priebe *et al.* 2005; Tarantino *et al.* 2011). The genetic premises of the experimental design were the isogenic backgrounds of the two inbred strains and potential parent-of-origin effects that can emerge in their reciprocal hybrids (Isles *et al.* 2001; Lawson *et al.* 2013). Indeed, the first-generation (F1) offspring of the two strains always presents the same complement of heterozygous individuals regardless of parental assortment. However, when there is an underlying parent-of-origin regulatory process, phenotypic differences between reciprocal crosses can be observed as a consequence of a particular parental assortment. Therefore, along with cohorts of C57BL/6N and BALB/c mice, we studied litters of C57BL/6N and BALB/c reciprocal hybrids. To isolate the effect of maternal care (environmental) from parent-of-origin (genomic imprinting) effects, we conducted a parallel investigation on the same four groups (C57BL/6N, BALB/c, F1 and F1r; Fig. 1a) that were raised by unrelated foster CD1 mothers.

To assess attachment behaviors in 1-year-old infants, Ainsworth devised the strange situation procedure (SSP) (Ainsworth *et al.* 1978), a behavioral test based on the natural tendency of infants to attach to caregivers, preferentially the mother. In the SSP, the exploratory and social behavior of the infant is recorded in a room during various short episodes during which the infant is in the presence of the



(b) **MOUSE STRANGE SITUATION PROCEDURE**

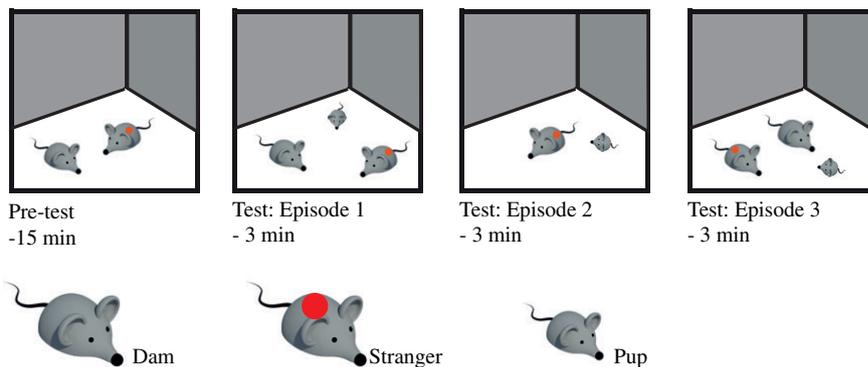


Figure 1: Reciprocal crosses and attachment test.

(a) Litters of C57BL/6N (group 'A') and BALB/c (group 'D') mice were derived from intercrosses within the same strain, while F1r (group 'B') and F1 (group 'C') mice were obtained from reciprocal crosses of the two strains. (b) Representation of the mouse strange situation procedure adapted from the human SSP. *Pretest episode:* familiarization between the mother and the stranger. *Test episode 1:* mother, pup and stranger are introduced in the arena. *Test episode 2:* pup and stranger are left alone in the arena. *Test episode 3:* the mother returns to the arena.

mother and/or a stranger. Briefly, after an assessment of the styles of interaction between the mother and the infant, the experiment assesses the response of the infant in presence of an unknown woman along with the mother and then in the presence of the stranger alone. At this point, the mother returns while the stranger leaves the room, and then the infant is left alone. The stranger then returns, and then the mother returns before the stranger leaves for good. Many psychological factors are taken into account as the infant goes through a total of eight situational episodes. However, the primary objective of this assessment is to determine the attachment style based on (1) the interest shown by the child toward the stranger and (2) the behavior of seeking comfort from the mother once infant and mother are reunited. The main outcome of this assessment is that the attachment

style is categorized as either secure or insecure (Ainsworth *et al.* 1978; Sheinbaum *et al.* 2015). Secure attachment is represented by the active exploration of the stranger, especially when the mother is in the room, and then by seeking consolation from the mother when she returns. Attachment is interpreted as insecure when the infant avoids the stranger and then shows either avoidance or indifference toward the mother when she returns to the room. We modified the SSP to study the attachment-like behavior in mouse pups. We could not faithfully mimic the SSP on account of objective specie-specific issues and the need to minimize stress on both the mother and the pups.

The results of our study show important genetic and gene-environment effects in determining particular attachment-like styles and adult behavioral traits.

