



Trans-generational influence of human disturbances in Japanese quail: egg quality influences male social and sexual behavior.

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25

26 **Abstract**

27 In many bird species prenatal exposure to yolk androgens of maternal origin has been found
28 to influence offspring behavioural phenotype. In contrast to altricial birds, far less is known
29 about maternal effects in precocial birds. In a previous experiment we found that female quail
30 (*Coturnix japonica*) that were not previously habituated to humans (NH) produced eggs with
31 less androgens (testosterone, androstenedione) and more progesterone when exposed to
32 human disturbances than females habituated to humans (H). Here, we analysed social
33 motivation and sexual behaviour of the male offspring of NH and H females. Classical
34 behavioural test procedures were applied including separation, runway, partner choice, and
35 female encounters tests. As chicks, offspring of the NH females spent more time far from
36 conspecifics than offspring of the H females. As adults, the same NH males showed less
37 crowing and courtship behaviour (ritual preening) in female encounter tests than H males.
38 Thus, maternal environment and egg quality may be key factors in the emergence of
39 individual variability of appetitive behaviour, such as social proximity and courtship
40 behaviour. Human disturbance of the mother seems to have triggered transgenerational effects
41 resulting in consistently reduced social and sexual motivation in offspring until adulthood.

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51 Introduction

52 Individual variability of sexual behaviour within a sex has been described in a wide
53 range of animals but little is known about the proximate factors responsible for this variability
54 (e.g. Gross 1996; Brooks & Endler 2001; Rhen & Crews 2002). In rodents, prenatal exposure
55 to hormones and particularly androgens has been identified as an important factor for the
56 ontogeny of individual reproductive phenotypes. For example, in the house mouse (*Mus*
57 *musculus*) or in the Mongolian gerbil (*Meriones unguicula*), steroid hormones were shown to
58 diffuse between foetuses. Therefore, intra-uterine position, between male or female siblings,
59 affects the reproductive phenotype of adults. Foetuses located between two male siblings will
60 be exposed to higher blood testosterone levels than foetuses embedded between two females
61 and this difference in prenatal exposure to hormones affects aggressive or sexual behaviour in
62 adulthood (Clark & Galef 1995; Ryan & Vanderbergh 2002, for reviews).

63 In rats and mice maternal stress during gestation (i.e. prenatal stress) can change
64 offspring testosterone patterns and affect their reproductive behaviour. Such individuals, for
65 example, show less attempts to copulate and longer latencies to mount than offspring from
66 mothers experiencing low stress levels during gestation (e.g. Ward 1972; Crump & Chevins
67 1989). Prenatal stress was also found to affect attraction to conspecifics. Indeed, whereas
68 naïve male mice usually show a preference for an oestrous female in a simultaneous test
69 choice paradigm, males born from dams which were prenatally stressed showed a preference
70 for sexually active males (Meek et al. 2006).

71 Bird embryos are also exposed to hormones of maternal origin that can affect
72 individual behavioural, physiological or morphological characteristics (Groothuis et al. 2005).
73 Consequences of maternal yolk androgens were mostly studied on early life history stages but
74 more recently also long term effects were reported to account for within-sex variation of
75 sexual traits or adult behaviour. In house sparrows (*Passer domesticus*), males which hatched

76 from testosterone-injected eggs were found to show larger badge sizes compared with control
77 males (Strasser & Schwabl, 2004). Yolk androgen levels scaled with the development of the
78 nuptial plumage and the frequency of aggressive or sexual behaviour in black-headed gulls
79 (*Larus ridibundus*; Eising et al. 2006) and with male and female spur length in ring-necked
80 pheasants (*Phasianus colchicus*; Rubolini et al. 2006). Thus, although prenatal exposure
81 particularly to androgens apparently was not involved in sexual differentiation (Carere &
82 Balthazart 2007), it may be at the origin of within-sex individual variability of traits.

