From social experience to social behaviour: hormonal and behavioural phenotypes during adolescence in male guinea pigs

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Abstract

Adolescence is the transition from juvenility to adulthood and is characterized by prominent endocrine, neural and behavioural alterations. Thus, adolescence represents a sensitive phase during which social experiences can shape endocrine and behavioural phenotypes. Although the influence of the social environment during adolescence has been widely investigated, most studies assessed such effects only before and after a given social experience, providing limited insight into how these phenotypes change within this ontogenetic phase. Our goal was therefore to examine potential effects of different social environments on the endocrine and behavioural phenotype in male guinea pigs across different time points within adolescence. For this approach, twenty domestic adolescent male guinea pigs (*Cavia aperea* f. *porcellus*) were housed in two distinct social environments: while males of both groups lived in heterosexual pairs, males of one group additionally received regular social stimulation through repeated short encounters with unfamiliar conspecifics, whereas males of the other group did not. This procedure increased the number of social interactions. Hormone concentrations and behavioural parameters were assessed repeatedly throughout the experiment. We hypothesized males from the two social conditions to differ in their hormonal and behavioural phenotype across adolescence. Males with additional social stimulation displayed initially elevated baseline cortisol concentrations, possibly enabling them to adequately react to the unpredictable social encounters. Over time, baseline cortisol concentrations slightly decreased again, suggesting conformance to the challenging environment. In contrast, differences in sociopositive behaviour became apparent only later during the experiment, with socially stimulated males showing higher levels of sociopositive behaviour than males without social stimulation. This may indicate that behavioural adjustment to a more complex social environment requires prolonged social experience. Taken together, these findings show that social experiences during adolescence can shape endocrine and behavioural phenotypes in male guinea pigs, but that these adjustments may differ in their temporal dynamics.

Keywords

Basal cortisol, basal testosterone, cortisol responsiveness, social environment, social niche conformance, sociopositive behaviour

1. Introduction

The social environment of an individual is defined by its conspecifics and how they interact with and affect the focal individual [1]. These social interactions can shape the individual's behavioural development profoundly [2]. Most research on the development of behavioural phenotypes has focused on early life phases, particularly the time from birth until weaning [3]. During this time, neural circuits are highly plastic and the organism is peculiarly susceptible to external influences from the (social) environment [3,4]. As shown in different rodent species, social environmental cues experienced by the mother can be transmitted to the offspring from conception to weaning, thereby shaping the offspring's phenotype [5–9]. However, in recent years it has become increasingly clear that adolescence is another sensitive phase during which behavioural trajectories can be shaped or reshaped in response to the social environment [3,10,11].

Adolescence is the ontogenetic phase encompassing the gradual transition from juvenility to adulthood [12]. In this phase, prominent alterations in the endocrine system, neural circuitry and behaviour occur [13,14]. Moreover, individuals gradually become independent from their parents, often disperse from their natal social environment and encounter unfamiliar conspecifics [11]. As sexual maturity emerges during adolescence, social interactions with conspecifics increasingly occur in a reproductive context [2,15]. Consequently, adolescence is often characterized by major changes in the social environment, requiring individuals to adjust their behavioural phenotype to the prevailing social conditions [16]. This is, for example, well-illustrated in guinea pig males (*Cavia aperea* f. *porcellus*), in which different group sizes during adolescence lead to distinct behavioural and reproductive strategies. Males reared in large groups, i.e., under high population density, usually display low-aggressive queuing strategy, whereas males reared in heterosexual pairs, i.e., under low population density, develop a high-aggressive resource defense strategy [2,3,10,17]. The queuing strategy is thought to be adaptive under socially complex conditions because it allows males to avoid reproductive competition until they are large enough to effectively challenge dominant males for mating access [2,3,10]. In contrast, the resource defense strategy may increase reproductive success under low-density conditions by allowing males to monopolize access to their female partner [3,10,17]. Similar patterns have also been reported in zebra finches (*Taeniopygia guttata*), where males reared in socially more complex environments during adolescence display lower aggressiveness and courtship behaviour than males reared with a single female and later integrated more successfully into unfamiliar mixed-sex groups [16,18]. Together, these examples demonstrate that it is essential that adolescent individuals acquire the social rules and behavioural patterns necessary for interacting with conspecifics and adjusting to their social environment [2,15,19].

Such behavioural adjustments to the social environment can be mediated through underlying endocrine mechanisms. During adolescence, both the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenocortical (HPA) axis undergo substantial maturation [20–22]. The HPG axis regulates the production of sex steroids such as testosterone in males [21], whereas the HPA axis regulates the secretion of glucocorticoids [23,24]. Glucocorticoids are released at low baseline levels to maintain metabolism and homeostasis and increase for example in response to unpredictable challenges. For social animals, this also includes challenges arising from the social environment [25]. This stressor-induced HPA function, i.e., HPA reactivity, primarily functions to mobilise energy and modulate different behaviours [26–30]. Interactions between the HPA and HPG axis have been shown to shape behavioural phenotypes and are modulated by the social environment [31,32]. In guinea pigs, the distinct behavioural and reproductive strategies described above are also associated with different endocrine phenotypes. The males raised in high population density during adolescence frequently engage in diverse social interactions [3,10,19]. These interactions- especially courting and aggressive encounters- trigger increased testosterone levels in male vertebrates [3,19,33]. Testosterone in turn has inhibiting effects on the HPA axis [34], thereby reducing HPA reactivity [3,10]. In contrast, males housed with only a same-age female during adolescence, i.e., low population density, experience fewer social interactions, leading to lower testosterone levels and higher HPA reactivity [3,10]. Also in rats (*Rattus norvegicus* f. *domestica*), social status within a group has been suggested to influence adolescent testosterone levels, which may contribute to a variation in glucocorticoid receptor expression and the organization of HPA axis regulation [35]. Thus, adolescence is a key phase for the adaptive shaping of endocrine and behavioural phenotypes in response to the social environment.

However, adolescence itself is not a uniform developmental phase but rather represents a highly dynamic and plastic developmental phase [3,10,11]. In line with this, studies in rodents have shown that the effects of social experience or stress on behavioural and endocrine parameters depend strongly on the specific timing within adolescence. For example, exposure to social defeat during early versus late adolescence can lead to opposite effects on aggressive behaviour later in life in male golden hamster (*Mesocricetus auratus* f. *domestica*) [36,37]. Similarly, in laboratory mice (*Mus musculus* f. *domestica*), exposure to chronic social stress during early adolescence has been associated with increased basal corticosterone levels [38,39], whereas exposure to a predator stress paradigm across mid- and late adolescence did not affect basal corticosterone levels [39,40]. These findings suggest that the impact of social challenges on hormonal and behavioural phenotypes is not uniform across adolescence but depends on the timing within adolescence at which these challenges occur. Although many studies have investigated such effects of the social environment on endocrine and behavioural phenotypes in adolescent guinea pigs [2,15,19,25,41,42], these studies have typically assessed responses before and

after a given social experience. As a consequence, they provide only limited insight into how these phenotypes change dynamically across adolescence.

The present study therefore aimed to examine effects of different social environments on the endocrine and behavioural phenotype across adolescence in male guinea pigs. By repeatedly assessing endocrine and behavioural parameters, we investigated how social environment and time interact in shaping these phenotypes during this dynamic developmental phase. As established in former work [19,41,43], the distinct social environments used in the present study were males living in heterosexual pairs versus males living in heterosexual pairs that received additional social stimulation via regular encounters with unfamiliar animals of both sexes (for details see methods). Regarding endocrine phenotypes, baseline testosterone and/ or cortisol (stress) responsiveness are known to be shaped by the social environment, as shown previously in juvenile, adolescent and adult male guinea pigs [17,19,41,43]. Baseline cortisol in turn often reflects more immediate responses to socially challenging environments [2,44]. For these reasons, we tested the hypothesis that baseline cortisol, baseline testosterone and cortisol responsiveness differ between males from the two social conditions. Concerning behavioural phenotype, we focused on sociopositive, courtship and sexual behaviour, as these are commonly known to be influenced by social environment during adolescence [2,11,15]. These behaviours were assessed in the home enclosure towards the female housing partner and in behavioural tests in which focus males were exposed to unfamiliar adult females and pre-weaned males, allowing the assessment of behavioural responses across different social contexts. In addition, we assessed risk-taking behaviour in another behavioural test, as adolescence is often characterized by increased risk-taking behaviour [45] which can furthermore be influenced by the social environment [46,47]. We hypothesized that behavioural measures would differ between males from the two social conditions. We had no a priori expectation in which direction an interaction of time and social conditions would shape endocrine and behavioural phenotype.

Besides the relation between social environment and endocrine and behavioural phenotype detectable by such group comparisons, recent work indicates that the social environment can further influence the stability of trait expression, i.e., repeatability [48]. Studies in guinea pigs provided first evidence that social complexity can affect the repeatability of hormonal traits [48]. Examining repeatability therefore offers insight into how consistently individuals express their endocrine phenotype under different social conditions, and whether additional social stimulation affects this stability. Against this background, we determined the repeatability of hormonal phenotype within the two social conditions to assess the stability of endocrine trait expression. We tested the hypothesis that repeatability of baseline cortisol, baseline testosterone, and cortisol responsiveness would differ between them without having an a priori expectation regarding the direction of these differences.

2. Material and methods

2.1 Animals and housing conditions

All animals included in this study were bred from a breeding program of multi-coloured shorthaired guinea pigs (*Cavia aperea* f. *porcellus*) at the Department of Behavioural Biology at the University of Münster. They were born and reared in a total of eight harem groups within one breeding room, each consisting of one male, one to three females and their pre-weaned offspring. The offspring was routinely taken out of the harems after weaning at post-natal day (PND) 21 (±1) and adults were removed and replaced at around 18-24 months of age. Each harem was kept in wooden enclosures on the ground with a base area of approximately 1.5 m^2 and a wall height of 0.5 m. The enclosures were filled with wood shavings (Tierwohl Super, J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany) as bedding and enriched with red plastic shelters and wooden bridges.

After weaning, the experimental animals were transferred to enclosures in a different housing room. These enclosures had a base area of 0.5 m^2, a wall height of 0.5 m, were also filled with wood shavings and enriched with a big and a small red plastic shelter. Food (hasfit Cavia C pellets, EQUOVIS GmbH, Münster, Germany) and water were available *ad libitum*. Since guinea pigs are incapable of synthesizing ascorbic acid [49,50] and therefore prone to vitamin C deficiency [51], a vitamin C supplement (100% L-ascorbic acid, altapharma, Dirk Rossmann GmbH, Burgwedel, Germany) was added to the water three times per week (approximately 120 mg vitamin C in 900 ml water shared between two animals). Additionally, hay was replenished daily and fresh fodder (carrots, cucumbers, apples) was fed regularly. All guinea pig housing rooms were kept under controlled conditions with a 12 h: 12 h light/ dark cycle (lights on at 07:00), temperature of approximately 22 °C and relative humidity of approximately 50 %.

2.2 Experimental design

For this study, twenty male guinea pigs were used. To investigate the influence of distinct social environments on hormonal and behavioural phenotypes, they were randomly assigned to one of two social conditions. All males lived in heterosexual pairs during adolescence, however, males of one group were additionally socially stimulated (see 2.3) regularly (pair-housed male with additionally social stimulation; PM+S condition), whereas males of the other group were not (pair-housed male without additional social stimulation; PM-S condition). The twenty males were organized into ten matched pairs, each consisting of one PM+S male and one PM-S male.

After weaning at PND 21 (±5), the two males within one matched pair were initially housed together. Their future female partners, which were about the same age, were housed together in an adjacent enclosure. Each male and his respective female partner stem from different harem groups, ensuring they were neither half nor full siblings. These pre-experimental housing conditions were maintained

until the focus males reached early adolescence at PND 53 (±5). At that time, the two males were separated and each one was housed with his assigned female partner.

Following a three-day habituation period, the experiments started at PND 56 (±5) and lasted seven weeks, meaning the animals were 102 (±5) days of age when the experiments ended. In guinea pig males, adolescence spans approximately from early adolescence (PND 55) to late adolescence (PND 120) [19], with sexual maturity usually being reached around PND 70 [52]. The home enclosures of both males within a matched pair, each now housed together with their respective female partner, were placed side by side in the same housing room. This was done to control for variability in housing conditions such as room temperature or humidity. All experimental procedures (cortisol response tests, body weight measurements, video recordings, behavioural tests) were conducted in parallel within each matched pair to minimise variability in timing of the experiments.

In total, four cortisol response tests (CRTs) to measure basal and reaction cortisol values (*see assessment of endocrine phenotypes*) were conducted within the seven week long experimental phase (**Fig. 1**). The first CRT was conducted before the social stimulation treatment started and is thus referred to as CRT0. CRT0 was conducted in the first experimental week three days after the focus males were housed together with their female partners. CRT1, CRT2 and CRT3 followed 14 (±2) days after the preceding one, with CRT1 being conducted approximately around the onset of sexual maturity (**Fig. 1**). To additionally assess behavioural changes over time, home enclosure behaviour was video-recorded from the second experimental week onwards. The six weeks of behavioural observations were divided into three consecutive two-week phases (phase 1 – phase 3), matching the timing of CRT1, CRT2 and CRT3 (**Fig. 1**). The social stimulation treatment started in the first experimental week after CRT0 and continued throughout the whole experiments. Both social stimulation sessions and video recordings were randomly distributed across the week to avoid possible habituation effects and to observe behaviour on different day times. In the final experimental week, an additional battery of behavioural tests was conducted to further evaluate social and risk-taking behaviour. These tests comprised the step-down test (SDT), the male-female interaction test (MFIT) and the social initiative test (SIT) (see 2.5.2). In addition, as part of another project, fur swabbing with PDMS (Polydimethylsiloxane) tubes to analyse chemical fingerprints was conducted. The procedural order of CRT3, behavioural tests and fur swabbing in the last experimental week varied across individuals, but was identical within each matched pair. The focus males never experienced more than one of these procedures per day.

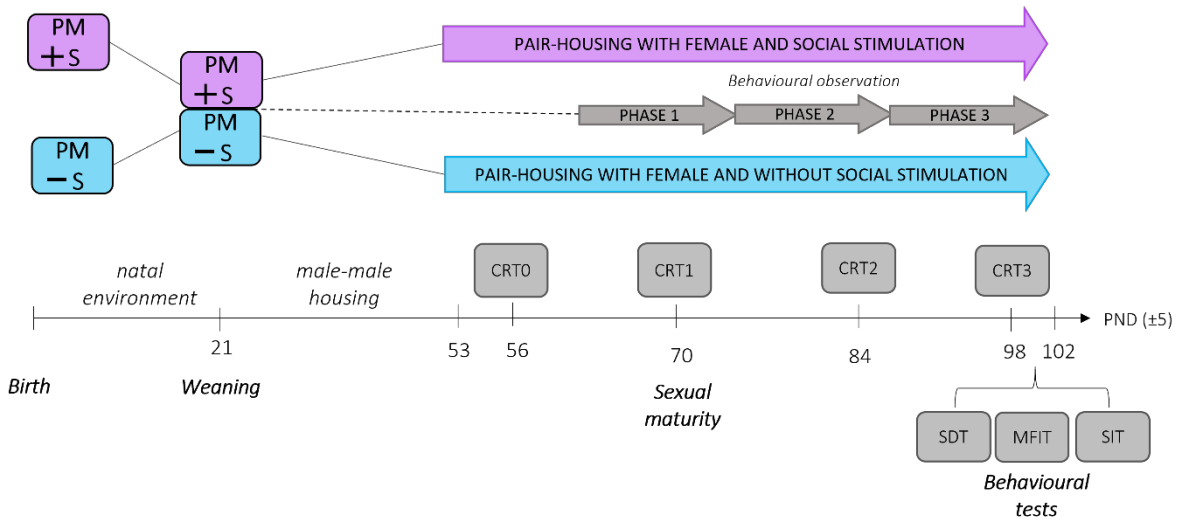

Figure 1: Experimental design. PM+S: pair-housed males with additional social stimulation; PM-S: pair-housed males without additional social stimulation. After weaning, one PM+S and one PM-S male were housed together. From post-natal day (PND) 53±5 onwards, the experimental phase started and males were pair-housed with another female. In total, four cortisol response tests (CRT0 – CRT3) were conducted to assess hormonal phenotypes. Social stimulation for PM+S males started after CRT0 and lasted until the experimental phase was finished at post-natal day (PND) 102±5. Video recordings of home enclosure behaviour (behavioural observation) were conducted from the second experimental week onwards and divided into three phases consisting of two weeks each (phase 1 – phase 3). In the final experimental week, additional behavioural tests (SDT: step-down test; MFIT: male-female interaction test; SIT: social initiative test) were performed. Developmental milestones are indicated along the timeline: birth (PND 0), weaning (PND 21), and sexual maturity (around PND 70).