Table 1: Number of animals tested for each experimental condition

Mother	Genotype	Number of litters	Number of pups per each litter	Total number of pups
Biological	(A) C57BL/6N	2	10, 10	20
	(B) F1r	2	10, 10	20
	(C) F1	2	10, 6	16
	(D) BALB/c	2	10, 10	20
Foster	(A) C57BL/6N	2	10, 6	16
	(B) F1r	2	10, 6	16
	(C) F1	2	10, 6	16
	(D) BALB/c	2	10, 6	16

The total number of animals tested was 140. We tested two litters for each genotype. Each litter size was either 6 or 10; 64 pups were fostered while 76 were raised by their biological mothers.

Materials and methods

Animal husbandry

C57BL/6N and BALB/c mice were imported from Charles River (Italy). Then at the Istituto Italiano di Tecnologia (IIT) mice were bred in order to obtain two cohorts of first generations (F1s) mice derived from intercrosses within each strain; yet, two additional cohorts of mice, F1 and F1r (reciprocal), were obtained from the reciprocal hybrids from C57BL/6N and BALB/c inbred strains. All mice were housed at the environmental temperature of 23°C, under a 12:12 light/dark cycle with lights on at 0800 h until 2000 h with food and water available *ad libitum*.

Animals were mated by introducing the female into the male cage and monitored for 1 h; then the mice were checked daily every morning and evening. Between E16 and E18 the dam was allocated an individual cage with paper for nesting. All dams were primiparae and both females and males were 12–15 weeks old when mated. Litters that were fostered were removed from their cage at birth, in the morning of P0, and smeared with sawdust from the cage of a lactating CD1 to which they were allocated. Cages were changed at weaning at P28 and every 2 weeks thereafter.

For each experimental condition we tested each pup of two litters (see Table 1). A total of 140 animals were tested; the size of the 2 litters was 10 and 6, respectively, for each genotype of the fostered litters. For all litters raised by their biological mother the litter size was 10 but for 1 F1 litter that had 6 pups. Only 1 pup of a litter of the foster condition had to be culled to downsize the litter to 10. All animal procedures were approved by the ethical national committee in Italy, for IIT Genova. The study followed ARRIVE guidelines (<http://www.nc3rs.org.uk/arrive-guidelines>).

Mouse strange situation procedure

In mice, to overcome species-specific differences, we devised only three consecutive episodes of 3 min each, preceded by a pretest phase in which the mother became acquainted with another age-matched virgin female. All pups were tested in a random order although we took care of not picking them when attached to the mother's nipple; after testing their tail was carefully colored with a marker. In the first episode, an 18-day-old pup was located in the arena with the mother and the stranger. In the second episode, the mother was removed from the arena and the pup is left alone with the stranger. In the third and final episodes, the mother was returned to the arena (Fig. 1b).

Accordingly, we classified the attachment-like style of all mice by defining three behavioral responses: (1) the '*maternal preference*', in which pups preferred the mother to the stranger; (2) the '*reunion*', defined as enhanced exploration of the mother by the pup in the third episode compared with the first episode and (3) the '*stranger effect*', expressed by an intensive exploration of the stranger by the pup during episode 2 compared with the other episodes. Both the mother and the stranger were monitored online and no aggressive behavior between the two adults or toward the pup was observed.

The mouse strange situation procedure (MSSP) was conducted, in the morning, in a grey arena (60 × 60 × 60 cm³) at P18. The pretest was conducted to habituate the dam and the stranger, an age- and sex-matched virgin C57BL/6J, to each other. For the stranger we have chosen a different substrain background to avoid effects due to the same strain. Several behavioral, cardiovascular and chemical phenotypic differences between C57BL/6J and C57BL/6N have been documented (Matsuo *et al.* 2010; Simon *et al.* 2013). Between pups the arena was thoroughly cleaned with water. All MSSPs were video recorded with a webcam and scored offline, for each episode of each mouse of each litter. Altogether 420 episodes, 3 per pup, were scored; for each episode we quantified the time (seconds) spent by the pup actively exploring (sniffing and touching) the stranger (t_S) and the mother (t_M) (Fig. 2).

Social interaction test

For this test we habituated each mouse (at 8 weeks of age) to the arena (60 × 60 × 60 cm³) for 5 min. Then we placed each mouse with a stranger (a sex- and age-matched C57BL/6J conspecific) for 1 min. We repeated this procedure with a second conspecific stranger after 3 min. The arena was thoroughly cleaned with water between pups. All trials were video recorded with a webcam and scored offline. All trials were visually scored; we measured the time spent by the mouse exploring the strangers (t_E) (Fig. 3a).