83 Our aim was to further investigate long-lasting effects of different prenatal exposure to
84 steroid hormones on social motivation and sexual behaviour of male Japanese quail. Steroids
85 are present in Japanese quail eggs (Hackl et al. 2003) and injection of testosterone into eggs
86 was found to affect emotional and social reactivity of chicks (Daisley et al. 2005; Okuliarová
87 et al. 2006; Okuliarová et al. 2007). The sexual behaviour of Japanese quail males is
88 particularly well described. Males signal their interest in a female by seeking proximity
89 irrespective of the female's sexual development. Conversely, females show no or only weak
90 tendency to approach males (Domjan & Hall 1986; Domjan & Ravert 1991; Mills et al.
91 1997). Under the control of androgens (e.g. Ball & Balthazart 2006; Shaw 2000), sexually
92 active males display both sexual vocalizations and courtship behaviour to attract females
93 (Adkins-Regan 1981, Guyomar'ch & Guyomar'ch 1996). Intra-individual stability and inter-
94 individual variability in crowing (courtship call) and postural courtship displays were found
95 (Lumineau et al. 2005) but the causal background for this variability remained poorly
96 understood. Recently we found that female quail not habituated (NH) to humans and exposed
97 to human disturbances laid eggs with less androgens (both testosterone and androstenedione)
98 and a higher proportion of immunoreactive progesterone compared to eggs of females
99 exposed to disturbances but previously habituated (H) to humans (Bertin et al. 2008). Growth
100 of chicks of NH females was delayed during the first week of age; they were also less reactive

101 in front of humans or a novel object. Here we present the analysis of the sexual behaviour of
102 the adult sons of NH and H females. We tested whether the quality of the pre-hatching
103 environment (i.e. human disturbances during the laying phase) would affect male offspring
104 behaviour in adulthood. We expected to find lower frequencies of sexual displays in the
105 offspring of NH females, which have been exposed to lower levels of yolk androgens during
106 early development.

107 Once adult and sexually mature, sons of NH and H females were tested for their sexual
108 behaviour in a simultaneous test choice paradigm and a direct confrontation with a sexually
109 active female. As proximity to females can also be influenced by the underlying social
110 motivation (i.e. the internal impulse that drives social animals to search for conspecifics,
111 Wheeler 1926, Launay 1993), the tendency of an isolated animal to establish contact with, to
112 move towards, and to remain close to a group of conspecifics was tested in runway tests when
113 males were juvenile and not yet sexually developed.

114

115 **Materials and methods**

116 **Animals and housing conditions**

117 Twenty nine male quail bred from eggs which were laid by differently treated mothers
118 were employed in our present experiments (Bertin et al. 2008 for a full description of the
119 procedure). Each bird was identified by a numbered leg ring. These males were obtained from
120 36 mothers (commercial strain). An effective “habituation” treatment as described in Bertin
121 and Richard-Yris (2004) was applied to half of the mothers (18 habituated females), whereas
122 the other half (18 non-habituated mothers) had no contact with humans except for
123 approximately 10 minutes a week during routine maintenance. The habituation consisted of a
124 treatment where the experimenter interacted daily with each quail by two different
125 procedures: in the morning the experimenter’s open hand was placed palm down for 30 s in

126 the home cage and no attempt was made to initiate contact with the quail; in the afternoon, the
127 experimenter picked up each quail, stroked it for 30 s and offered attractive food for 10 s
128 while holding it in her hand. Additionally, in the habituated group, maintenance took place
129 daily. The group of non-habituated quail received the same quantity of food, but was not
130 exposed to humans except for brief weekly maintenance. Birds of both groups were housed in
131 the same room but were separated by an opaque plastic curtain so that birds of NH group
132 could not have any visual contact with the experimenter during the habituation treatment.
133 Male quail were randomly introduced every two days into each female's cage in order to
134 fertilize any eggs. After the habituation treatment, when birds were 61 days-old and for a 15
135 day period, both NH and H birds were exposed to identical human disturbances. One
136 experimenter ran back and forth past the cages, caught and carried each bird for 30 s, opened
137 and closed each home cage door, or waved one hand for 30 s inside each home cage.