2.3 Social stimulation

The social stimulation procedure applied in the present study was adapted from earlier studies, where additional social stimulation successfully influenced hormonal profiles in juvenile and adolescent guinea pig males [19,41,43]. The social stimulation for the respective males (PM+S) started after CRT0 and was applied three times per week for the whole experimental phase of seven weeks. Each social stimulation session consisted of introducing an unfamiliar conspecific into the home enclosure of the focus male and his female partner for up to ten minutes. Per week, two social stimulation sessions were done with another male and one with a female, resulting in a total of 14 male and seven female stimulation sessions per focus male. The female stimulation animals always came from the harems to ensure they were pregnant and thus in the same reproductive stadium, preventing a confounding influence of oestrus. In total, 30 stimulus animals were used (14 males, 16 females). If the focus male was stimulated more than once with the same stimulus animal, there was always a minimum interval of seven days between these stimulation sessions.

PM+S males never experienced more than one social stimulation session per day. To reduce potential habituation effects, both the day of the week and time of day of stimulation sessions were varied. Prior to each session, the red plastic shelters were temporarily removed from the home enclosure of the focus male and the video camera was turned on, as all stimulation sessions were recorded. After the stimulation animals were introduced into the home enclosure, a timer was started as the sessions had

maximum length of ten minutes. Sessions were terminated earlier if aggressive behaviour escalated in order to minimise the risk of injury. Out of a total of 140 stimulation sessions using male stimulus animals, 21 were aborted due to escalating aggression.

2.4 Assessment of hormone concentrations

Hormones were measured using blood samples obtained in cortisol response tests (CRTs), a standardized test in which the endocrine stress response was assessed at different time points following exposure to a novel environment as stressor [43,53,54]. The test started between 12:30 and 13:30, as plasma cortisol concentrations fluctuate throughout the day and a peak is observed at 13:00 [55]. Prior to that, the animals were undisturbed for one hour.

At the start of the CRT, the male was taken out of his home enclosure and placed on the experimenter's lap outside of the housing room. To facilitate blood flow, a muscle salve (Finalgon® Wärmesalbe DUO, Zentiva Pharma GmbH, Frankfurt am Main, Germany) for expanding the blood vessels was applied to the guinea pig's ear and wiped off again. After that, the marginal ear vessel was punctured with a lancet (Solofix® Blutlanzetten, B. Braun Melsungen AG, Melsungen, Germany) and blood was collected in heparinized capillary tubes (Capillary tubes for microhaematocrits, 100 µl, Paul Marienfeld GmbH & Co KG, Lauda Königshofen, Germany) to later on determine basal cortisol (c0) and basal testosterone (t) levels. For cortisol, this procedure had to be completed within 3 minutes to prevent the sampling process itself from influencing the hormone values in the obtained sample itself [55]. Such stress-induced changes in hormone values appear later in testosterone than in cortisol [55] and collecting a sufficient amount of blood for testosterone assays requires slightly more time. Therefore, blood samples for testosterone were collected within 6 minutes, which represents a compromise between sample quality and animal welfare and follows the procedure applied in earlier studies (e.g., [2,15,17,43,56]). Then, the guinea pig was singly placed into an unfamiliar enclosure in a different housing room where it stayed for a total of two hours. This enclosure had a size of 1 m^2, wall height of 0.5 m and was equipped with wood shavings, food and water. Exactly one and two hours after the first one, blood sampling was repeated to determine first (c1) and second (c2) cortisol responsiveness. The guinea pigs were weighed after each blood sampling and returned to their home enclosure after the last one.

To separate the blood plasma, the sample was centrifugated (13,000 × g for 5 min), transferred into a 1.5 mL Eppendorf tube and deep frozen at -20°C until assayed. Hormone concentrations were determined in duplicate using enzyme-linked immunosorbent assays (ELISA) (cortisol: RE52061, IBL International, Hamburg, Germany; antibody cross-reactivity: cortisol (100%), prednisolone (30%), 11-deoxycortisol (20%), cortisone (10.7%), prednisone (6.5%), 17 α-hydroxyprogesterone (5.4%), 6β-hydroxycortisol (4.4%), corticosterone 3.8%, desoxycorticosterone (1.8%); testosterone: RE52151, IBL International, Hamburg, Germany; antibody cross-reactivity: testosterone 100%, 11β-OH-testosterone

8.7%, 11α OH-testosterone, 3.2%, dihydrotestosterone 1.9%). Intra- and inter-assay CVs were determined 2.09% and 3.98% for cortisol and 4.7% and 5.7% for testosterone.

In some cases, it was not possible to collect a sufficient amount of blood for the ELISA, resulting in a decreased sample size. For each CRT, the sample size per group ranged between n = 4 and n = 10. Detailed sample sizes for each hormone measurement and CRT are provided in the supplementary material (Tab. S4)

2.5 Assessment of behavioural parameters

2.5.1 Home enclosure behaviour

To examine how distinct social environments influence social behaviour, home enclosure behaviour of the focus males in both conditions was recorded (outside of social stimulation sessions for PM+S males). Video recordings were conducted twice per week for one hour each, starting from the second experimental onwards when PM+S males already experienced some social stimulation sessions. For this purpose, a video camera (Panasonic HC-V785 or SONY HDR-CX405) was installed approximately 1.5 m above each experimental home enclosure. The day and time (between 08:00 and 17:00) at which the videos were recorded was randomized. In total, 12 h of home enclosure behaviour was collected for each individual. Video analysis was done with the program Interact (Interact, Lab Suite Version 2022, Program version 20.8.3.0, Mangold International GmbH, Arnstorf, Germany). The videos were blinded and randomized, ensuring ID and treatment of the respective individual as well as the time of recording were unknown to the observer. Behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt) were analysed. The full ethogram can be found in the supplementary material (Tab. S1). As the plastic shelters remained in the enclosure during recordings, animals were occasionally not visible, resulting in some zero entries when behaviour could not be observed.

2.5.2 Behavioural tests

A battery of behavioural tests was performed across three days during the seventh (final) experimental week, when males were approximately at PND 100 ± 5. These tests included the step-down test (SDT), male-female interaction test (MFIT) and social initiative test (SIT). They were adapted from previous studies implementing these tests in guinea pigs and wild cavies [57,58]. Each behavioural test was conducted on a separate day. On a given test day, both males within a matched pair were tested consecutively in that same test, with the order of testing (PM+S first vs. PM-S first) randomized. Similar to the CRT, all behavioural tests were conducted in an unfamiliar enclosure in a different housing room. This test enclosure had a size of 1 m^2, wall height of 0.5 m and was equipped with wood shavings. For the SDT, the experimenter stayed in the room, whereas the MFIT and SIT were videotaped and

conducted without the experimenter being present. For this purpose, a video camera (Panasonic HC-V785 or SONY HDR-CX405) was installed approximately 1.5 m above the test enclosure. Before a test started, the focus male was taken out of his home enclosure and carried to the test enclosure in a Makrolon type II cage. The individual tests are described in more detail below.

2.5.2.1 Step-down test (SDT)

The SDT is a test to assess risk-taking behaviour [57,58]. An elevated, sheltered, square platform (900 cm^2 base area; 23 cm height) was placed in the centre of the test enclosure. The platform was covered with wood shavings. The focus male was removed from the transport cage and placed on the platform facing away from the experimenter. The focus male was gently held still until his reflex to escape the experimenter subsided. The experimenter then stepped back and measured the focus male's latency to step down from the platform. This was defined as the time point at which the animal touched the floor of the enclosure with all four paws. A maximum test duration of 15 minutes was applied. If the focus male did not step down within this time frame, the test was terminated and latency was recorded as 15 minutes (900 seconds).

2.5.2.2 Male-female interaction test (MFIT)

The MFIT was performed to assess how males with and without additional social stimulation interact with an unfamiliar female conspecific. Similar to social stimulation, the females used for the MFIT stem from the harem groups and were therefore not in oestrus during the tests. Within each matched pair, the same female was used. Prior to the test, the test enclosure was divided into two equal halves by a mesh partition. The female was placed in one half of the enclosure and the focus male in the other. The experimenter stepped back and the animals had an acclimatation period of 60 seconds to initiate first visual and olfactory contact through the mesh. Afterward, the partition was removed and the animals could freely interact for 30 minutes. During this time, the experimenter left the room. The entire session was recorded and videos were analysed with the program Interact (Interact, Lab Suite Version 2022, Program version 20.8.3.0, Mangold International GmbH, Arnstorf, Germany). The videos were blinded and randomized, ensuring ID and treatment of the respective individual were unknown to the observer. The analysis started after the acclimation period was over and the mesh removed. Latency until first contact with female and behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing, time spent near female), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt) were analysed (Tab. S2). Due to technical issues with the camera, one MFIT video was missing in the PM-S group; thereby reducing the sample size to n = 9 in that group.

2.5.2.3 Social initiative test (SIT)

The SIT was conducted to investigate general motivation of the focus males to initiate social contact [58]. For this purpose, an unfamiliar male infant (5 to 15 days of age) was used as interaction partner to minimize the likelihood that the behaviour of the focus males was driven by either sexual or agonistic motivation [58]. Within each matched pair, the same male infant was used. Prior to the test, the infant was placed into the test enclosure under a small grated metal basket (25 x 21 x 15 cm) turned upside down to prevent approaches initiated by the infant. As control, an identical but empty basket was also placed into the enclosure. Both baskets were positioned in the centre of the enclosure, approximately 20 cm apart and about 20 cm from the left or right wall, respectively. The focus male was then placed into the enclosure facing away from the experimenter and towards the baskets. The experimenter left the room and the focus male was allowed to make contact to the infant for 15 minutes (basket phase). After this time, the experimenter quietly re-entered the room, removed both baskets and the animals were allowed to freely interact for an additional 15 minutes (no basket phase), during which the experimenter again left room. The entire session was recorded and videos were analysed with the program Interact (Interact, Lab Suite Version 2022, Program version 20.8.3.0, Mangold International GmbH, Arnstorf, Germany). The videos were blinded and randomized, ensuring ID and treatment of the respective individual were unknown to the observer. The analysis was divided into basket phase and no basket phase. During the no basket phase, the following parameters were analysed: frequency of empty basket sniffing, frequency of infant basket sniffing, time spent near empty basket and time spent near infant basket. For the no basket phase, latency until first contact with infant and behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing, time spent near infant), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt) were analysed (Tab. S3). Due to technical issues with the camera, one SIT video was missing in the PM+S group; thereby reducing the sample size to n = 9 in that group.

2.6 Statistics

Data analysis was carried out with RStudio version 2022.07.0 [59]. A priori sample-size calculation was conducted using the software G*Power version 3.1.9.7 [60]. The calculations were based on baseline and response cortisol values. Previous studies showed that effects of the social environment on cortisol concentrations are large, with estimated effect size of f = 0.69 [53,61]. To detect effects with f = 0.69 with an α error probability of 0.05 and a power of 80% a total sample size of at least 19 animals would be needed. Thus, we decided to use a total sample size of n = 20 animals with n = 10 animals per treatment group.

Descriptive statistics, model summaries and detailed test statistics for all analyses can be found in the supplementary material.

2.6.1 Hormone concentrations

Linear mixed-effect models were used to analyse the influence of the social condition on hormone concentrations using the *lme4* [62] and *lmerTest* package [63]. In total, four models were fit with 1) baseline cortisol, 2) baseline testosterone, 3) increase in cortisol responsiveness after 1 hour and 4) increase in cortisol responsiveness after 2 hours as a respective response variable. Increase in cortisol responsiveness after 1 hour and after 2 hours was calculated by subtracting baseline cortisol values from the absolute values of cortisol responsiveness after 1 hour and after 2 hours. This was done to eliminate confounding effects caused by different cortisol baseline values. To investigate changes in hormone concentrations over time, we added the interaction between social condition (additional social stimulation versus no additional stimulation) and the variable CRT, representing the first, second and third CRT conducted after treatment, as a fixed effect. As the focus of this study was to investigate the effects of additional social stimulation on hormone concentrations over time, data from CRT0 was excluded from the models as it was conducted before the social stimulation treatment started. However, hormone concentrations at CRT0 were still compared between the treatment groups using Wilcoxon rank-sum test to confirm there were indeed no differences between the groups prior to treatment. Furthermore, the continuous variable body weight was first mean-centered and then included as a fixed effect because earlier studies in guinea pigs have shown that body weight can influence hormone concentrations [25,48]. Last, we fitted ID as a random effect. We used the *performance* [64] and *DHARMa* package [65] to check model assumptions. Marginal and conditional R^2 values were calculated using the *performance* package [64], while partial R^2 values for individual predictors were calculated using the *sensemakr* package [66]. Pair-wise comparisons for treatment, CRT and treatment*CRT interaction were done by applying Tukey's adjustment for multiple comparison using the *emmeans* package [67].

Adjusted repeatability estimates of hormone concentrations were calculated for each of the treatment groups using the *rptR* package [68]. 95% confidence intervals were determined by parametric bootstrapping (N = 1000), and likelihood ratio tests were used for significance testing. The models used to estimate adjusted repeatability were the same as mentioned before, with the only exception that treatment was removed as fixed effect.

2.6.2 Behaviour

2.6.2.1 Home enclosure behaviour

For the analysis of the home enclosure behaviour, count data of behaviours from the coded videos were first aggregated on a weekly level by summing the counts from the two recordings per week for each individual. To account for observation time, these values were subsequently transformed into

406 frequencies (occurrence per hour). Behaviour was pooled into the categories sociopositive behaviour
407 (naso-nasal sniffing, naso-anal sniffing) and courtship behaviour (ano-genital licking, rumba) with
408 individual behaviours being summed within each category. Behaviour from the category sexual
409 behaviour (mating and mating attempt) was only observed in 4 out of 240 videos in total and therefore
410 excluded from statistical analysis. For both sociopositive and courtship behaviour, zero-inflation of data
411 was assessed using the *performance* package [64].

412 For sociopositive behaviour, no significant zero-inflation was detected. Therefore, a generalized linear
413 mixed-effect model with negative binomial distribution was fit.

414 Courtship behaviour showed a significant zero-inflation. Thus, a hurdle approach (two-part modelling
415 approach) [69–71] was used. In a first step, courtship frequencies were converted into binary variables
416 indicating whether a behaviour occurred during an observation period or not (0 = did not occur; 1 = did
417 occur). This data was then analysed using generalized linear mixed-effects model with binomial
418 distribution. In a second step, the analysis was restricted to observations in which the respective
419 behaviour occurred, meaning zero observations were excluded. For the remaining observations, the
420 original behavioural frequencies were retained, log-transformed to improve model fit and analysed
421 using linear mixed-effects models.