Statistical analysis

The unit of observation of this study was the single pup. The pups were grouped according to their genotype (four in total: C57BL/6N, F1, F1r and BALB/c) and to the maternal breeding (biological or foster). We used the paired two tails *t*-test for comparisons between t(M₃) and t(S₃) to assess '*maternal preference*' and for comparisons between t(M₃) and t(M₁) to assess the '*reunion*', within each group. We run repeated-measures one-way ANOVAs for the comparison of t(S_{1–3}) for the '*stranger effect*', followed by *post hoc* analysis using the Newman–Keuls multiple comparison test. In addition, we have run two one-way ANOVAs, one to test the mean difference [t(M₃) – t(S₃)] and the other to test the mean difference [t(M₃) – t(M₁)] across strains for each condition (biological and foster mother). Finally, for the social interaction test analysis, we use paired two tails *t*-tests for comparisons between t(E₁) and t(E₂) within each experimental group and a one-way ANOVA to test the mean difference [t(E₁) – t(E₂)] across strains for each condition.

Results

In our study, we propose a modified version of the classical eight-episode human SSP, called the mouse SSP, the MSSP (see *Materials and methods* and Fig. 1b).

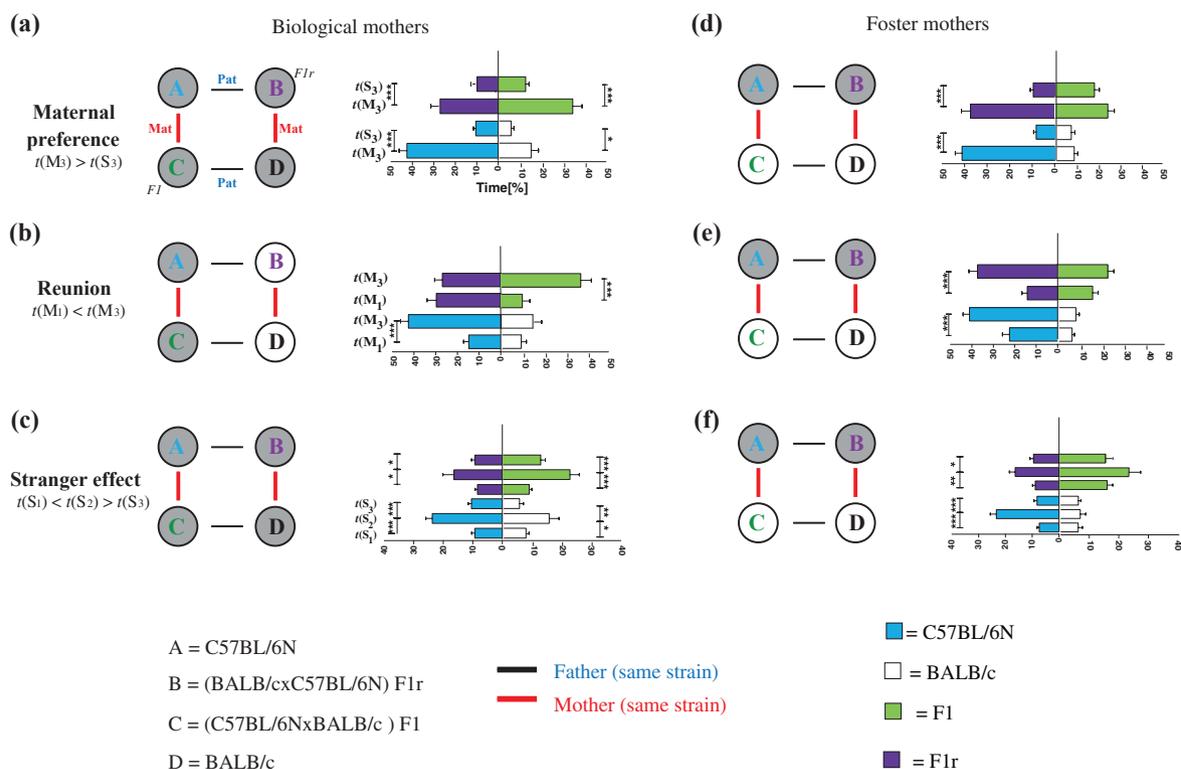


Figure 2: Attachment-like behaviors in mice raised by biological (a–c) and foster (d–f) mothers. We plot behavioral responses of all groups for ‘maternal preference’ (a and d), ‘reunion’ (b and e) and ‘stranger effect’ (c and f). Circles represent the groups (‘A’ = C57BL/6N; ‘B’ = BALB/cxC57BL/6N F1r; ‘C’ = C57BL/6NxBALB/c F1 and ‘D’ = BALB/c). Gray circles represent the presence of the behavioral response in the group, while open circles represent the absence of the response. Each behavioral response is calculated as the difference between time spent exploring the mother, $t(M)$, and time spent exploring the stranger, $t(S)$. *Maternal preference* is defined as greater exploration of the mother than the stranger during episode 3 (tM_3 and tS_3 , respectively). A *reunion* rebound effect is defined as greater exploration of the mother during episode 3 (tM_3) than during episode 1 (tM_1). Finally, the *stranger effect* is defined as greater exploration of the stranger during episode 2 (tS_2) than during the other two episodes (tS_1 and tS_3). The lines that connect the