138 Egg collecting started nine days after the beginning of the 15-day human disturbances
139 period. In each group, eggs from each hen were collected every day for six consecutive days.
140 Each day and in each group around half the eggs was stored for hormonal assay and the other
141 half was stored for incubation (all females were sampled). Eggs were placed in an incubator
142 and maintained at 37.6 ± 0.1 °C and 60% humidity while being turned automatically. Inside
143 the incubator we used cardboard divisions to identify the group origin (H or NH mothers) and
144 the date of collection of hatched chicks. Identifying which chick hatched from which specific
145 egg was not feasible without interfering with the physical requirement of hatching. However,
146 from the opened egg shells we knew that on average 2 ± 1.6 chicks had hatched per female.
147 Sex was determined via plumage and inspection for the male cloacal foam glands. We
148 obtained 37 chicks (29 females and 14 males) from 18 habituated mothers and 34 chicks (19
149 females and 15 males) from the 18 non-habituated mothers. Only the 29 male offspring (14 H
150 and 15 NH) were kept for this study. All birds were socially raised in two groups of

151 equivalent sex ratio in two 282 x 64 x 69 cm cages with saw dust on the floor. Water and food
152 were available ad libitum during a 13:11 h light:dark cycle.

153

154 **Behavioural procedures**

155 To characterize social motivation and sexual behaviour of animals we applied behavioural
156 tests well established in this species:

157

158 *Separation test:*

159 Eight days after hatching we tested the reaction of the chicks to a separation from conspecifics
160 according to Launay (1993). Each bird was taken from its home cage, gently carried in a
161 transport box (15 x 15 x 15 cm) and placed into a cage (94 x 64 x 69 cm) to which they were
162 all familiarized before. The experimenter, out of sight of the bird, recorded latency of first
163 distress call and number of distress calls during a 3-min period.

164

165 *Runway test:*

166 When the chicks were 13 days of age we tested their social motivation in a classical runway
167 test. The apparatus was a straight 120 cm-long wire-mesh tunnel with a cage (42 x 36 x 22
168 cm) at the end of the tunnel into which one male chick and one female chick were placed. The
169 tunnel was divided into three zones of equal sizes (40 x 36 x 22 cm), “non-social” (far from
170 the conspecifics), “middle”, and “social” (close to the conspecifics). Tested males were
171 individually introduced in the non-social area and were observed for a 5-min period beginning
172 after the latency to their first step. NH and H males were alternatively introduced. During the
173 five minute period, the experimenter hidden behind a curtain with observation windows
174 recorded the latency to the first step, the latency of distress calling, the number of calls and

175 the time spent in each area. The occurrence of agonistic behaviour (attacks, attack calls, peck
176 and avoidance) was also recorded.

177

178 *Choice test:*

179 When birds were 68 days of age, they were all fully developed which was manifested by the
180 presence of cloacal foam and crowing behaviour. To test their sexual preference, we used the
181 same tunnel that had been used for the runway test (above) but one cage was placed at both
182 end of the tunnel. One of the two cages contained a sexually developed male and the other
183 cage a sexually developed female both unfamiliar to the focal male. To avoid a specific
184 individual effect, six males and six females from a commercial strain were alternatively used
185 for the 29 test sessions. Again, the tunnel was divided into three zones of equal sizes (40 x 36
186 x 22 cm), “non-social” (far from the conspecifics), “female area” (close to the female), and
187 “male area” (close to the male). The side of the male and the female was switched half way
188 the 29 testing sessions. Males were individually introduced in the middle of the tunnel and
189 were observed for a 5-min period beginning with the first step they showed after initial
190 immobility. NH and H males were alternatively introduced. During the five minute period, the
191 experimenter recorded the latency to the first step as well as latencies to enter each area and
192 the time spent in each area. The occurrence of agonistic (attacks, attack calls, peck and
193 avoidance) or sexual behaviour (see description below) directed against any of the two target
194 individuals was also recorded.

195

196 *Female encounter test:*

197 All males were exposed to an unfamiliar female in a wooden cage (94 x 64 x 69 cm)
198 partitioned in two halves by a wire mesh grid at 69 days of age when they all were fully
199 sexually developed. All birds had been previously exposed to the same cage, and were

200 familiar with it. Six sexually developed adult females from a commercial farm were used for
201 all encounters. After a female was introduced in the cage she was given a 2-min habituation
202 period. Males were then individually introduced in the other half of the cage and were
203 observed for a 5-min period beginning after they did their first step. NH and H males were
204 alternatively introduced. All the encounters were staged in the morning when male sexual
205 activity is greatest (Ottinger et al. 1982). To avoid the risk of being injured by females (which
206 may be aggressive towards males when sexually developed) and because the direct encounter
207 with a female resulted in decreasing the time spent courting in favour of copulation (Beani et
208 al. 2000), males and females were not given direct access to each other (i.e. the wire mesh
209 partition stayed). Furthermore, as copulation success depends more on the female receptivity
210 and behaviour, we focused on courtship behaviour preceding copulation attempts only.