422 All models for sociopositive and courtship behaviour were fitted using the *lme4* [62] and *lmerTest*
423 package [63]. Again, interaction between treatment and time was used as fixed effect in the models to
424 investigate the influence of treatment over time. Time was categorized into "Phase 1" (1st and 2nd week
425 of video recordings), "Phase 2" (3rd and 4th week of video recordings),) and "Phase 3" (5th and 6th week
426 of video recordings). ID was again fitted as a random effect. Model assumptions as well as the estimation
427 of the different R^2 values were conducted in the same manner as for the analysis of hormone
428 concentrations. Pair-wise comparisons for treatment, phase and treatment*phase interaction were
429 done by applying Tukey's adjustment for multiple comparison using the *emmeans* package [67].

430 ### 2.6.2.2 Behavioural tests

431 Prior to statistical analyses, normality of the data was assessed used Shapiro-Wilk tests. Depending on
432 the distribution of the data, either parametric (Welch's t-test) or non-parametric tests (Wilcoxon rank-
433 sum tests) were used for pair-wise comparisons.

434 Data of the step-down test (SDT) was analysed in two steps. First, the proportion of animals stepping
435 down from the platform within the maximum test duration was compared between the two social
436 conditions. For this purpose, a binary variable was created indicating whether an individual stepped
437 down during the test (latency < 900 s) or not (latency = 900 s). Differences between social conditions
438 were analysed using Fisher's exact test. In a second step, the latency to step down from the platform

was analysed only for individuals that stepped down during the test (latency < 900 s). Differences in latency between the two social conditions were assessed using a Wilcoxon rank-sum test.

Data from the male-female interaction test (MFIT) was analysed by comparing multiple behavioural measures between the two social conditions. These variables included latency to first contact with the female, time spent near the female as well as behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt). Within each category, individual behaviours were summed prior to analysis. Each variable was analysed using either Welch's t-tests (time spent near female, sociopositive behaviour, courtship behaviour) or Wilcoxon rank-sum tests (latency to first contact with female, sexual behaviour). To account for multiple testing, the Benjamini-Hochberg correction was applied for controlling the false discovery rate. Raw p-values are reported, while statistical significance was determined based on the corrected significance levels.

Data from the social initiative test (SIT) was analysed separately for the basket phase and the no basket phase. For the basket phase, the influence of social condition and basket type (empty vs. infant) on sniffing frequency and duration of time spent near the baskets was analysed. Sniffing frequency was analysed using a generalized linear mixed-effects models with Poisson distribution, whereas the duration of time spent near the baskets was analysed using a linear mixed-effects model. As duration data was not normally distributed, it was log-transformed prior to analysis to improve model fit. Models were fitted using the *lme4* [62] and *lmerTest* package [63]. Treatment, basket type and their interaction were included as fixed effects, while ID was fitted as a random effect. We used the *performance* [64] and *DHARMa* package [65] to check model assumptions. Marginal and conditional R^2 values were calculated using the *performance* package [64], while partial R^2 values for individual predictors were calculated using the *sensemakr* package [66]. Pair-wise comparisons for treatment, basket type and treatment*basket type interaction were done by applying Tukey's adjustment for multiple comparison using the *emmeans* package [67]. For the no basket phase, the analysed variables included latency to first contact with the infant, time spent near the infant as well as behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing, time spent near infant) and courtship behaviour (ano-genital licking, rumba). Sexual behaviour (mating and mating attempt) was excluded from statistical analysis as it occurred only in 5 out of 19 individuals and at very low frequencies. Within each behavioural category, individual behaviours were summed prior to analysis. Each variable was analysed using either Welch's t-tests (time spent near infant, sociopositive behaviour, courtship behaviour) or Wilcoxon rank-sum tests (latency to first contact with infant). To account for multiple testing, the Benjamini-Hochberg correction was applied for controlling the false discovery rate. Raw p-values are reported, while statistical significance was determined based on the corrected significance levels.

3. Results

3.1 Effects of social environment on hormone concentrations

The comparison of hormone concentrations (c0, c1, c2, t) at CRT0 using Wilcoxon rank-sum tests revealed no significant differences between social conditions prior to treatment (see supplementary material, **Tab. S9**).

Regarding baseline cortisol levels, a significant treatment-by-time interaction effect was found between CRT1 and CRT3 (ß = 110.79 ± 40.31, t = 2.75, p = 0.012), where c0 values slightly decreased for the PM+S group and increased for the PM-S group (**Fig. 2a**). For baseline testosterone levels (**Fig. 2b**), neither a significant effect of treatment or time (CRT), nor a significant treatment-by-time interaction effect was found (see supplementary material, **Tab. S17**).

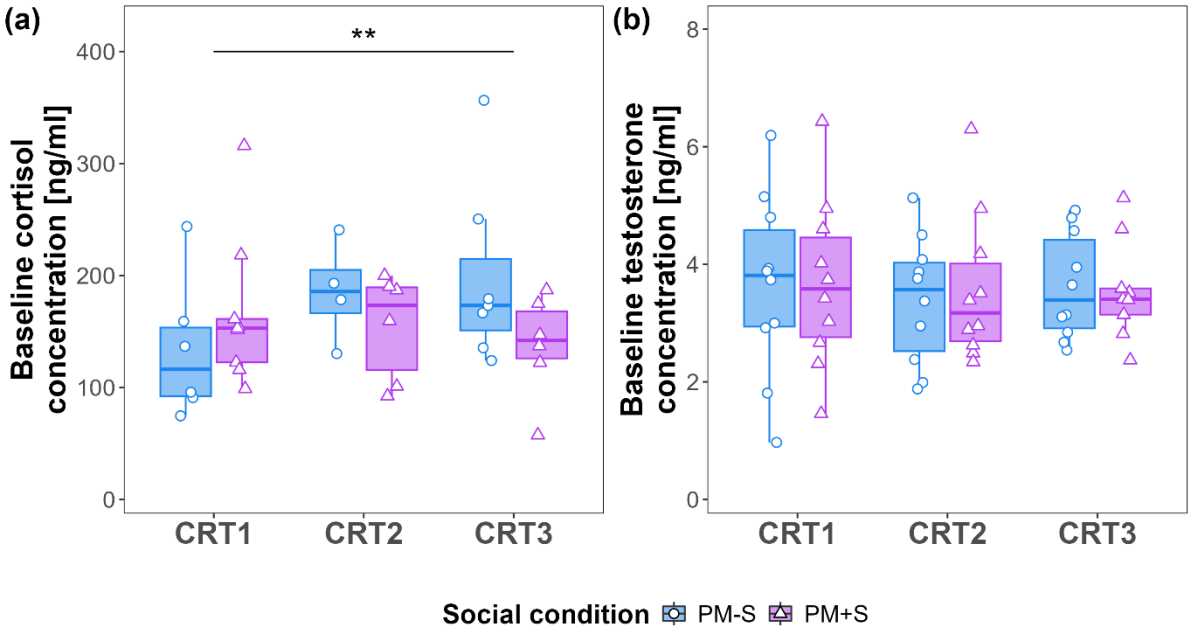

Figure 2: Baseline cortisol **(a)** and testosterone **(b)** concentrations (ng ml^{-1}) two weeks (CRT1), four weeks (CRT2) and six weeks (CRT3) after treatment start. Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points. Significance line indicates significant treatment-by-time interaction effect. **p < 0.01.

Regarding increase in cortisol responsiveness after 1 hour (c1) and 2 hours (c2) of exposure to a novel environment, neither a significant effect of treatment or time (CRT), nor a significant treatment-by-time interaction effect was found (see supplementary material, **Tab. S15+S16**) (**Fig. 3**).

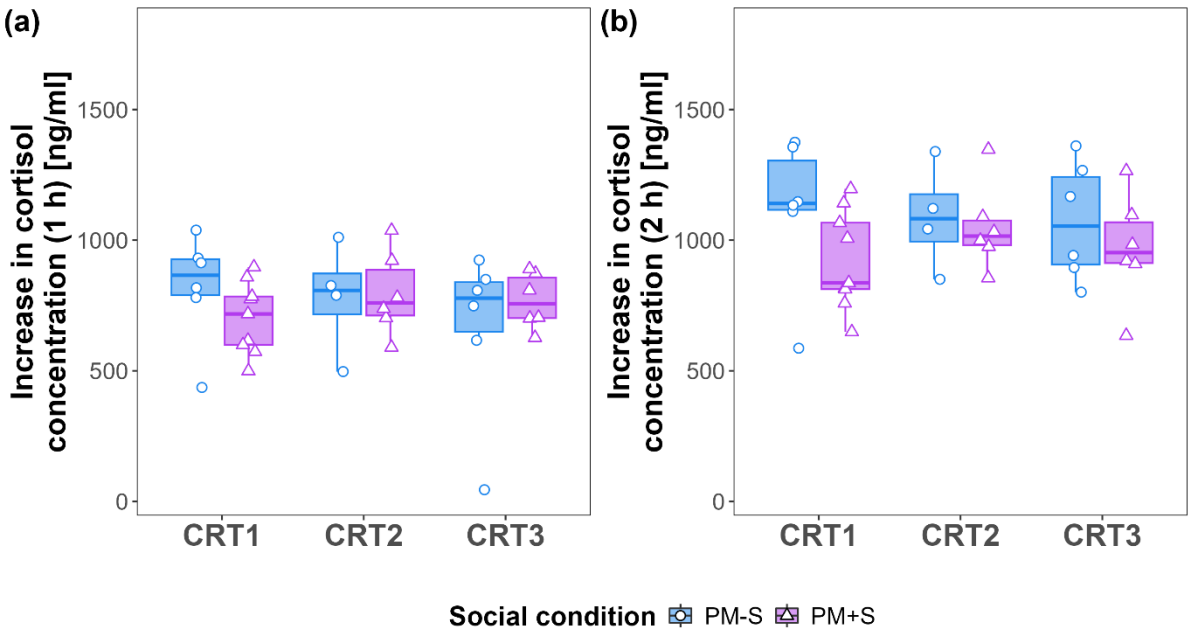

Figure 3: Increase in cortisol concentrations (ng ml^{-1}) at one hour **(a)** and two hours **(b)** of exposure to a novel environment two weeks (CRT1), four weeks (CRT2) and six weeks (CRT3) after treatment start. Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points.

Adjusted repeatability was analysed for hormone concentrations (baseline cortisol, baseline testosterone, cortisol responsiveness after 1 and 2 hours) in both social conditions (**Fig. 4**). Baseline cortisol (c0) was not significantly repeatable under any social condition (PM+S group: R = 0.076, CI = [0, 0.685], p = 0.392; PM-S group: R = 0.124, CI = [0, 0.833], p = 0.401). Similarly, baseline testosterone was not repeatable in either of the two social conditions (PM+S group: R = 0.244, CI = [0, 0.669], p = 0.153; PM-S group: R = 0.346, CI = [0, 0.732], p = 0.076). In contrast, repeatability of increase in cortisol responsiveness after 1 hour (c1) differed between the two social conditions: while it showed high and significant repeatability in the PM+S group (R = 0.721, CI = [0.233, 0.945], $p < 0.001$), it showed very low and non-significant repeatability in the PM-S group (R = 0.121, CI = [0, 0.877], p = 0.432). For increase in cortisol responsiveness after 2 hours (c2) we found high and significant repeatability in the PM-S group (R = 0.782, CI = [0.321, 0.980], p = 0.007). In contrast, repeatability in the PM+S group was moderate but not statistically significant (R = 0.418, CI = [0, 0.864], p = 0.056).

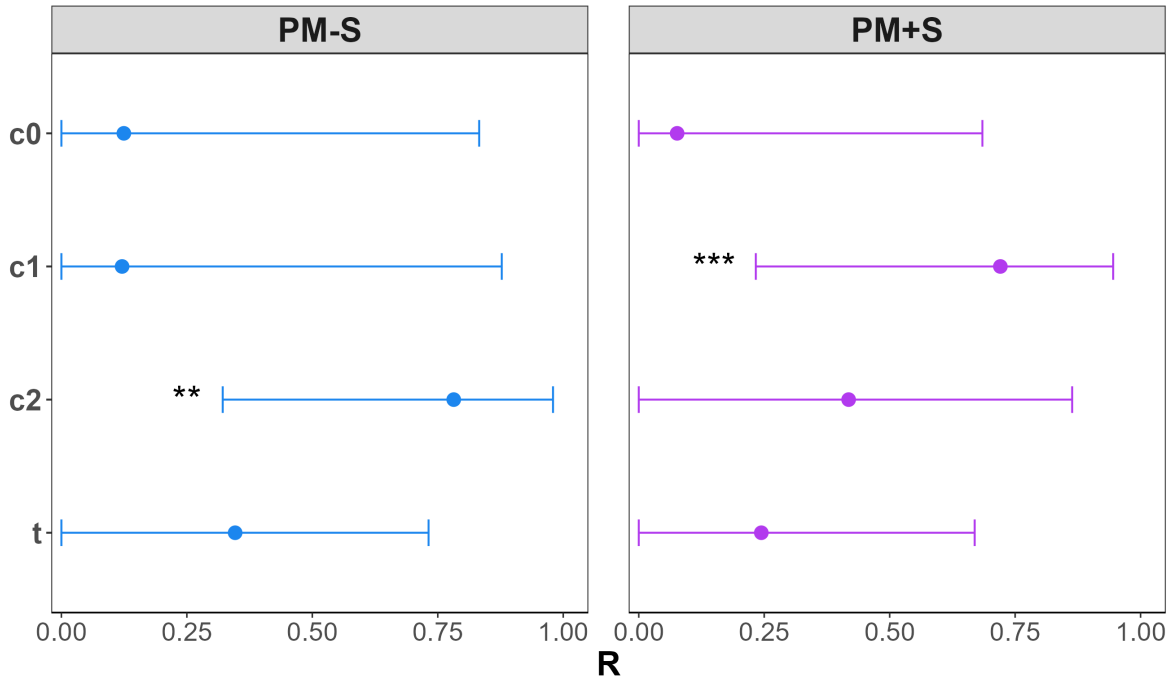

Figure 4: Repeatability (R) of baseline cortisol (c0), increase in cortisol responsiveness after 1 (c1) and 2 hours (c2) of exposure to a novel environment and baseline testosterone (t). Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are adjusted repeatability (data points) and confidence intervals (whisker). **p < 0.01, *** p < 0.001.

3.2 Effects of social environment on behaviour

For sociopositive behaviour, pairwise comparisons revealed a significant effect of treatment during phase 3, with higher frequencies in the PM+S group compared to the PM-S group ($\beta = 0.46 \pm 0.16$, $z = -2.26$, $p = 0.024$) (**Fig. 5a**). Courtship behaviour was analysed using a two-step hurdle approach. In a first step, we analysed whether courtship behaviour occurred at all (**Fig. 5b**). Neither a significant effect of treatment or time (phase), nor a significant treatment-by-time interaction effect was found (see supplementary material, **Tab. S23**). In a second step, we analysed the frequency of behaviours only in those data in which courtship behaviour occurred, meaning zero-entries were excluded (**Fig. 5c**). However, also here neither a significant effect of treatment or time (phase), nor a significant treatment-by-time interaction effect was found (see supplementary material, **Tab. S24**).