circles indicate the parental strains. Black lines indicate the strain of the father, and red lines indicate the strain of the mother. All data are reported as percentages of time spent exploring during the trial for the group. All graphs are presented as the means \pm SEM. Significant differences are indicated as follows: * P -value < 0.05, ** P -value < 0.01, *** P -value < 0.001.

All t -values, F -values, P -values and degrees of freedom are summarized in Tables S1 and S2, Supporting Information.

Maternal preference is paternally driven

All pups in the first experiment, which were raised by their biological mothers, showed a significant ‘maternal preference’ effect (C57BL/6N: $t_{(15)} = 8.36$, P -value < 0.001; F1: $t_{(19)} = 4.11$, P -value < 0.001; F1r: $t_{(19)} = 4.70$, P -value < 0.001; BALB/c: $t_{(19)} = 2.43$, P -value = 0.025; Fig. 2a). In the second experiment, in which foster mothers raised the mice, C57BL/6N mice presented a ‘maternal preference’ effect, whereas BALB/c mice did not (C57BL/6N: $t_{(15)} = 8.88$, P -value < 0.001; Fig. 2d). Furthermore, the two hybrid cohorts expressed a behavioral response that was consistent with paternal inheritance. In particular, F1r mice that had C57BL/6N fathers showed a ‘maternal preference’ effect ($t_{(15)} = 5.94$, P -value < 0.001), while F1 mice that had BALB/c fathers showed no ‘maternal preference’ effect. This

result provides evidence of the role of gene-environment interaction in the response to mother-pup separation. However, the behavioral response for this trait is paternally driven. Overall the mean difference of time spent exploring the mother with the time spent exploring the stranger across strains was significant [($F_{3,72}$) = 4.40, P -value = 0.007; ($F_{3,60}$) = 18.67, P -value \leq 0.001; see Table S2]. The *post hoc* tests showed only a significant difference, for the pups raised by their biological mothers, between the C57BL/6N and BALB/c mice (P -value < 0.01). For the fostered pups, the only non-significant differences were between C57BL/6N and F1r and between BALB/c and F1 mice (all significant differences P -values < 0.001; see Table S2).

Reunion effect with the mother is paternally driven

In the analysis of the second behavioral response, the ‘reunion’, we observed in the first experiment a positive rebound effect (i.e. the pup spent more time exploring the