211 During the five minute period, the experimenter noted the occurrence of sexual behavioural
212 items previously described in (Guyomarc'h 1974; Guyomarc'h & Guyomarc'h 1986;
213 Guyomarc'h & Guyomarc'h 1996; Nichols 1991; Lumineau et al. 2005). Behaviours recorded
214 include vocalisations, such as crowing and growling, but also courtship displays including:

- 215 - The body shaking (BS): in a standing position, the male ruffles all his feathers and shakes
216 them.
- 217 - The curtsy (Ct): looking towards the female, the male bends his leg, thus lowering his
218 body.
- 219 - The shoulder-feathers-smoothing or ritual preening (RP): a rapid movement of the head
220 towards a shoulder or the breast, the beak briefly smoothing some feathers.
- 221 -The circling (C): very often associated with growling. The neck is obliquely stretched out
222 ahead, the wings lowered and legs extended. The male finally encircles the female and a
223 mounting attempt may occur.

224 - The flattening on the ground (F): The male lies flat on the ground, stretching his neck and
225 his head.

226 -The side display (SD): practically always associated with a bisyllabic contact call. The male
227 extends his neck with his head lightly inclined, so that the female can see his sexually
228 dimorphic chin feathers. The legs are fully extended, and the wings drawn in tightly to the
229 body.

230 -The nest invitation ceremony (NI): the male ritually scratches the ground in front of the
231 female and utters rhythmic nest call.

232 -The repeated down-up head movement (HM). The male nods his head rapidly with no
233 vocalization associated.

234 Aggressive behaviours, such as attacks, runaway calls, or pecking at the wire mesh were also
235 recorded.

236 All work was carried out in accordance with Austrian guidelines on animal research.

237

238 **Statistical analyses**

239 Kolmogorov-Smirnov tests were used to determine whether the data sets were normally
240 distributed. As the parametric requirements were not obtained, non-parametric statistics were
241 used. We used Mann-Whitney U-tests for comparisons between H and NH quail, Wilcoxon
242 signed rank tests for comparisons within H and NH birds and Chi-square tests (Siegel &
243 Castellan 1988). Data are presented as mean \pm SE. All analyses were performed using
244 Statview software (SAS, Cary, NC), with significance accepted at $p \leq 0.05$.

245

246 **Results**

247 When separated from conspecifics at 8 days of age, NH males tended to show a longer
248 latency to start distress calling as compared to H males (Table 1). The total number of distress
249 calls during the 3-minutes “separation test” was similar in NH and H chicks.

250 In the runway test, NH young spent twice as much time in the “non-social area” than
251 H birds (Table 1). The other parameters did not differ significantly.

252 In the choice test, NH males left the “non-social area” with a longer latency than H
253 birds (123.86 ± 31.35 s and 21.21 ± 6.94 s respectively, Mann-Whitney, $U = 55$, $p = 0.02$) and
254 spent more than twice as much time in the “non-social area” than H birds (Fig. 1). However,
255 the proportion of time spent in the area close to the female or the area close to the male did
256 not differ significantly between the two groups. H birds spent significantly more time close to
257 the male and/or female than in the “non-social area” (227.14 ± 21 s vs 72.85 ± 21 s,
258 Wilcoxon, $z = 2.35$, $p = 0.018$) whereas no difference was observed in NH birds ($139.60 \pm$
259 28.65 s vs 160.40 ± 28.65 s, Wilcoxon, $z = 0.5$, $p = 0.58$). The latency to the first step did not
260 differ between NH and H birds (17.33 ± 6.18 s and 20.5 ± 11.99 s respectively, Mann-
261 Whitney, $U = 94$, $p = 0.62$). Aggressive interactions were rare; only one NH male expressed
262 one attack call toward one female.