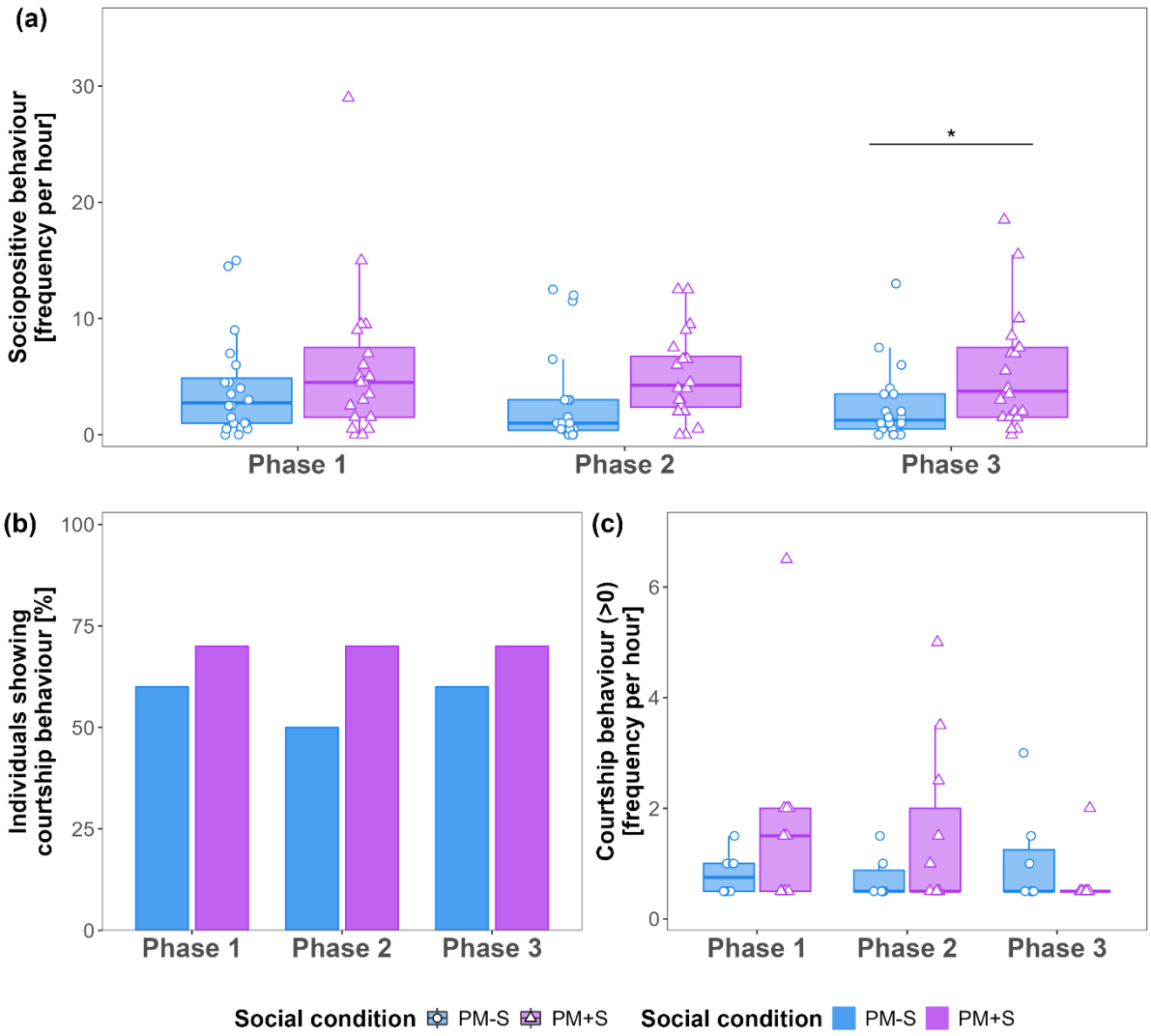

Figure 5: Home enclosure behaviour. **(a)** Frequency (per h) of sociopositive behaviour, **(b)** proportion of individuals showing courtship behaviour, and **(c)** frequency (per h) of courtship behaviour (>0; only if it was shown) in "Phase 1" (1st and 2nd week of video recordings), "Phase 2" (3rd and 4th week of video recordings), and "Phase 3" (5th and 6th week of video recordings) in the home enclosure. Males were either additionally socially stimulated (PM+S) or not (PM-S). **(a) (c)** Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points. **(b)** Bars represent the proportion of individuals per group. *$p < 0.05$

Data from the step-down test (SDT) was analysed in a similar two-step approach. First, we analysed whether individuals stepped down from the platform within the maximum test duration or not. No significant difference between the two social conditions was found in the proportion of individuals stepping down (see supplementary material, **Tab. S25** and **Fig. S1a**). In a second step, we analysed the latency to step down from the platform for those individuals that stepped down during the test. Also here, no significant difference between the two social conditions was detected (see supplementary material, **Tab. S25** and **Fig. S1b**).

In the male-female interaction test (MFIT), males from the two social conditions did not differ in latency to approach the female or in the time spent near the female (see supplementary material, **Tab. S26** and **Fig. S2a** + **S2b**). Also, for sexual behaviour, no significant difference between social conditions was found

(see supplementary material, **Tab. S26** and Fig. **S2C**). Males from the PM+S group exhibited sociopositive and courtship behaviour more often than males from the PM-S group (sociopositive behaviour: t = −2.43, d = 1.09, p = 0.029; courtship behaviour: t = −2.36, d = 1.06, p = 0.032) (**Fig. 6**), but these differences were not statistically significant after correction for multiple comparisons.

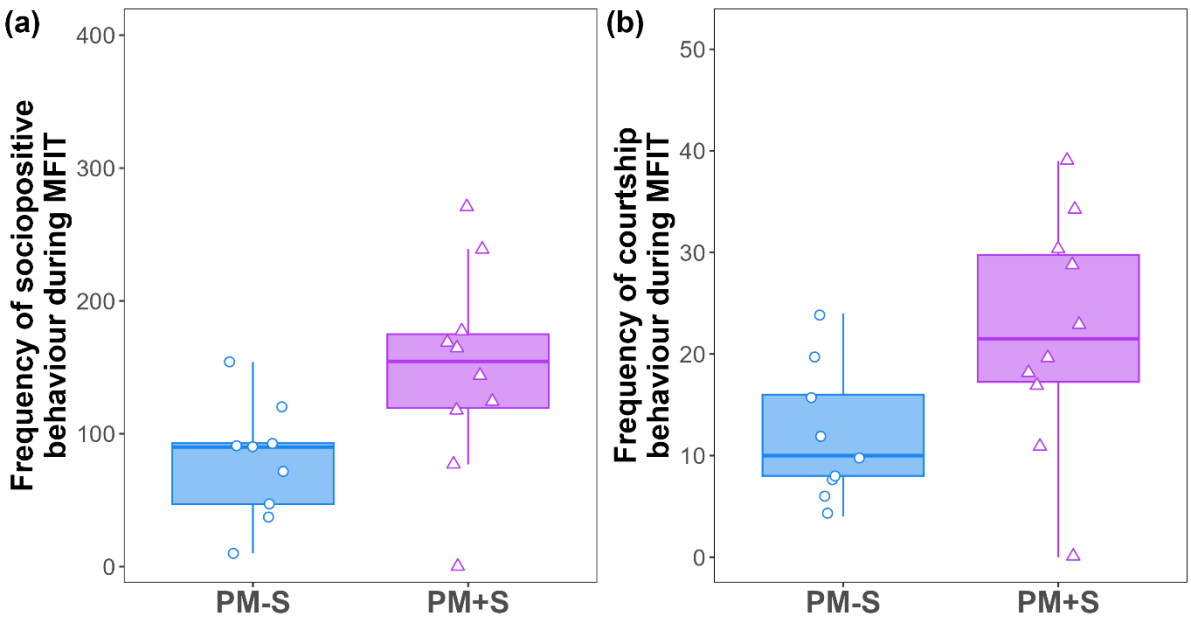

Figure 6: Male-female interaction test (MFIT). **(a)** frequency of sociopositive behaviour and **(b)** frequency of courtship behaviour towards female. Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points.

Data from the social initiative test (SIT) was analysed separately for the basket phase and the no basket phase. During the basket phase, no significant differences between the two social conditions were found for sniffing behaviour or time spent near the baskets (see supplementary material, **Tab. S30+S31**). However, a significant effect of basket type was detected for both variables, with higher sniffing frequencies and more time spent near the infant basket compared to the empty basket in both social conditions (sniffing: PM-S: ß = −22.20 ± 3.77, t = −5.90, p < 0.001; PM+S: ß = −20.44 ± 3.97, t = −5.15, p < 0.001; time spent near baskets: PM-S: ß = −1.32 ± 0.26, t = −5.16, p < 0.001; PM+S: ß = −1.47 ± 0.27, t = −5.46, p < 0.001) (see supplementary material, **Fig. S3**). No significant treatment-by-basket interaction effect was found for either variable (see supplementary material, **Tab. S30+S31**). During the no basket phase, males from the two social conditions did not differ in latency to approach the infant or time spent near the infant (see *supplement*ary material, **Tab. S27** and **Fig. S4**). Males from the PM+S group exhibited more sociopositive behaviour than males from the PM-S group (t = −2.81, d = 1.33, p = 0.016) (**Fig. 7a**), but this difference was not statistically significant after correction for multiple comparisons. Regarding courtship behaviour, no significant difference between treatment groups was found (**Fig. 7b**).

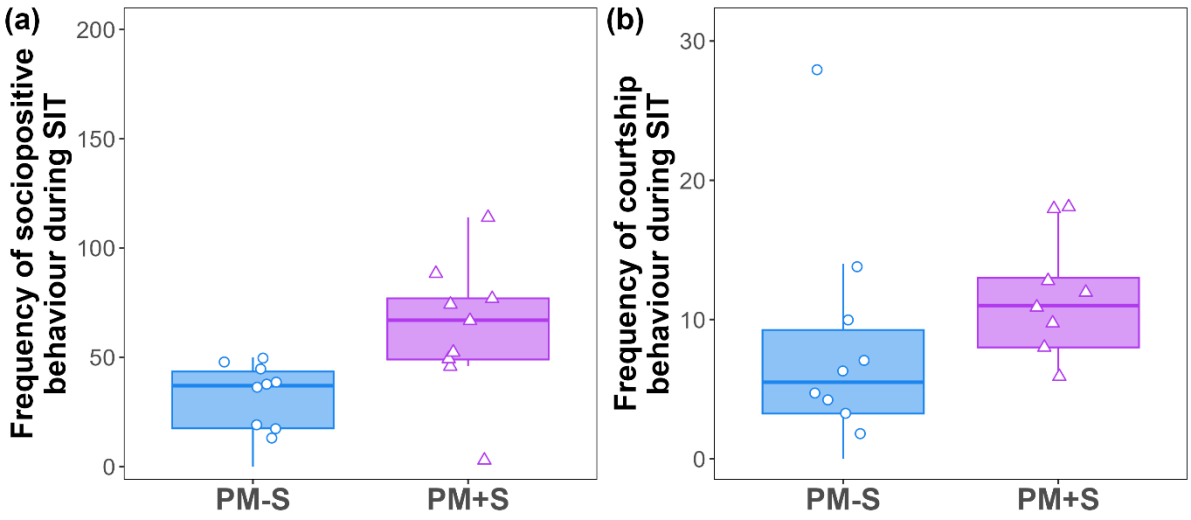

Figure 7: Social initiative test (SIT), no basket phase. **(a)** Frequency of sociopositive behaviour and **(b)** frequency of courtship behaviour towards infant. Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points

4. Discussion

In this study, we examined potential effects of different social environments on the endocrine and behavioural phenotype in adolescent male guinea pigs. By repeatedly analysing hormonal and behavioural parameters during adolescence, we aimed to explore how the social environment might shape these phenotypes within adolescence. For this purpose, male guinea pigs kept under pair-housing conditions with one female only (PM-S) were compared with males that were also housed with one female, but received additional social stimulation through interactions with unfamiliar males and females (PM+S).

Regarding endocrine parameters, we found a significant treatment-by-time interaction effect for baseline cortisol levels (c0), with c0 values slightly decreasing over time in the PM+S group and increasing in the PM-S group. In contrast, no significant differences were found for baseline testosterone concentrations or increase in cortisol responsiveness after 1 h (c1) or 2 h (c2). However, repeatability of increase in cortisol responsiveness differed between groups; with c1 being repeatable in PM+S males and c2 in PM-S males. Regarding behaviour, socially stimulated males displayed higher levels of sociopositive behaviour toward their female housing partner as well as towards unfamiliar conspecifics in the behavioural tests. No differences between social conditions were found in risk-taking behaviour.

4.1. Effects of social environment on hormone concentrations

Baseline cortisol and testosterone

Baseline cortisol and its change over time differed between social conditions. Males with additional social stimulation (PM+S) displayed initially elevated baseline cortisol concentrations compared to PM-S males in the cortisol response test conducted two weeks after the start of social stimulation. Stimulated males were confronted with unpredictable interactions with unfamiliar conspecifics which might have constituted a more challenging or demanding environment than pair-housing only. Similarly, other rodent studies have shown that short-term (one to two weeks) exposure to chronic mild or unpredictable stress increased basal serum corticosterone levels [72–75]. Although glucocorticoids such as cortisol are elevated in response to stressors in vertebrates, they should not be equated directly with stress, as their primary function is energy mobilization [28,76]. In a study by Zimmermann and colleagues, male guinea pigs reared in heterosexual pair-housing conditions were transferred to mixed-sex colonies in late adolescence and displayed substantial elevations in baseline cortisol levels during the first few days [2]. It was argued that this pronounced HPA activity is involved in mobilizing the required energy for courtship, sexual and aggressive interactions since these are associated with high energetic costs [2,77]. Yet, prolonged elevation of glucocorticoids can also have detrimental effects, such as suppressing immune function, which can impact health [15,30]. This could explain why the initially elevated baseline cortisol levels in PM+S males in this study slightly decreased over time again. It is also plausible that males might have learned that social stimulations were not inherently harmful, as only ten percent of social stimulation sessions had to be terminated due to escalated aggression. In males without additional social stimulation, however, baseline cortisol levels increased over time, so that PM-S showed significantly higher levels than PM+S males in the cortisol response test conducted in the last experimental week. As PM-S males were housed with a female partner and did not experience additional social challenges throughout the experimental phase, the observed increase in cortisol concentrations is unlikely to reflect increasing stress levels over time. In a study by Lürzel and colleagues, male guinea pigs also showed an increase in baseline cortisol concentrations from early to late adolescence which was interpreted as developmental effect [41]. Similarly, studies in mice have shown that periadolescent males exhibit higher basal corticosterone levels compared to adults, again suggesting an age-related elevation glucocorticoid levels during adolescence [78,79]. This pattern may be linked to adolescence being a developmental phase characterized by complex alterations in brain structures and endocrine systems which also affect the regulation of the HPA axis [2,12,80,81]. Still, it remains unclear, why such an age-related increase was not observed in PM+S males. In the context of the additional social stimulation experienced by these males, developmental processes and influences

of the social environment may have acted simultaneously, contributing to the slight decrease in baseline cortisol observed in PM+S males rather than the increase seen in PM-S males.