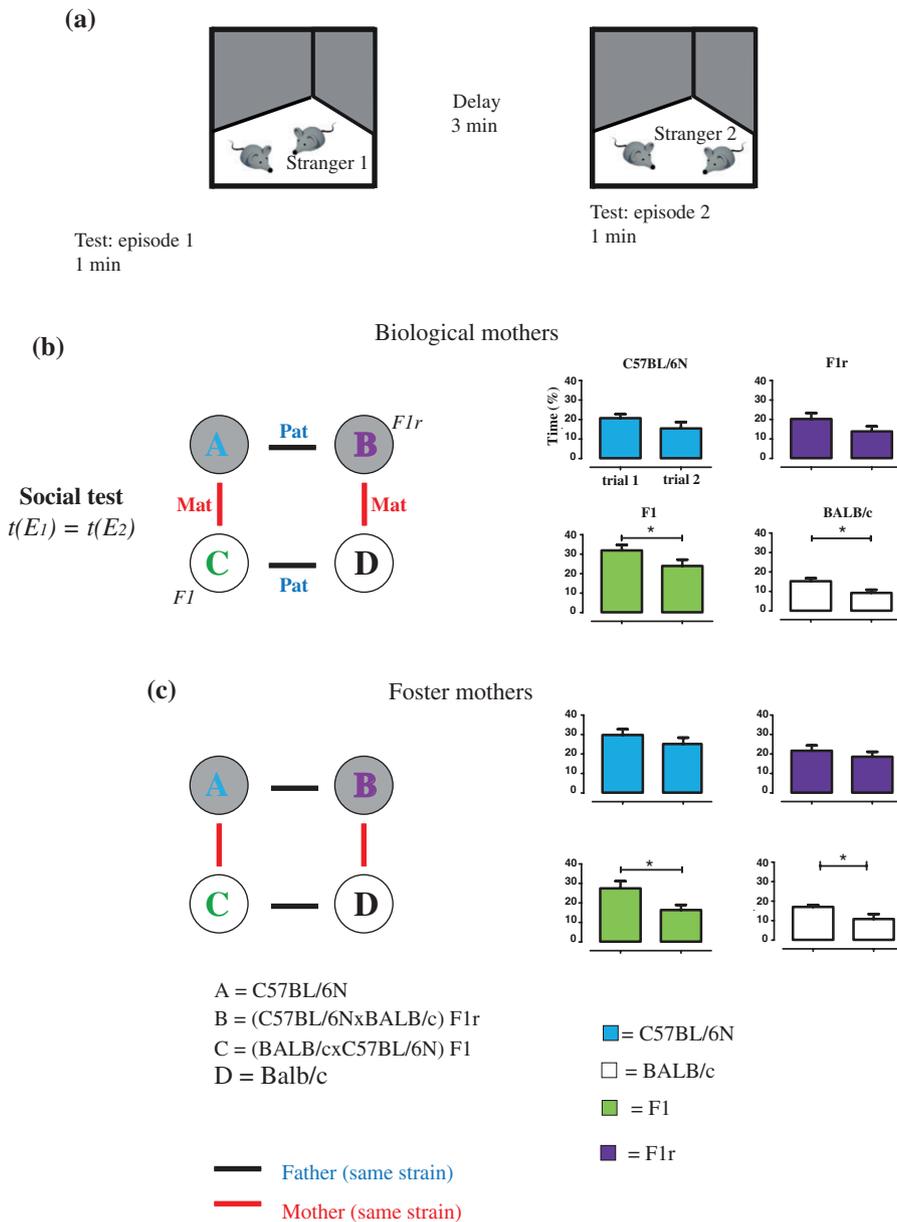


Figure 3: Social interaction in mice. (a) Here, we represent the social test in which each mouse is exposed to two conspecific strangers in the arena, each encounter lasts 1 min with an interval of 3 min. Experiment 1 (b) represents the behavior of mice raised by their biological mother, while experiment 2 (c) represents the behavior of mice raised by a foster mother. When the explorations of conspecific strangers (tE_i) are similar between the two episodes, the effect is represented by a gray circle. If the exploration decreases in episode 2, the effect is represented by an open circle. The groups are 'A' = C57BL/6N; 'B' = BALB/cxC57BL/6N F1r; 'C' = C57BL/6NxBALB/c F1 and 'D' = BALB/c. All data are reported as percentages of time spent exploring during the two trials for each group. Graphs are presented as the means \pm SEM. Significant differences are indicated as follows: * P -value < 0.05.

mother during episode 3 than during episode 1) in C57BL/6N mice ($t_{(15)} = 8.84$, P -value < 0.001) but not in BALB/c mice (Fig. 2b). However, F1 mice with C57BL/6N mothers exhibited rebound explorations during the reunion ($t_{(19)} = 5.68$, P -value < 0.001), while F1r mice with BALB/c mothers had no rebound (Fig. 2b). This finding indicated a maternal influence on the rebound measure. In the second experiment, where the effect of maternal care was equalized for all mice, the parental strains maintained the same differences as for the first experiment. In particular, we observed a rebound effect in C57BL/6N mice ($t_{(15)} = 4.85$, P -value < 0.001) but not in BALB/c mice (Fig. 2e). However, in this experiment, the fostering factor across all groups led to differences in the behavioral responses of F1s. Specifically, while F1r mice

expressed rebound behavior during the reunion with the mother ($t_{(15)} = 5.05$, P -value < 0.001), similar to C57BL/6N mice, F1 mice did not, indicating a paternal effect. The results for the 'reunion' phenotype showed an additional gene-environment interaction. Interestingly, in the first experiment the good maternal care of the C57BL/6N dams interacted with the paternally driven attachment-like style to enhance the maternal preference in the F1 mice (i.e. the 'reunion'), while the F1r mice raised by BALB/c mothers did not exhibit this behavior. Overall the mean difference of time spent exploring the stranger with the time spent exploring the mother across strains was significant [$(F_{3,72}) = 9.30$, P -value < 0.001; $(F_{3,60}) = 7.01$, P -value < 0.001]. The *post hoc* tests showed significant differences, for the pups