263 In the “female encounter” tests, the total frequency of sexual behaviour was lower in
264 NH than in H males (5.33 ± 4.43 and 6.35 ± 2.58 respectively, Mann-Whitney, $U = 60.5$, $p =$
265 0.04). Also the frequencies of crowing and ritual preening were lower in NH than in H birds
266 (Fig. 2). Significantly fewer NH than H birds were observed crowing (one bird versus six
267 birds, Chi-square, $p = 0.02$). Ritual preening was not observed in any of the NH males, but
268 was seen in four H birds (Chi-square, $p = 0.02$). Tests sessions without observable sexual
269 behaviour were more frequent in NH than H males (9 vs 3, respectively, Chi-square, $p =$
270 0.02). In both groups, some of the courtship displays, such as curtsy, flattening on the ground

271 and the repeated down-up head movement items, as well as aggressive behaviour were never
272 observed.

273

274 **Discussion**

275 In the current study we showed that sons coming from mothers not habituated to humans
276 showed a longer latency before distress calling at the age of eight days, they spent more time
277 in “non-social areas” as juveniles and as adults, and expressed less sexual behaviour towards
278 a mature female as adults than sons of mothers habituated to humans. Thus, maternal
279 experience in the domain of predator-related stressors has trans-generational effects and can
280 result in reduced social motivation in the offspring. The behavioural performance in various
281 tests was persistent from early age into adulthood, which suggests regarding it as a
282 behavioural phenotype.

283 Also as juveniles, NH males tended to show a longer latency to distress calling in a
284 test of separation and spent twice as much time than H males in the “non-social zone” in the
285 runway test. Once adults, in the choice test, NH males clearly took much longer to leave the
286 non-social area, spent more than twice as much time than H males in the non-social area and
287 did not show any preference between non-social or social areas as H males did. Two
288 explanations may account for the observed patterns of behaviour in NH sons: first, NH males
289 may have had a lower level of social motivation than H males. When isolated, Japanese quail
290 chicks selected for a high level of social motivation distress called earlier than chicks selected
291 for a low level of social motivation (Launay et al. 1991). In runway tests, quail with a high
292 level of social motivation joined their conspecifics faster than quail with a low level of social
293 motivation (Mills & Faure 1990; Formanek et al. 2008). Alternatively, the behaviour
294 observed in NH sons may have been due to a particularly high degree of emotional reactivity.
295 A higher emotional reactivity with a longer freezing time would also result in more time spent

296 in the non-social area. Yet, in that case H males would have been expected to stay longer in
297 the non-social area as they were found to be more emotional based on tonic immobility,
298 human observer or novel object tests (Bertin et al. 2008). Furthermore, the latency of first
299 step, which reflects the initial phase of disinhibition following freezing (Jones 1980) did not
300 differ between NH and H birds in any test.

301 In our study, consistent patterns were observed both when animals were juveniles or
302 adults, which could indicate a stable trait of their temperament. We hypothesize that this
303 difference in social behaviour could be due to a variation of early exposure to yolk androgens
304 in the eggs laid by H or NH females. Bertin et al. (2008) found that the eggs of NH mothers
305 contained lower androgen levels and indeed, in Japanese quail low levels of social motivation
306 as chicks and adults were correlated with low levels of yolk testosterone (Gil & Faure 2007).
307 Injecting testosterone into quail eggs also affected the social motivation of quail but,
308 contrarily to Gil & Faure (2007), low levels of social motivation were associated with high
309 testosterone levels (Daisley et al. 2005). The difference between correlational and
310 experimental designs may explain the contradiction observed. It may also likely be due to
311 different androgen dosages and the non-linear shape of dose-response curves usually found
312 with steroid hormones.

313 In female encounter tests, fewer NH birds than H birds expressed sexual behaviour
314 and also the frequency of sexual behaviour was lower in NH than in H birds. Our data are in
315 accordance with Eising et al. (2006) and Partecke & Schwabl (2008) reporting enhanced
316 sexual display frequencies with maternal androgens in eggs. If NH male embryos were
317 exposed to lower androgen levels than H male embryos, this subtle difference of early
318 hormone exposure could be responsible for the variation of behaviour from chick until
319 adulthood.