Regarding baseline testosterone, no differences between treatment groups were found. At first glance, this result may appear surprising, as the "challenge" hypothesis proposes that increased social interactions with conspecifics- especially agonistic interaction with other males as well as courtship and sexual interaction with females- can elevate testosterone levels during adolescence in male vertebrates [33,82,83]. Accordingly, one might expect socially stimulated males, which were regularly exposed to such interactions, to exhibit higher testosterone levels than pair-housed males without additional social stimulation. However, previous studies in guinea pigs have shown that increases in basal testosterone primarily occur under colony-housing conditions, where individuals are constantly exposed to social challenges such as male-male competition in a reproductive context and the presence of potential mating partners [19]. In contrast, social stimulation during adolescence appears to induce only an acute increase in circulating testosterone levels without affecting basal gonadal activity [19,41]. Thus, a similar pattern may have occurred in the present study, although testosterone levels were not measured directly after social stimulation. Testosterone can also have inhibiting effects on HPA function. In rats, for example, high circulating levels of testosterone have been shown to reduce basal corticosterone as well as corticosterone responsiveness [34,84]. In the study by Lürzel and colleagues, socially stimulated males displayed decreased cortisol responsiveness in late adolescence [19,41]. The authors suggested that this might be due to the acute surges in testosterone following social stimulation, which could have had organizational effects on HPA responsiveness [19,41]. Similarly, potentially transient increases in testosterone following social stimulation in the present study may have influenced HPA activity and thereby contributed to the lower baseline cortisol concentrations observed at the end of the experimental phase.

Cortisol responsiveness

It still remains unclear, why no differences in increase in cortisol 1 h (c1) or 2 h (c2) were found, since a majority of guinea pig studies reported that different social experiences plastically shape cortisol responsiveness during adolescence [3,10,19,41]. However, adolescence itself represents a highly dynamic and plastic developmental phase [3,10,11], and previous studies in rodents have shown that the impact of (social) challenges on HPA (re)activity is not uniform across adolescence but depends on the developmental stage at which these challenges occur [36–40]. Therefore, the absence of differences in cortisol responsiveness in the present study may reflect stage-specific sensitivity of the HPA axis. In the study by Lürzel and colleagues, for example, social stimulation started later in adolescence (PND 80 [41] or PND 86 [19]), whereas in the present study males were exposed to social stimulation at a considerably earlier stage (around PND 57). Although the repeated endocrine measurements in this

study allowed us to assess changes within the experimental period, this period did not cover adolescence in its entirety and may therefore not have included all sensitive windows for the modulation of HPA reactivity. In addition, rearing conditions differed substantially between studies. Recent findings in juvenile guinea pigs have shown that different social environments can already shape hormonal and behavioural phenotypes during earlier developmental stages such as juvenility [43]. While males in Lürzel and colleagues' study were born into large mixed-sex groups and subsequently housed with a female from PND 30 onward [19,41] , males in the present study were born in smaller harem groups and were pair-housed with another male from weaning (PND 21) until being housed with a female partner at around PND 53.

4.2. Effects of social environment on the repeatability of hormone concentrations

While no treatment differences in c1 and c2 were detected, differences in repeatability of these values were found between social conditions. Repeatability of hormone concentrations was investigated to assess the stability of endocrine trait expression within individuals and to examine whether social environment affects this stability, as suggested by previous studies [48,85–87]. Cortisol responsiveness after 1 h (c1) was highly and significantly repeatable in PM+S males but not in PM-S males, whereas cortisol responsiveness after 2 h (c2) was highly and significantly repeatable in PM-S males but not in PM+S males. In guinea pigs, cortisol responsiveness after 1 hour reflects the speed of the stress response, whereas cortisol responsiveness after 2 hours indicates its magnitude [25,88]. One possible interpretation is that repeated exposure to short-term social challenges (social stimulation) in PM+S males might have stabilized individual differences in the initial phase of the stress response (c1, i.e., speed). This could be advantageous in an environment characterized by frequent social challenges, where a consistent and predictable initial stress response could facilitate efficient coping [89]. At the same time, the absence of repeatability in the later phase of the stress response (c2, i.e., magnitude) may indicate that the magnitude of the stress response remains flexible in these males, allowing individuals to adjust their stress responsiveness according to the severity of a given environmental challenge [89]. In contrast, in PM-S males, which were not exposed to such repeated social challenge, stable individual differences may have been more pronounced in the later phase of the response (c2, i.e., magnitude). In a more stable and less variable social environment, maintaining flexibility in the initial stress response while showing consistency in the overall magnitude of the stress response may be advantageous, as it could reduce unnecessary energetic costs [89]. Although previous studies have examined different aspects of the stress response, such as its speed and magnitude (e.g., [88]), studies specifically addressing the repeatability of these components are currently lacking, and interpretations therefore remain speculative and do not yet allow conclusions about their potential adaptive significance.

A broader framework to interpret such patterns is provided by the concept of individualized social niches, which has gained prominence in behavioural ecology [1,90,91]. These describe the unit that is shaped by social interactions of a focal individual with conspecifics [1,48]. Occupying different social niches within a social group is assumed to avoid social conflict and reduce unpredictability in social encounters [48,92]. A repeatability study conducted by Mutwill and colleagues, for example, found that in adolescent and adult male guinea pigs, baseline testosterone was repeatable in males housed in mixed-sex colonies but not in males housed in mixed-sex pairs [48]. This finding was attributed to the occupation of individualized social niches by males under more complex colony-housing conditions [48]. In this context, the distinct repeatability patterns of cortisol responsiveness observed in the present study may indicate that different social environments promote stability in different components of the stress response and could therefore reflect social niche specification. Specifically, frequent short-term social interactions in PM+S males may favour stability in the initial response, whereas the more stable conditions in PM-S males may favour stability in the overall magnitude of the stress response. Nevertheless, our findings align with a general pattern reported in a meta-analysis, showing that repeatability estimates tend to be higher for peak hormone levels than for baseline levels [93]. The reason for this might be elevated hormone responses (e.g., through stress) capturing a more defined aspect of endocrine function, while baseline hormone levels can represent multiple different biological functions [85,94].

4.3. Effects of social environment on (social) behaviour

Risk-taking behaviour was assessed in the step-down test and no differences between social conditions were detected. This contrasts with findings in other studies, where the social environment has been shown to influence behavioural domains such as risk-taking, for example in mice and rats [46,47]. One possible explanation is that risk-taking behaviour may be less sensitive to variation in the social environment due to domestication-related factors, as previous studies have reported higher levels of risk-taking behaviour in wild cavies compared to domesticated guinea pigs [58]. In their natural habitats, exploration is essential for wild cavies to access resources but inherently involves risk-taking due to high predation pressure [58,95], whereas in domesticated guinea pigs, the constant availability of resources likely reduced the selection pressure for high levels of risk-taking behaviour [58].

Regarding social behaviour, stimulated males exhibited significantly more sociopositive behaviour than non-stimulated males towards their female housing partner during the final phase of video recordings, i.e., in the last two weeks of the experiment. Additionally, stimulated males also showed more sociopositive behaviour than non-stimulated males in the male-female interaction test (MFIT) and the social initiative test (SIT). These behavioural tests were also conducted at the end of the experimental phase. However, these differences were no longer statistically significant after correction for multiple

689 testing. Nevertheless, the overall pattern suggests that males receiving prolonged additional social
690 stimulation tend to exhibit more sociopositive behaviour across different social contexts, including
691 interaction with their female partner, an unfamiliar female (MFIT) and an unfamiliar male infant (SIT).
692 Thus, social stimulation appears to promote a shift towards more sociopositive behaviour in adolescent
693 males.

694 This shift may be beneficial for several reasons. Socially stimulated males were regularly exposed to
695 unfamiliar conspecifics of both sexes, which may have simulated a higher-density social environment
696 compared to PM-S males, which interacted with a single female only. Under such socially more complex
697 conditions, a low-aggression phenotype associated with a queuing strategy, may be more adaptive for
698 adolescent males, as it allows them to avoid reproductive competition until they are large enough to
699 effectively challenge dominant males for mating access [2,3,10]. Importantly, subordinate males can
700 still achieve mating success by investing in social relationships with females, as female choice may favour
701 males displaying higher amounts of sociopositive and courtship behaviour towards them [96,97]. In line
702 with this, Machatschke and colleagues showed that male guinea pigs less involved in male-male conflicts
703 displayed significantly more socio-sexual behaviour towards females and had more success in female
704 choice than males exhibiting a lot of inter-male aggression [98]. Based on these findings, the increased
705 sociopositive behaviour observed in socially stimulated males in the present study may contribute to
706 the avoidance of male-male conflict and the establishment of social relationships with females.

707 Such an influence of social environment on behavioural phenotypes has also been discussed within the
708 framework of social niche conformance. Social niche conformance refers to the process by which
709 individuals adjust to an existing social environment through shaping of their hormonal and/ or
710 behavioural phenotype [1,90,91,99]. In this context, the higher levels of sociopositive behaviour
711 observed in socially stimulated males may reflect such a social niche conformance process, helping
712 individuals to adjust to a more complex social environment characterized by both increased potential
713 mating opportunities and mating competition.

714 Such social niche conformance processes underlying behavioural adjustments have already been
715 described in adolescent guinea pigs: males living in different social environments during adolescence
716 adopt distinct behavioural strategies, such as the resource defense or queuing strategy [1,2,10,15].
717 These behavioural adjustments have largely been explained by endocrine mechanisms. In particular,
718 increased numbers of social interactions in more complex social environments trigger increased
719 testosterone levels [3,19,33], which in turn have inhibiting effects on the HPA axis [34] and thereby
720 reduce HPA reactivity (cortisol responsiveness) promoting a low-aggression phenotype [3,10]. In
721 contrast, reduced social interactions under low-density conditions are associated with lower
722 testosterone levels and higher HPA reactivity, promoting a high-aggression phenotype [3,10]. However,

in the present study, neither baseline testosterone concentrations nor HPA reactivity differed between social conditions, suggesting that the observed increase in sociopositive behaviour is unlikely to be mediated by these endocrine mechanisms. While this contrasts with previous findings in guinea pigs, it cannot be fully explained yet and may depend on factors such as social context, developmental stage or the specific behaviours considered. More generally, behavioural adjustments to the social environment can also occur independently of endocrine changes. For example, in zebra finches, males adjusted their courtship and competitive behaviour to the social environment without corresponding changes in testosterone or corticosterone levels, suggesting that such adjustments of behavioural phenotype can occur independently of endocrine modulation [99]. An alternative explanation is that the observed behavioural adjustment in this study was mediated by social learning processes. Social learning is known to play a crucial role during behavioural development in male guinea pigs [97,100]. Males raised in socially complex environments acquire appropriate behavioural responses through interactions with conspecifics, whereas limited social experience can result in learning deficiencies during behavioural development [100]. Recent findings in juvenile guinea pig males further support the idea that social experiences can shape behavioural phenotypes through learning processes[43]. Together, these findings suggest that the increased sociopositive behaviour in socially stimulated males in the present study could also reflect social learning rather than solely endocrine-mediated mechanisms.

Conclusion

The present study demonstrated that distinct social environments during adolescence influence both hormonal and behavioural phenotypes in male guinea pigs. Males exposed to additional social stimulation displayed changes in baseline cortisol concentrations over time as well as increased sociopositive behaviour across different social contexts, which could possibly reflect social niche conformance processes to a more complex social environment. Notably, changes in baseline cortisol concentrations emerged already after a relatively short period of social stimulations, whereas differences in sociopositive behaviour became apparent only later during the experimental phase. This suggests that endocrine adjustments to the social environment may occur more rapidly, whereas behavioural adjustments may require prolonged social experience. In addition, repeatability analyses revealed social condition-specific patterns in different components of cortisol responsiveness, suggesting that adolescent social environments may not only influence endocrine phenotypes themselves but also the stability with which these phenotypes are expressed. Together, these findings emphasize adolescence as a dynamic developmental phase during which social experiences can shape behavioural and endocrine phenotypes.

Ethics

All procedures complied with the regulations covering animal experimentation within Germany (Animal Welfare Act) and the EU (European Communities Council Directive 2010/ 63/ EU), and were approved by the local and federal authorities (Landesamt für Verbraucherschutz und Ernährung Nordrhein-Westfalen "LAVE (ehemals LANUV) NRW", reference number: 81-02.04.2022.A080).

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CRediT authorship contribution statement

Melanie Gleske: Methodology; writing – original draft; investigation; formal analysis; visualization; data curation. S. Helene Richter: Conceptualization; writing – review and editing. Sylvia Kaiser: Conceptualization; methodology; supervision, writing – review and editing; funding acquisition. All authors critically revised the manuscript and gave final approval for publication.

Declaration of competing interests

The authors declare no conflict of interests.

Data availability

Open data/code are not available yet but will be accessible with peer-reviewed publication.

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Supplementary material

From social experience to social behaviour: hormonal and behavioural phenotypes during adolescence in male guinea pigs

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Supplementary material

Material and Methods: Ethogram

Table S1: Ethogram used for the observation of home enclosure behaviour. The abbreviation "FA" stands for "focus animal", e.g., the experimental male.

Category	Behaviour	Description	Measurement
Courtship behaviour	Ano-genital licking	The FA stretches its snout towards or touches the female's ano-genital region and lick or nuzzles the other female's region. The distance between the two animals is less than one snout-width.	Frequency
Courtship behaviour	Rumba	The FA approaches the female slowly and visibly shifts its weight from one hind leg to the other and back, it can also move forward while doing so. This is often accompanied by a low purring noise. Behaviour ends when the FA stops for more than 3s.	Frequency
Sexual behaviour	Mating and mating attempt	The FA moves the forepart of its body onto the back of the female from behind. Subsequently, the FA either proceeds with pelvic thrusting (defined as fast, rhythmic movements of the lower body) or is prevented from doing so by the female.	Frequency
Sociopositive behaviour	Naso-nasal sniffing	The FA stretches its nose towards the female's nose or snout. The distance between the two animals is less than one snout-width.	Frequency
Sociopositive behaviour	Naso-anal sniffing	The FA stretches its nose towards or touches the female's anal region with its nose. The distance between the two animals is less than one snout-width.	Frequency

Table S2: Ethogram used for the male-female interaction test (MFIT). The abbreviation "FA" stands for "focus animal", e.g., the experimental male.

Category	Behaviour	Description	Measurement
Courtship behaviour	Ano-genital licking	The FA stretches its snout towards or touches the female's ano-genital region and lick or nuzzles the other female's region. The distance between the two animals is less than one snout-width.	Frequency
Courtship behaviour	Rumba	The FA approaches the female slowly and visibly shifts its weight from one hind leg to the other and back, it can also move forward while doing so. This is often accompanied by a low purring noise. Behaviour ends when the FA stops for more than 3s.	Frequency
Sexual behaviour	Mating and mating attempt	The FA moves the forepart of its body onto the back of the female from behind. Subsequently, the FA either proceeds with pelvic thrusting (defined as fast, rhythmic movements of the lower body) or is prevented from doing so by the female.	Frequency
Sociopositive behaviour	First contact	The FA stretches its nose towards the female (at any body part) for the first time. The distance between the two animal is less than one snout-width.	Latency
Sociopositive behaviour	Naso-nasal sniffing	The FA stretches its nose towards the female's nose or snout. The distance between the two animals is less than one snout-width.	Frequency
Sociopositive behaviour	Naso-anal sniffing	The FA stretches its nose towards or touches the female's anal region with its nose. The distance between the two animals is less than one snout-width.	Frequency
Sociopositive behaviour	Being near female	The FAs head is within less than one head length of the female for at least 3s. Behaviour ends when not shown for at least 3s.	Duration

Table S3: Ethogram used for the social initiative test (SIT). The abbreviation "FA" stands for "focus animal", e.g., the experimental male.