raised by their biological mothers, between: C57BL/6N and BALB/c (P -value < 0.05), F1r and F1 (P -value < 0.001), F1r and C57BL/6N (P -value < 0.001), and F1 and BALB/c (P -value < 0.01). For the fostered pups the *post hoc* tests showed significant differences between C57BL/6N and BALB/c (P -value < 0.01), F1 and C57BL/6N (P -value < 0.05), F1r and F1 (P -value < 0.05), F1r and BALB/c (P -value < 0.001) (see Table S2).

Exploration of the stranger is paternally driven

In the first experiment, all groups showed a significant 'stranger effect', represented by increased exploration of the stranger by the pup in episode 2 (C57BL/6N: P -value < 0.001; F1: P -value < 0.001; F1r: P -value = 0.02; BALB/c: P -value = 0.006; Fig. 2c). Instead, in experiment 2, the behavioral response of the mice was expressed through paternal inheritance (C57BL/6N: P -value < 0.001; F1: P -value = 0.058; F1r: P -value = 0.007; BALB/c: P -value = 0.900; Fig. 2f). In particular, only C57BL/6N and F1r mice showed an increased exploration of the stranger, while BALB/c and F1 mice did not show a 'stranger effect'. Once again, the good C57BL/6N maternal care in the first experiment masked the paternally driven effect.

Attachment-like styles predict adult social interaction

Early life experiences have important effects across the life span of an individual (Roth & Sweatt 2011). Various clinical approaches have suggested that adult psychological life is often an expression of the attachment style during infancy (Sheinbaum *et al.* 2015). In addition, long-term studies have shown that a particular attachment style in humans is relatively stable from infancy to early adulthood (Waters *et al.* 2000). Therefore, we tested the social interactions of the same mice for which we had characterized attachment-like styles. Standard social behavioral testing in mice includes either the exploration of conspecifics across different spatial conditions or repeated exposures to the same animal. In order to avoid the burden of long and repeated behavioral procedures, we simplified our study by exposing each mouse to only two trials and with two different mice. Because of the novelty of the conspecifics at each trial a normal mouse is spontaneously motivated to interact with each conspecific. In the assessment of social behavior, we measured the time that each mouse (now at 8 weeks of age) spent exploring the two age- and sex-matched conspecific C57BL/6J mice during the two consecutive trials. In both experiments with biological and foster mothers, we observed that all mice with C57BL/6N fathers explored similarly the two conspecific strangers by showing the same level of interest in the first trial as well as in the second one (Fig. 3b,c). This behavior suggested a sustained interest in social interaction. In comparison, mice with BALB/c fathers showed significantly less concern for the second encounter with a conspecific stranger (BALB/c: P -value = 0.008; F1: P -value = 0.028; BALB/c: P -value = 0.028; F1: P -value = 0.020; for mice raised by biological and foster mothers, respectively; Fig. 3b,c). The ANOVAS to compare mean differences [$t(E_1) - t(E_2)$] across strains were not significant in both the biological and foster mother conditions.

Discussion

Taken together, the behaviors of our mice led us to define different attachment-like styles according to genetic, epigenetic and environmental differences. In particular, we observed that C57BL/6N mice presented all three behavioral markers (i.e. maternal preference, reunion and stranger effect) of a secure attachment-like style, whereas BALB/c mice showed only two of the three positive markers. The latter group did not exhibit rebound exploration of mothers during their reunion; seeking for the mother in the human version of the test testifies the secure attachment of the infant (Ainsworth *et al.* 1978; Sheinbaum *et al.* 2015). Therefore, while most pups raised by their biological mothers showed signs of secure attachment-like, pups raised by foster mothers showed either secure or insecure attachment-like behaviors according to their genotype. Furthermore, we observed that when foster mothers raised their pups, all behavioral responses to define specific attachment-like styles were paternally driven, with secure attachment-like occurring in the offspring of C57BL/6N fathers, while insecure attachment-like occurred in the offspring of BALB/c fathers. A limitation of this study is that we did not monitor the stress-response system in the partners of the dyad when they were assessed; further studies could assess, e.g. the plasma cortisol level in pups and mothers before and after the MSSPs. Moreover, further studies in the future should be dedicated to investigate additional effects because of the genetic background as specific paternally driven effects differentially influence the development of attachment-like behaviors in mice.

Interestingly, it has been observed in other rodents, such as guinea pigs, that pups prefer being in proximity of the mothers rather than other adults (Hennessy *et al.* 2003) although they would also explore other female adult conspecific (Graves & Hennessy 2000) and the presence of the mother is more effective in lowering stress levels, compared with other female adults, when the pup is placed in a novel environment. Attachment has been also widely explored, in the rat, by Regina Sullivan's group. Her group has described the neurobiology of attachment and assigned a primary role to the amygdala (Sullivan *et al.* 2000). They showed that attachment occurs even with trauma and/or an abusive care giver; in fact they found that the presence of the dam suppressed the pup's response to a threat or an aversive learning (Perry & Sullivan 2014; Shionoya *et al.* 2007) thus allowing the young individual to survive although with long-term psychological effects that may become evident with maturation.

Imprinted genes, such as Peg3, Mest, Gnas and Gnasxl, play a pivotal role in many aspects of maternal behavior (Isles & Holland 2005); however, a direct effect of an imprinted gene on the development of attachment behavior has not been determined. The intragenomic conflict theory of genomic imprinting advocates for a particular role for paternally imprinted genes (Haig 2014; Haig & Wilkins 2000). Paternally expressed genes are thought to maximize the allocation of resources to the pups, while maternally expressed genes are thought to lead to an equal distribution among the offspring. The evolutionary explanation for this difference resides in greater maternal than paternal relatedness among

developing pups. Consequently, the expression of a gene according to parental origin may promote the exploitation of maternal resources by the pup (or infant). In fact, while the key role of the mother is to provide nutrients, prenatally (Reik *et al.* 2003) and perinatally (Plagge *et al.* 2004), in the postnatal period it becomes pivotally important to promote positive signals in the sensory system of the mother (Wilkinson *et al.* 2007). The results of this study also converge with the coadaptation theory of genomic imprinting (Cowley *et al.* 2014; Wolf & Hager 2006), in which allele matching between the mother and the pup reinforces the relationship between the infant and the mother.