Phase	Category	Behaviour	Description	Measurement
Basket phase		Sniffing empty basket	The FA stretches its nose towards the empty basket. The distance between the FA and basket is less than one snout-width.	Frequency
Basket phase		Sniffing infant basket	The FA stretches its nose towards the infant basket. The distance between the FA and basket is less than one snout-width.	Frequency
Basket phase		Being near empty basket	The FAs head is within less than one head length of the empty basket for at least 3 seconds. Behaviour ends when not shown for at least 3s.	Duration
Basket phase		Being near infant basket	The FAs head is within less than one head length of the infant basket for at least 3 seconds. Behaviour ends when not shown for at least 3s.	Duration
No basket phase	Courtship behaviour	Ano-genital licking	The FA stretches its snout towards or touches the infant's ano-genital region and lick or nuzzles the other infant's region. The distance between the two animals is less than one snout-width.	Frequency
No basket phase	Courtship behaviour	Rumba	The FA approaches the infant slowly and visibly shifts its weight from one hind leg to the other and back, it can also move forward while doing so. This is often accompanied by a low purring noise. Behaviour ends when the FA stops for more than 3s.	Frequency
No basket phase	Sexual behaviour	Mating and mating attempt	The FA moves the forepart of its body onto the back of the infant from behind. Subsequently, the FA either proceeds with pelvic thrusting (defined as fast, rhythmic movements of the lower body) or is prevented from doing so by the infant.	Frequency

No basket phase	Sociopositive behaviour	First contact	The FA stretches its nose towards the infant (any body part) for the first time. The distance between the two animal is less than one snout-width.	Latency
No basket phase	Sociopositive behaviour	Naso-nasal sniffing	The FA stretches its nose towards the infant's nose or snout. The distance between the two animals is less than one snout-width.	Frequency
No basket phase	Sociopositive behaviour	Naso-anal sniffing	The FA stretches its nose towards or touches the infant's anal region with its nose. The distance between the two animals is less than one snout-width.	Frequency
No basket phase	Sociopositive behaviour	Being near infant	The FAs head is within less than one head length of the infant for at least 3s. Behaviour ends when not shown for at least 3s.	Duration

Results: Descriptive statistics

Table S4: Descriptive statistics for baseline cortisol (c0), increase in cortisol responsiveness after 1 hour (c1) and 2 hours (c2) of exposure to a novel environment and baseline testosterone (t). All hormones were measured as concentration in blood plasma (ng/ml).

Social condition	Hormone	Time point	n	mean	median	SD	min	max
PM+S	c0	CRT0	7	150.10	108.43	107.51	92.03	389.11
		CRT1	9	165.72	152.92	65.82	98.88	315.89
		CRT2	6	155.01	173.31	47.25	92.28	199.98
		CRT3	6	137.67	142.06	46.03	57.50	187.16
	c1	CRT0	7	677.41	575.80	186.91	542.20	1030.21
		CRT1	9	702.34	717.74	137.14	499.31	897.08
		CRT2	6	795.08	759.45	160.51	589.78	1037.14
		CRT3	6	767.32	756.47	105.84	626.95	889.90
	c2	CRT0	7	1033.46	930.67	234.25	810.56	1467.88
		CRT1	9	920.50	836.74	188.36	648.80	1196.53
		CRT2	6	1049.78	1015.50	165.20	855.15	1347.36
		CRT3	6	968.48	952.22	210.60	634.91	1265.25
	t	CRT0	10	4.43	3.77	2.36	1.09	9.18
		CRT1	10	3.66	3.58	1.43	1.46	6.43
		CRT2	10	3.56	3.17	1.26	2.34	6.30
		CRT3	9	3.55	3.41	0.85	2.37	5.13
PM-S	c0	CRT0	9	163.35	149.57	83.86	44.26	320.62
		CRT1	6	133.48	116.27	62.46	74.69	243.73
		CRT2	4	185.59	185.69	45.50	130.25	240.73
		CRT3	7	197.90	173.20	80.83	124.02	356.45
	c1	CRT0	9	612.71	635.60	222.04	315.66	955.42
		CRT1	6	819.55	865.56	208.62	436.30	1038.31
		CRT2	4	780.84	807.73	212.61	497.01	1010.90
		CRT3	6	665.24	778.13	321.08	44.81	923.92
	c2	CRT0	9	932.78	991.03	333.41	342.41	1361.44
		CRT1	6	1118.21	1140.65	285.15	586.50	1374.79
		CRT2	4	1088.44	1082.20	202.36	850.14	1339.20
		CRT3	6	1072.11	1054.22	224.58	801.01	1360.82
	t	CRT0	9	4.97	4.65	2.50	1.35	8.69
		CRT1	10	3.64	3.81	1.55	0.97	6.19
		CRT2	10	3.39	3.57	1.08	1.88	5.13
		CRT3	10	3.62	3.40	0.90	2.54	4.92

Table S5: Descriptive statistics for behavioural parameters assessed in the home enclosure towards the female housing partner. All behaviours were measured as frequencies per hour.

Social condition	Behaviour	Time point	n	mean	median	SD	min	max
PM+S	Sociopositive	Phase 1	20	5.85	4.5	6.69	0	29.00
		Phase 2	20	5.10	4.25	3.75	0	12.50
		Phase 3	20	5.35	3.75	5.00	0	18.50
	Courtship	Phase 1	20	0.95	0.5	1.49	0	6.50
		Phase 2	20	0.80	0.25	1.36	0	5.00
		Phase 3	20	0.33	0.25	0.47	0	2.00
	Sexual	Phase 1	20	0.03	0	0.11	0	0.50
		Phase 2	20	0.03	0	0.11	0	0.5
		Phase 3	20	0.00	0	0.00	0	0.00
PM-S	Sociopositive	Phase 1	20	4.08	2.75	4.38	0	15.00
		Phase 2	20	2.93	1	4.22	0	12.50
		Phase 3	20	2.48	1.25	3.21	0	13.00
	Courtship	Phase 1	20	0.30	0	0.44	0	1.50
		Phase 2	20	0.23	0	0.41	0	1.50
		Phase 3	20	0.35	0.00	0.65	0	2.50
	Sexual	Phase 1	20	0.03	0	0.11	0	0.5
		Phase 2	20	0.00	0	0.00	0	0.00
		Phase 3	20	0.03	0	0.11	0	0.5

Table S6: Descriptive statistics for the step-down test.

Social condition	Variable	n	mean	median	SD	min	max
PM+S	Latency to step down	10	409.8	319	350.845	1	900
PM-S	Latency to step down	10	391.2	724.5	435.177	1	900

Table S7: Descriptive statistics for the male-female interaction test.

Social condition	Variable	n	mean	median	SD	min	max
PM+S	Latency first contact female	10	186.8	9.5	566.834	1	1800
	Time spent near female	10	949	1052.5	385.698	57	1414
	Sociopositive behaviour	10	148.4	154.5	77.04	0	271
	Courtship behaviour	10	22.1	21.5	11.532	0	39
	Sexual behaviour	10	11.5	1	25.33	0	79
PM-S	Latency first contact female	9	188.111	6	462.504	2	1406
	Time spent near female	9	874.778	861	331.512	245	1339
	Sociopositive behaviour	9	79.333	90	43.824	10	154
	Courtship behaviour	9	12	10	6.708	4	24
	Sexual behaviour	9	16.889	1	30.945	0	93

Table S8: Descriptive statistics for the social initiative test.

Social condition	Variable	n	mean	median	SD	min	max
PM+S	Sniffing empty basket	9	15.444	17	6.784	3	22
	Sniffing infant basket	9	35.889	39	12.82	15	53
	Time spent near empty basket	9	83.111	105	46.948	20	139
	Time spent near infant basket	9	321.333	267	156.179	121	644
	Latency first contact infant	9	31.444	20	39.627	1	132
	Time spent near infant	9	463	459	260.379	22	806
	Sociopositive behaviour	9	63.333	67	31.153	3	114
	Courtship behaviour	9	10.667	11	5.679	0	18
	Sexual behaviour	9	1	0	2	0	6
PM-S	Sniffing empty basket	10	17.9	16.5	8.293	3	28
	Sniffing infant basket	10	40.1	44.5	16.381	4	57
	Time spent near empty basket	10	89.7	63.5	78.014	3	248
	Time spent near infant basket	10	261.4	285	132.831	33	440
	Latency first contact infant	10	129.1	20.5	276.444	1	900
	Time spent near infant	10	543.7	620	255.198	0	815
	Sociopositive behaviour	10	30.5	37	17.018	0	50
	Courtship behaviour	10	7.9	5.5	8.13	0	28
	Sexual behaviour	10	0.6	0	1.265	0	3

Results: Wilcoxon test for treatment comparisons of hormone concentrations at CRT0

Table S9: Wilcoxon rank-sum test of hormone concentrations calculated for the first cortisol response test (CRT) conducted before treatment (PM+S; PM-S) (N = 16).

Wilcoxon rank-sum test (CRT0)	W	r	p-value
Baseline cortisol	42	0.237	0.290
Increase in cortisol responsiveness, 1h	26	0.118	0.597
Increase in cortisol responsiveness, 2h	31	0	1
Baseline testosterone	51	0.1	0.653

Results: Model summaries of linear mixed effect models for hormone concentrations

Table S10: Model summary from mixed effect model used to analyse baseline cortisol (N = 38). The model included the interaction between treatment (social condition) and time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Baseline cortisol	Estimate	Std. error	[95% CI]	t-value	p-value	R^2
					Full model: Marginal R^2	0.332
					Full model: Conditional R^2	0.393
Intercept	107.550	23.176	[60.249, 154.851]	4.640	< 0.001	
Fixed effects						
Treatment (social condition)	39.402	28.268	[-18.272, 97.075]	1.394	0.173	0.057
CRT1- CRT2 (time)	84.091	35.223	[11.747, 156.436]	2.387	0.024	0.160
CRT1- CRT3 (time)	123.500	33.840	[54.384, 192.616]	3.650	0.001	0.290
Body weight	-0.510	0.157	[-0.837,-0.184]	-3.242	0.004	0.269
Treatment*CRT1-CRT2	-69.042	43.185	[-158.289, 20.205]	-1.599	0.123	0.077
Treatment*CRT1-CRT3	-110.785	39.731	[-193.198,-28.371]	-2.788	0.011	0.182

Table S11: Model summary from mixed effect model used to analyse increase in cortisol responsiveness after 1 hour of exposure to a novel environment (N = 37). The model included the interaction between treatment (social condition) and time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Increase in Cortisol responsiveness, 1h	Estimate	Std. error	[95% CI]	t-value	p-value	R^2
					Full model: Marginal R^2	0.096
					Full model: Conditional R^2	0.329
Intercept	843.856	84.350	[671.226, 1016.485]	10.004	< 0.001	
Fixed effects						
Treatment (social condition)	-119.282	102.704	[-329.480, 90.916]	-1.161	0.255	0.048
CRT1- CRT2 (time)	-48.859	120.608	[-297.811, 200.093]	-0.405	0.689	0.015
CRT1- CRT3 (time)	-204.688	122.679	[-455.437, 46.061]	-1.668	0.106	0.099
Body weight	0.604	0.601	[-0.646, 1.854]	1.006	0.326	0.051
Treatment*CRT1-CRT2	107.959	145.557	[-195.027, 410.945]	0.742	0.467	0.025
Treatment*CRT1-CRT3	205.816	136.520	[-78.626, 490.257]	1.508	0.147	0.076

Table S12: Model summary from mixed effect model used to analyse increase in cortisol responsiveness after 2 hours of exposure to a novel environment (N = 37). The model included the interaction between treatment (social condition) and time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Increase in cortisol responsiveness, 2h	Estimate	Std. error	[95% CI]	t-value	p-value	R^2
					Full model: Marginal R^2	0.136
					Full model: Conditional R^2	0.642
Intercept	1138.070	92.394	[947.499, 1328.640]	12.318	< 0.001	
Fixed effects						
Treatment (social condition)	-195.930	113.459	[-430.793, 38.934]	-1.727	0.098	0.094
CRT1- CRT2 (time)	-42.610	109.464	[-269.986, 184.767]	-0.389	0.701	0.004
CRT1- CRT3 (time)	-82.035	122.256	[-331.803, 167.733]	-0.671	0.507	0.011
Body weight	0.588	0.699	[-0.862, 2.039]	0.841	0.409	0.010
Treatment*CRT1-CRT2	105.315	126.988	[-162.354, 372.985]	0.829	0.418	0.027
Treatment*CRT1-CRT3	56.782	118.903	[-193.958, 307.522]	0.478	0.639	0.012

Table S13: Model summary from mixed effect model used to analyse baseline testosterone (N = 59). The model included the interaction between treatment (social condition) and time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Baseline testosterone	Estimate	Std. error	[95% CI]	t-value	p-value	R^2
					Full model: Marginal R^2	0.076
					Full model: Conditional R^2	0.354
Intercept	3.831	0.389	[3.047, 4.616]	9.858	< 0.001	
Fixed effects						
Treatment (social condition)	-0.068	0.529	[-1.135, 0.999]	-0.129	0.898	< 0.001
CRT1- CRT2 (time)	-0.546	0.473	[-1.499, 0.408]	-1.154	0.255	0.019
CRT1- CRT3 (time)	-0.584	0.548	[-1.684, 0.516]	-1.065	0.292	0.019
Body weight	0.005	0.003	[-0.001, 0.010]	1.726	0.099	0.081
Treatment*CRT1-CRT2	0.073	0.624	[-1.194, 1.340]	0.117	0.907	< 0.001
Treatment*CRT1-CRT3	-0.039	0.633	[-1.324, 1.246]	-0.062	0.951	< 0.001

Results: Multiple comparisons of linear mixed effect models of hormone concentrations

Table S14: Multiple comparisons (Tukey's) of linear mixed effect model to determine effects of treatment (social condition), time (CRT) and treatment*time interaction on baseline cortisol. Significant ($p < 0.05$) results are indicated in bold.

Baseline cortisol	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
CRT1	-39.402	28.540	30.697	[-97.633, 18.830]	-1.381	0.177
CRT2	29.641	35.427	30.976	[-42.615, 101.896]	0.837	0.409
CRT3	71.383	30.279	30.911	[9.621, 133.146]	2.357	**0.025**
Pair-wise comparison (between time points)						
CRT 1- CRT 2 (PM-S)	-84.091	36.311	25.881	[-174.345, 6.163]	-2.316	0.071
CRT 1- CRT 3 (PM-S)	-123.500	34.531	29.792	[-208.658,-38.342]	-3.577	**0.003**
CRT 2- CRT 3 (PM-S)	-39.409	34.394	26.842	[-124.715, 45.897]	-1.146	0.495
CRT 1- CRT 2 (PM+S)	-15.049	28.760	25.184	[-86.652, 56.554]	-0.523	0.861
CRT 1- CRT 3 (PM+S)	-12.715	30.621	30.044	[-88.199, 62.768]	-0.415	0.910
CRT 2- CRT 3 (PM+S)	2.334	30.500	21.967	[-74.293, 78.960]	0.077	0.997
Interaction contrasts (social condition * time point)						
CRT1- CRT2	69.042	44.179	22.607	[-22.437, 160.521]	1.563	0.132
CRT1- CRT3	110.785	40.313	21.024	[26.955, 194.614]	2.748	**0.012**
CRT2- CRT3	41.742	45.179	22.864	[-51.749, 135.234]	0.924	0.365

Table S15: Multiple comparisons (Tukey's) of linear mixed effect model to determine effects of treatment (social condition), time (CRT) and treatment*time interaction on increase in cortisol responsiveness after 1 hour of exposure to a novel environment.