References

- Ainsworth, M.D.S., Blehar, M.C., Waters, E. & Wall, S. (1978) *Patterns of Attachment: A Psychological Study of the Strange Situation*. Erlbaum, Hillsdale, NJ.
- Bartolomei M.S. & Ferguson-Smith A.C. (2011) Mammalian genomic imprinting. *Cold Spring Harb Perspect Biol.* **3**(7).
- Bremner, J.D. (2003) Long-term effects of childhood abuse on brain and neurobiology. *Child Adolesc Psychiatr Clin N Am* **12**, 271–292.
- Bucci, S., Seymour-Hyde, A., Harris, A. & Berry, K. (2016) Client and therapist attachment styles and working alliance. *Clin Psychol Psychother* **23**, 155–165.
- Calatayud, F., Coubard, S. & Belzung, C. (2004) Emotional reactivity in mice may not be inherited but influenced by parents. *Physiol Behav* **80**, 465–474.
- Cowley, M., Garfield, A.S., Madon-Simon, M., Charalambous, M., Clarkson, R.W., Smalley, M.J., Kendrick, H., Isles, A.R., Parry, A.J., Carney, S., Oakey, R.J., Heisler, L.K., Moorwood, K., Wolf, J.B. & Ward, A. (2014) Developmental programming mediated by complementary roles of imprinted Grb10 in mother and pup. *PLoS Biol* **12**, e1001799.
- Graves, F.C. & Hennessy, M.B. (2000) Comparison of the effects of the mother and an unfamiliar adult female on cortisol and behavioral responses of pre- and postweaning guinea pigs. *Dev Psychobiol* **36**, 91–100.
- Haig, D. (2014) Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity (Edinb)* **113**, 96–103.
- Haig, D. & Wilkins, J.F. (2000) Genomic imprinting, sibling solidarity and the logic of collective action. *Philos Trans R Soc Lond B Biol Sci* **355**, 1593–1597.
- Heim, C. & Nemeroff, C.B. (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* **49**, 1023–1039.
- Hennessy, M.B., Young, T.L., O'Leary, S.K. & Maken, D.S. (2003) Social preferences of developing guinea pigs (*Cavia porcellus*) from the preweaning to the periadolescent periods. *J Comp Psychol* **117**, 406–413.
- Isles, A.R. & Holland, A.J. (2005) Imprinted genes and mother-offspring interactions. *Early Hum Dev* **81**, 73–77.
- Isles, A.R., Baum, M.J., Ma, D., Keverne, E.B. & Allen, N.D. (2001) Urinary odour preferences in mice. *Nature* **409**, 783–784.
- Landers, M.S. & Sullivan, R.M. (2012) The development and neurobiology of infant attachment and fear. *Dev Neurosci* **34**, 101–114.
- Lawson, H.A., Cheverud, J.M. & Wolf, J.B. (2013) Genomic imprinting and parent-of-origin effects on complex traits. *Nat Rev Genet* **14**, 609–617.
- Matsuo, N., Takao, K., Nakanishi, K., Yamasaki, N., Tanda, K. & Miyakawa, T. (2010) Behavioral profiles of three C57BL/6 sub-strains. *Front Behav Neurosci* **4**, 29.
- Perry, R. & Sullivan, R.M. (2014) Neurobiology of attachment to an abusive caregiver: short-term benefits and long-term costs. *Dev Psychobiol* **56**, 1626–1634.
- Plagge, A., Gordon, E., Dean, W., Boiani, R., Cinti, S., Peters, J. & Kelsey, G. (2004) The imprinted signaling protein XL alpha s is required for postnatal adaptation to feeding. *Nat Genet* **36**, 818–826.
- Priebe, K., Romeo, R.D., Francis, D.D., Sisti, H.M., Mueller, A., McEwen, B.S. & Brake, W.G. (2005) Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/cJ mice: a cross-fostering study. *Dev Psychobiol* **47**, 398–407.
- Reik, W., Constancia, M., Fowden, A., Anderson, N., Dean, W., Ferguson-Smith, A., Tycko, B. & Sibley, C. (2003) Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *J Physiol* **547**, 35–44.
- Roth, T.L. & Sweatt, J.D. (2011) Annual research review: epigenetic mechanisms and environmental shaping of the brain during sensitive periods of development. *J Child Psychol Psychiatry* **52**, 398–408.
- Sheinbaum, T., Kwapil, T.R., Ballestri, S., Mitjavila, M., Chun, C.A., Silvia, P.J. & Barrantes-Vidal, N. (2015) Attachment style predicts affect, cognitive appraisals, and social functioning in daily life. *Front Psychol* **6**, 296.
- Shionoya, K., Moriceau, S., Bradstock, P. & Sullivan, R.M. (2007) Maternal attenuation of hypothalamic paraventricular nucleus norepinephrine switches avoidance learning to preference learning in preweaning rat pups. *Horm Behav* **52**, 391–400.
- Simon, M.M., Greenaway, S., White, J.K. *et al.* (2013) A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. *Genome Biol* **14**, R82.
- Sullivan, R.M. (2012) The neurobiology of attachment to nurturing and abusive caregivers. *Hastings Law J* **63**, 1553–1570.
- Sullivan, R.M., Landers, M., Yeaman, B. & Wilson, D.A. (2000) Good memories of bad events in infancy. *Nature* **407**, 38–39.
- Tarantino, L.M., Sullivan, P.F. & Meltzer-Brody, S. (2011) Using animal models to disentangle the role of genetic, epigenetic, and environmental influences on behavioral outcomes associated with maternal anxiety and depression. *Front Psychiatry* **2**, 44.
- Tyrer, P., Mulder, R., Crawford, M., Newton-Howes, G., Simonsen, E., Ndeti, D., Koldobsky, N., Fossati, A., Mbatia, J. & Barrett, B. (2010) Personality disorder: a new global perspective. *World Psychiatry* **9**, 56–60.
- Waters, E., Weinfield, N.S. & Hamilton, C.E. (2000) The stability of attachment security from infancy to adolescence and early adulthood: general discussion. *Child Dev* **71**, 703–706.
- Wilkinson, L.S., Davies, W. & Isles, A.R. (2007) Genomic imprinting effects on brain development and function. *Nat Rev Neurosci* **8**, 832–843.
- Wolf, J.B. & Hager, R. (2006) A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biol* **4**, e380.

Acknowledgments

We would like to thank Professor Giorgio Vallortigara and Professor David Haig for critical reading of the manuscript and for comments and suggestions.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Table S1. The *t*-values, degrees of freedom and *P*-values for each genotype and mothers condition.

Table S2. The *F*-values, degrees of freedom and *P*-values for each genotype and mothers condition.