Increase in Cortisol responsiveness, 1h	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
CRT1	119.282	103.565	28.423	[-92.720, 331.283]	1.152	0.259
CRT2	11.323	126.805	29.834	[-247.708, 270.353]	0.089	0.929
CRT3	-86.534	113.299	29.460	[-318.100, 145.032]	-0.764	0.451
Pair-wise comparison (between time points)						
CRT 1- CRT 2 (PM-S)	48.859	124.080	23.515	[-261.418, 359.136]	0.394	0.918
CRT 1- CRT 3 (PM-S)	204.688	125.851	29.377	[-105.908, 515.284]	1.626	0.251
CRT 2- CRT 3 (PM-S)	155.829	124.364	26.479	[-152.868, 464.527]	1.253	0.434
CRT 1- CRT 2 (PM+S)	-59.100	98.659	24.842	[-304.940, 186.740]	-0.599	0.822
CRT 1- CRT 3 (PM+S)	-1.127	108.624	29.810	[-269.002, 266.748]	-0.010	1.000
CRT 2- CRT 3 (PM+S)	57.973	102.650	20.376	[-201.356, 317.301]	0.565	0.840
Interaction contrasts (social condition * time point)						
CRT1- CRT2	-107.959	148.686	20.114	[-418.001, 202.083]	-0.726	0.476
CRT1- CRT3	-205.816	138.997	19.796	[-495.949, 84.318]	-1.481	0.154
CRT2- CRT3	-97.856	157.156	21.715	[-424.026, 228.313]	-0.623	0.540

Table S16: Multiple comparisons (Tukey's) of linear mixed effect model to determine effects of treatment (social condition), time (CRT) and treatment*time interaction on increase in cortisol responsiveness after 2 hours of exposure to a novel environment.

Increase in Cortisol responsiveness, 2h	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
CRT1	195.930	113.912	23.207	[-39.599, 431.458]	1.720	0.099
CRT2	90.615	132.299	27.838	[-180.458, 361.688]	0.685	0.499
CRT3	139.148	121.005	25.705	[-109.720, 388.015]	1.150	0.261
Pair-wise comparison (between time points)						
CRT 1- CRT 2 (PM-S)	42.610	111.627	21.946	[-237.853, 323.072]	0.382	0.923
CRT 1- CRT 3 (PM-S)	82.035	125.794	29.773	[-228.203, 392.272]	0.652	0.793
CRT 2- CRT 3 (PM-S)	39.425	115.210	24.112	[-248.199, 327.049]	0.342	0.938
CRT 1- CRT 2 (PM+S)	-62.705	93.518	27.213	[-294.470, 169.060]	-0.671	0.782
CRT 1- CRT 3 (PM+S)	25.253	112.834	29.679	[-253.069, 303.575]	0.224	0.973
CRT 2- CRT 3 (PM+S)	87.958	90.749	20.324	[-141.349, 317.265]	0.969	0.604
Interaction contrasts (social condition * time point)						
CRT1- CRT2	-105.315	128.611	17.860	[-375.668, 165.038]	-0.819	0.424
CRT1- CRT3	-56.782	120.430	17.761	[-310.041, 196.477]	-0.471	0.643
CRT2- CRT3	48.533	137.907	18.801	[-240.315, 337.382]	0.352	0.729

Table S17: Multiple comparisons (Tukey's) of linear mixed effect model to determine effects of treatment (social condition), time (CRT) and treatment*time interaction on baseline testosterone.

Baseline testosterone	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
CRT1	0.068	0.529	43.036	[-1.000, 1.136]	0.129	0.898
CRT2	-0.005	0.536	42.475	[-1.086, 1.075]	-0.010	0.992
CRT3	0.107	0.543	43.671	[-0.987, 1.202]	0.198	0.844
Pair-wise comparison (between time points)						
CRT 1- CRT 2 (PM-S)	0.546	0.474	43.393	[-0.604, 1.696]	1.152	0.488
CRT 1- CRT 3 (PM-S)	0.584	0.551	51.979	[-0.745, 1.913]	1.060	0.543
CRT 2- CRT 3 (PM-S)	0.038	0.467	41.861	[-1.097, 1.173]	0.082	0.996
CRT 1- CRT 2 (PM+S)	0.472	0.492	46.912	[-0.718, 1.663]	0.961	0.605
CRT 1- CRT 3 (PM+S)	0.623	0.567	51.856	[-0.744, 1.991]	1.100	0.518
CRT 2- CRT 3 (PM+S)	0.151	0.471	40.782	[-0.994, 1.296]	0.321	0.945
Interaction contrasts (social condition * time point)						
CRT1- CRT2	-0.073	0.624	35.162	[-1.340, 1.194]	-0.117	0.907
CRT1- CRT3	0.039	0.634	35.317	[-1.247, 1.326]	0.062	0.951
CRT2- CRT3	0.113	0.635	35.293	[-1.176, 1.401]	0.177	0.860

Results: Adjusted repeatability analysis of hormone concentrations

Table S18: Adjusted repeatability analysis of linear mixed effects models of baseline cortisol (c0), increase in cortisol responsiveness after 1 hour of exposure to a novel environment (c1), increase in cortisol responsiveness after 2 hours of exposure to a novel environment (c2) and baseline testosterone (t). Significant ($p < 0.05$) results are indicated in bold.

Repeatability	PM-S				PM+S			
	Std. error	[95% CI]	R	p-value	Std. error	[95% CI]	R	p-value
c0	0.270	[0, 0.833]	0.124	0.401	0.220	[0, 0.685]	0.076	0.392
c1	0.292	[0, 0.877]	0.121	0.432	0.185	[0.233, 0.945]	0.721	**< 0.001**
c2	0.164	[0.321, 0.980]	0.782	**0.007**	0.248	[0, 0.864]	0.418	0.056
t	0.203	[0, 0.732]	0.346	0.076	0.202	[0, 0.669]	0.244	0.153

Results: Model summaries of (generalized) linear mixed effect models for behaviour

Table S19: Model summary from generalized linear mixed effect model used to analyse sociopositive behaviour (N = 60). The model included the interaction between treatment (social condition) and time (phase) with individual ID as random effect. Phase 1 (time) and PM-S (treatment) were set as reference level by default.

Sociopositive behaviour	Estimate	Std. error	[95% CI]	z-value	p-value	R^2
					Full model: Marginal R^2	0.124
					Full model: Conditional R^2	0.147
Intercept	1.384	0.246	[0.903, 1.865]	5.638	< 0.001	
Fixed effects						
Treatment (social condition)	0.363	0.331	[-0.285, 1.011]	1.098	0.272	0.013
Phase 1- Phase 2 (time)	-0.333	0.337	[-0.992, 0.327]	-0.988	0.323	0.005
Phase 1- Phase 3 (time)	-0.499	0.341	[-1.168, 0.170]	-1.462	0.144	0.010
Treatment*Phase 1- Phase 2	0.213	0.469	[-0.707, 1.132]	0.454	0.650	< 0.001
Treatment*Phase 1- Phase 3	0.415	0.468	[-0.504, 1.332]	0.885	0.376	0.002

Table S20: Model summary from generalized linear mixed effect model used to analyse the binary occurrence (0/1) of courtship behaviour (N = 120). The model included the interaction between treatment (social condition) and time (phase) with individual ID as random effect. Phase 1 (time) and PM-S (treatment) were set as reference level by default.

Occurrence courtship behaviour (0/1)	Estimate	Std. error	[95% CI]	z-value	p-value	R^2
					Full model: Marginal R^2	0.056
					Full model: Conditional R^2	0.282
Intercept	-0.487	0.592	[-1.646, 0.673]	-0.823	0.411	
Fixed effects						
Treatment (social condition)	0.984	0.848	[-0.679, 2.646]	1.159	0.246	0.014
Phase 1- Phase 2 (time)	-0.518	0.719	[-1.927, 0.891]	-0.721	0.471	0.004
Phase 1- Phase 3 (time)	-0.252	0.706	[-1.636, 1.131]	-0.357	0.721	< 0.001
Treatment*Phase 1- Phase 2	0.004	1.013	[-1.983, 1.990]	0.004	0.997	< 0.001
Treatment*Phase 1- Phase 3	-0.263	1.005	[-2.233, 1.707]	-0.261	0.794	< 0.001

Table S21: Model summary from linear mixed effect model used to analyse courtship behaviour (>0). Data was log-transformed (N = 53). The model included the interaction between treatment (social condition) and time (phase) with individual ID as random effect. Phase 1 (time) and PM-S (treatment) were set as reference level by default.

Courtship behaviour (>0)	Estimate	Std. error	[95% CI]	t-value	p-value	R^2
					Full model: Marginal R^2	0.133
					Full model: Conditional R^2	NA
Intercept	-0.383	0.241	[-0.867, 0.102]	-1.590	0.119	
Fixed effects						
Treatment (social condition)	0.500	0.311	[-0.125, 1.125]	1.611	0.114	0.052
Phase 1- Phase 2 (time)	-0.012	0.368	[-0.752, 0.728]	-0.033	0.974	< 0.001
Phase 1- Phase 3 (time)	0.175	0.352	[-0.533, 0.884]	0.498	0.621	0.005
Treatment*Phase 1- Phase 2	-0.034	0.469	[-0.978, 0.910]	-0.073	0.942	< 0.001
Treatment*Phase 1- Phase 3	-0.848	0.457	[-1.767, 0.072]	-1.854	0.070	0.068

Results: Multiple comparisons of (generalized) linear mixed effect models of behaviour

Table S22: Multiple comparisons (Tukey's) of generalized linear mixed effect model to determine effects of treatment (social condition), time (phase) and treatment*time interaction on sociopositive behaviour. Significant ($p < 0.05$) results are indicated in bold.

Sociopositive behaviour	Estimate	Std. error	df	[95% CI]	z-value	p-value
Pair-wise comparison (between social conditions)						
Phase 1	0.696	0.230	Inf	[0.364, 1.330]	-1.098	0.272
Phase 2	0.562	0.194	Inf	[0.286, 1.106]	-1.668	0.095
Phase 3	0.460	0.158	Inf	[0.234, 0.902]	-2.258	**0.024**
Pair-wise comparison (between time points)						
Phase 1- Phase 2 (PM-S)	1.395	0.469	Inf	[0.634, 3.070]	0.988	0.584
Phase 1- Phase 3 (PM-S)	1.647	0.562	Inf	[0.740, 3.665]	1.462	0.310
Phase 2- Phase 3 (PM-S)	1.181	0.413	Inf	[0.520, 2.680]	0.476	0.883
Phase 1- Phase 2 (PM+S)	1.127	0.367	Inf	[0.526, 2.419]	0.368	0.928
Phase 1- Phase 3 (PM+S)	1.088	0.349	Inf	[0.513, 2.306]	0.263	0.963
Phase 2- Phase 3 (PM+S)	0.965	0.313	Inf	[0.452, 2.062]	-0.110	0.993
Interaction contrasts (social condition * time point)						
Phase 1- Phase 2	0.808	0.379	Inf	[0.322, 2.027]	-0.454	0.650
Phase 1- Phase 3	0.661	0.309	Inf	[0.264, 1.655]	-0.885	0.376
Phase 2- Phase 3	0.817	0.390	Inf	[0.321, 2.081]	-0.423	0.672

Table S23: Multiple comparisons (Tukey's) of generalized linear mixed effect model to determine effects of treatment (social condition), time (phase) and treatment*time interaction on the binary occurrence (0/1) of courtship behaviour.

Occurrence courtship behaviour (0/1)	Estimate	Std. error	df	[95% CI]	z-value	p-value
Pair-wise comparison (between social conditions)						
Phase 1	0.374	0.317	Inf	[0,071, 1,972]	-1.159	0.246
Phase 2	0.373	0.320	Inf	[0,069, 2,003]	-1.150	0.250
Phase 3	0.486	0.412	Inf	[0,093, 2,555]	-0.852	0.394
Pair-wise comparison (between time points)						
Phase 1- Phase 2 (PM-S)	1.679	1.207	Inf	[0,311, 9,055]	0.721	0.751
Phase 1- Phase 3 (PM-S)	1.287	0.908	Inf	[0,246, 6,729]	0.357	0.932
Phase 2- Phase 3 (PM-S)	0.766	0.556	Inf	[0,140, 4,191]	-0.367	0.928
Phase 1- Phase 2 (PM+S)	1.673	1.199	Inf	[0,312, 8,976]	0.718	0.753
Phase 1- Phase 3 (PM+S)	1.673	1.199	Inf	[0,312, 8,976]	0.718	0.753
Phase 2- Phase 3 (PM+S)	1.000	0.707	Inf	[0,191, 5,245]	0.000	1.000
Interaction contrasts (social condition * time point)						
Phase 1- Phase 2	0.996	1.010	Inf	[0,137, 7,262]	-0.004	0.997
Phase 1- Phase 3	1.300	1.307	Inf	[0,181, 9,324]	0.261	0.794
Phase 2- Phase 3	1.305	1.322	Inf	[0,179, 9,500]	0.263	0.793

Table S24: Multiple comparisons (Tukey's) of linear mixed effect model to determine effects of treatment (social condition), time (phase) and treatment*time interaction on courtship behaviour (>0).

Frequency courtship behaviour (>0)	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
Phase 1	-0.500	0.316	39.330	[-1,139, 0,138]	-1.584	0.121
Phase 2	-0.466	0.360	42.745	[-1,192, 0,259]	-1.297	0.202
Phase 3	0.347	0.340	43.023	[-0,339, 1,034]	1.020	0.313
Pair-wise comparison (between time points)						
Phase 1- Phase 2 (PM-S)	0.012	0.376	46.815	[-0,897, 0,921]	0.032	0.999
Phase 1- Phase 3 (PM-S)	-0.175	0.356	40.877	[-1,042, 0,692]	-0.492	0.876
Phase 2- Phase 3 (PM-S)	-0.187	0.386	45.974	[-1,123, 0,748]	-0.485	0.879
Phase 1- Phase 2 (PM+S)	0.046	0.299	44.074	[-0,679, 0,771]	0.154	0.987
Phase 1- Phase 3 (PM+S)	0.672	0.294	37.992	[-0,044, 1,389]	2.289	0.070
Phase 2- Phase 3 (PM+S)	0.626	0.309	40.544	[-0,126, 1,379]	2.026	0.119
Interaction contrasts (social condition * time point)						
Phase 1- Phase 2	0.034	0.480	46.122	[-0,932, 1,000]	0.071	0.944
Phase 1- Phase 3	0.848	0.462	39.734	[-0,086, 1,781]	1.835	0.074
Phase 2- Phase 3	0.814	0.495	44.289	[-0,183, 1,811]	1.644	0.107

Results: Statistical tests for behavioural tests (SDT, MFIT, SIT)

Table S25: Fisher's exact test and Wilcoxon rank-sum test results for the step-down test (SDT) (N = 20).

Fisher's exact test (SDT)	Odds ratio	[95% CI]	p-value
Proportion of individuals stepping down	11	[0.405, 53.810]	0.350
Wilcoxon rank-sum test (SDT)	**W**	**r**	**p-value**
Latency to step down	11	0.347	0.211

Table S26: Wilcoxon rank-sum test and Welch's t-test results for the male-female interaction test (MFIT) (N = 19). P-values were adjusted for multiple testing using the Benjamini–Hochberg correction. Significant ($p < 0.05$) results are indicated in bold.

Wilcoxon rank-sum test (MFIT)	**W**	**r**	**p-value**	**p-value (adjusted)**
Latency first contact with female	46.5	0.019	0.935	0.935
Sexual behaviour	48	0.048	0.833	0.935
Welch's t-test (MFIT)	**t**	**Cohen's d**	**p-value**	**p-value (adjusted)**
Time spent near female	-0.45	0.205	0.658	0.935
Sociopositive behaviour	-2.43	1.086	**0.029**	0.081
Courtship behaviour	-2.36	1.055	**0.032**	0.081

Table S27: Wilcoxon rank-sum test and Welch's t-test results for the social initiative test (SIT) during the no basket phase (N = 19). P-values were adjusted for multiple testing using the Benjamini–Hochberg correction. Significant ($p < 0.05$) results are indicated in bold.

Wilcoxon rank-sum test (SIT no basket phase)	**W**	**r**	**p-value**	**p-value (adjusted)**
Latency first contact with infant	47.5	0.037	0.870	0.870
Welch's t-test (SIT no basket phase)	**t**	**Cohen's d**	**p-value**	**p-value (adjusted)**
Time spent near infant	0.681	0.313	0.505	0.674
Sociopositive behaviour	-2.807	1.329	**0.016**	0.063
Courtship behaviour	-0.867	0.391	0.399	0.674

Results: Model summaries of (general) linear mixed effect models for the social initiative test (SIT) during the basket phase

Table S28: Model summary from general linear mixed effect model used to analyse the frequency of basket sniffing in the social initiative test (SIT) during the basket phase (N = 19). The model included the interaction between treatment (social condition) and basket type (empty or infant) with individual ID as random effect. Empty basket (basket type) and PM-S (treatment) were set as reference level by default.

Sniffing empty and infant basket (SIT)	**Estimate**	**Std. error**	**[95% CI]**	**z-value**	**p-value**	**R^2**
					Full model: Marginal R^2	0.427
					Full model: Conditional R^2	0.915
Intercept	2.784	0.162	[2.466, 3.101]	17.182	< 0.001	
Fixed effects						
Treatment (social condition)	-0.103	0.236	[-0.566, 0.360]	-0.437	0.662	0.006
Basket type	0.807	0.090	[0.631, 0.982]	9.005	< 0.001	0.343
Treatment*Basket type	0.037	0.135	[-0.228, 0.301]	0.271	0.786	0.002

Table S29: Model summary from linear mixed effect model used to analyse the duration of time spent near the basket in the social initiative test (SIT) during the basket phase. Data was log-transformed (N = 19). The model included the interaction between treatment (social condition) and basket type (empty or infant) with individual ID as random effect. Empty basket (basket type) and PM-S (treatment) were set as reference level by default.

Time spent near empty and infant basket (SIT)	**Estimate**	**Std. error**	**[95% CI]**	**t-value**	**p-value**	**R^2**
					Full model: Marginal R^2	0.400
					Full model: Conditional R^2	0.744
Intercept	4.045	0.277	[3.474, 4.615]	14.583	< 0.001	
Fixed effects						
Treatment (social condition)	0.149	0.403	[-0.680, 0.978]	0.370	0.714	0.004
Basket type	1.321	0.256	[0.781, 1.862]	5.156	< 0.001	0.250
Treatment*Basket type	0.153	0.372	[-0.633, 0.939]	0.411	0.686	0.002

Results: Multiple comparisons of (generalized) linear mixed effect models for the social initiative test (SIT) during the basket phase

Table S30: Multiple comparisons (Tukey's) of generalized linear mixed-effects model to determine effects of treatment (social condition), basket type (infant or empty) and treatment*basket type interaction on the frequency of basket sniffing in the social initiative test (SIT) during the basket phase. Significant ($p < 0.05$) results are indicated in bold.

Sniffing empty and infant basket during SIT	Estimate	Std. error	df	[95% CI]	z-value	p-value
Pair-wise comparison (between social conditions)						
Empty basket	0.103	0.236	Inf	[-0.360, 0.566]	0.437	0.662
Infant basket	0.067	0.221	Inf	[-0.366, 0.499]	0.302	0.763
Pair-wise comparison (between basket types)						
PM-S	-0.807	0.090	Inf	[-0.982,-0.631]	-9.005	**< 0.001**
PM+S	-0.843	0.101	Inf	[-1.041,-0.645]	-8.345	**< 0.001**
Interaction contrasts (social condition * basket type)	0.037	0.135	Inf	[-0.228, 0.301]	0.271	0.786

Table S31: Multiple comparisons (Tukey's) of linear mixed-effects model to determine effects of treatment (social condition), basket type (infant or empty) and treatment*basket type interaction on the duration of time spent near baskets in the social initiative test (SIT) during the basket phase. Significant ($p < 0.05$) results are indicated in bold.

Time spent near empty and infant basket during SIT	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
Empty basket	-0.149	0.403	25.591	[-0.978, 0.680]	-0.370	0.714
Infant basket	-0.302	0.403	25.591	[-1.131, 0.527]	-0.750	0.460
Pair-wise comparison (between basket types)						
PM-S	-1.321	0.256	17	[-1.862,-0.781]	-5.156	**< 0.001**
PM+S	-1.474	0.270	17	[-2.044,-0.904]	-5.458	**< 0.001**
Interaction contrasts (social condition * basket type)	0.153	0.372	17	[-0.633, 0.939]	0.411	0.686

Results: Figures for behaviour tests (SDT, MFIT, SIT)

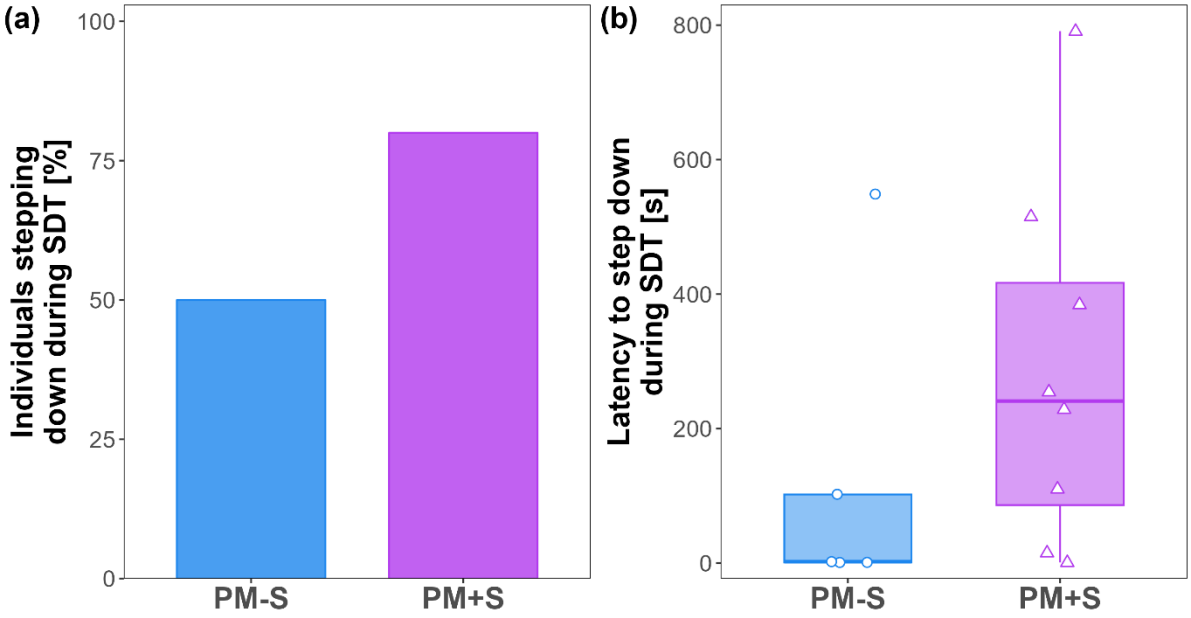

Figure S1: Step-down test (SDT). **(a)** Percentage of individuals stepping down from the platform within the maximum test duration (900 s) and **(b)** latency to step down from the platform for individuals that stepped down. Males were either additionally socially stimulated (PM+S) or not (PM-S). In **(a)**, bars represent the proportion of individuals per group. In **(b)**, boxplots display medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points.

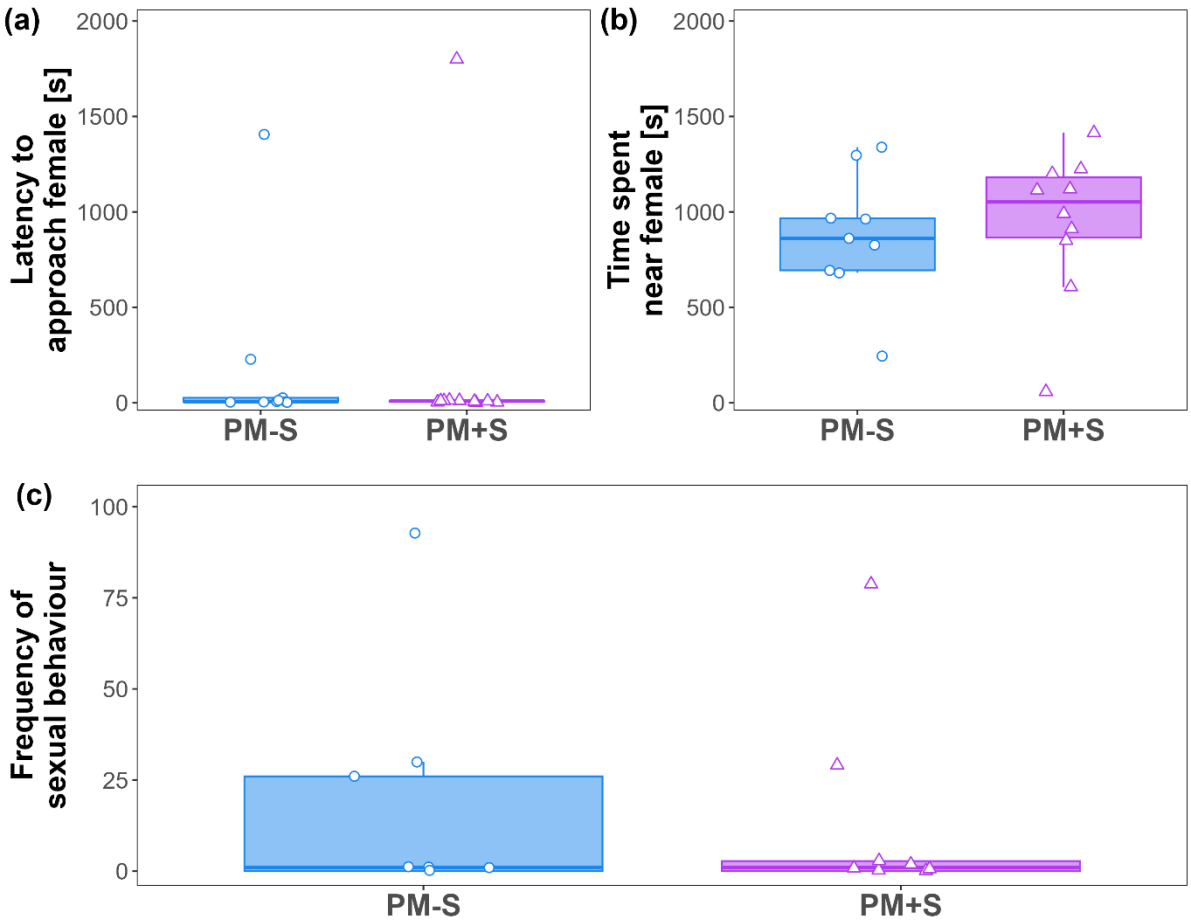

Figure S2: Male-female interaction test (MFIT). **(a)** Latency to first contact with female, **(b)** time spent near female and **(c)** frequency of sexual behaviour towards female. Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points.

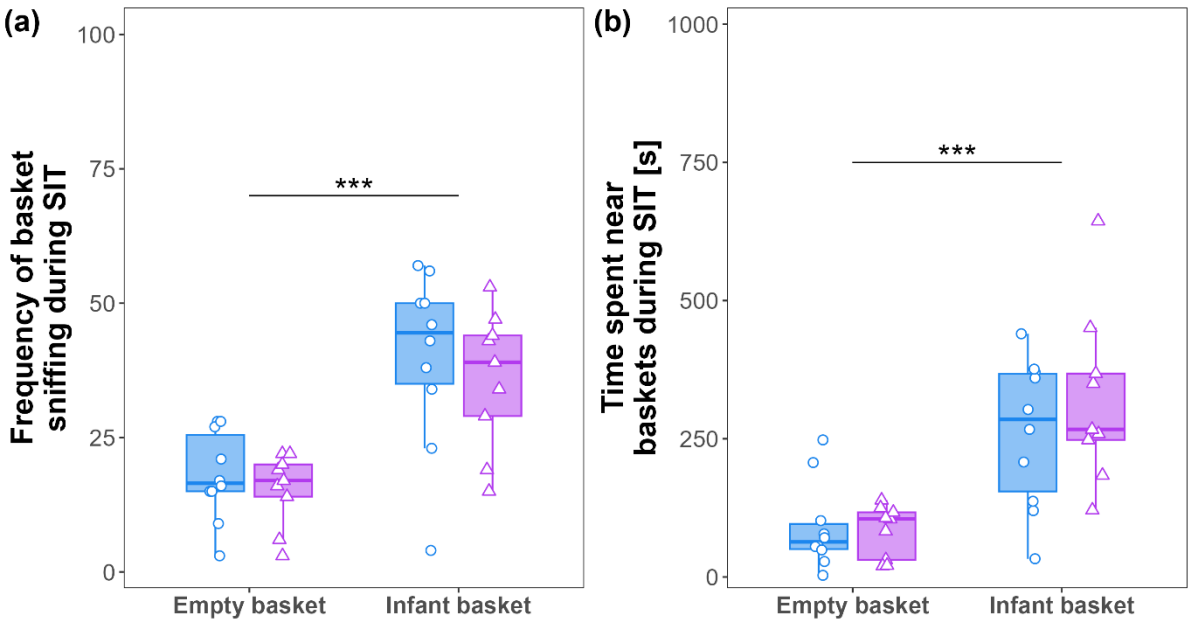

Figure S3: Social initiative test (SIT), basket phase. **(a)** Sniffing frequency towards the empty and infant basket and **(b)** time spent near the empty and infant basket. Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points. ***p < 0.001.

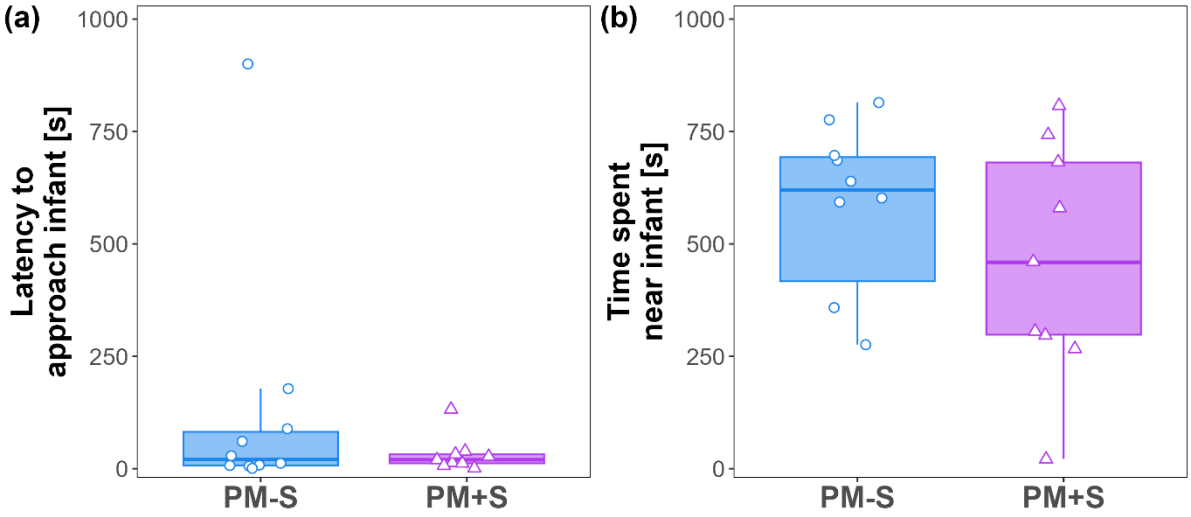

Figure S4: Social initiative test (SIT), no basket phase. **(a)** Latency to first contact with infant and **(b)** time spent near infant. Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are medians (horizontal lines in boxes), first to third quartiles (boxes), whiskers and all data points.