320 NH males also crowed less than H males when confronted to a mature female. Male
321 crowing is characteristic of the family Phasianidae. This courtship call is produced by
322 sexually mature males and is thought to attract females (Goodson & Adkins-Regan 1997).
323 Crowing in quail is an androgen dependent vocalization (Adkins-Regan 1981). Both acoustic
324 and postural components of crowing involve the activation of the midbrain vocal nucleus,
325 *Nucleus intercollicularis* (Shaw 2000). This behaviour has a clear daily pattern with activity
326 peaks and males singing in choruses at dawn and at dusk (Ottinger et al. 1982, Pincemy &
327 Guyomarc'h 2005). Despite the stereotypy of this behaviour, the singing rate greatly differed
328 within a population with high- and low-caller males (Beani et al. 2000, Pincemy &
329 Guyomarc'h 2005). Lumineau et al. (2005) also report intraindividual stability and
330 interindividual variability in the expression of quail courtship behaviour suggesting stable
331 behavioural profiles or personalities within populations. Our data suggest that early exposure
332 to maternal hormones could be part of the processes responsible for this inter-individual
333 variability of sexual behaviour. However, although male crowing is considered to be part of
334 their reproductive repertoire, Chiba & Hosokawa (2006) suggested that other underlying
335 motivations may also influence crowing rate. Indeed, crowing rate increases when males are
336 isolated; thus, motivations similar to those responsible for eliciting distress calls may thus be
337 involved. Consequently, we hypothesize that a lower motivation to interact with females may
338 also be responsible for the lower crowing rate observed in NH males. We also observed less
339 ritual preening in NH males than in H males. The function and determinism of ritual preening
340 is far less known. This courtship behaviour was more frequently observed in front of a real
341 female than in front of a lure and its frequency decreased drastically when males were
342 confronted with aggressive females compared to non-aggressive females (Lumineau et al.
343 2005). The difference we observed in ritual preening frequencies may thus also, as crowing,
344 have reflected a lower motivation to interact with females in NH males.

345 Here we showed that the sons of females that were not habituated to humans and
346 exposed to human disturbances had a lower motivation to interact with conspecifics and
347 expressed less sexual behaviour. Maternal sex steroids may likely be a key factor in the
348 emergence of within sex inter-individual variability. The results are in accordance with the
349 growing body of literature showing that early exposure to maternal hormones can influence
350 secondary sexual traits in birds (Strasser & Schwabl 2004; Eising et al. 2006; Partecke &
351 Schwabl 2008; Rubolini et al. 2007). Also in mammals, sons of females that were exposed to
352 social stress during pregnancy showed less sexual performance (e.g. Kaiser & Sachser 2001).
353 In male quail, appetitive behaviour (social proximity, courtship behaviour) and consummatory
354 sexual behaviour (mounting and copulation) were both activated by the aromatisation of
355 estrogen from testosterone in the brain, however, regulated by different subregions of the
356 preoptic area (Balthazart et al. 1998). Prenatal exposure to maternal hormones may have long
357 term organizational effects by modulating either the production of androgen or the sensitivity
358 to androgens later in life (Groothuis et al. 2005; Carere & Balthazart 2007). NH males were
359 also exposed to a higher proportion of progesterone (Bertin et al. 2008). Consequences of
360 prenatal exposure to this steroid remain unknown despite the large amounts observed in eggs
361 (Hackl et al. 2003). Also Bottoni et al. (1985) reported a depression of quail sexual behaviour
362 after administration of progesterone. Therefore, we should not exclude a potential influence of
363 this steroid. On the other hand progesterone may act as a physiological precursor molecule for
364 the formation of other (gonadal or adrenal) steroid metabolites. In sum, NH males developed
365 a “bold” or “proactive” behavioural profile, i.e. less emotional and less socially dependent
366 (Koolhaas et al. 1999). Accordingly, mothers may potentially affect their offspring
367 behavioural phenotype, i.e. from early age into adulthood, by variably providing hormonal
368 yolk contents as a function of (perceived) present environment during laying. Our results have

369 obvious implications in the field, as well as in the lab or in farm animals, where the
370 consequences of anthropogenic disturbances should be taken into account at multiple levels.

371

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For Peer Review

567 **Figure captions**

568 Figure 1: NH and H adult Japanese quail males mean duration scores in the non-social area,
569 female area or male area in choice tests. * Mann-Whitney U test, $p < 0.05$.

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571 Figure 2: Mean frequencies of sexual displays of NH and H Japanese quail males in female
572 encounter tests. *Mann-Whitney U test, $p < 0.05$.

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592 **Table 1.** Young Japanese quail males results in tests of separation and runway tests.

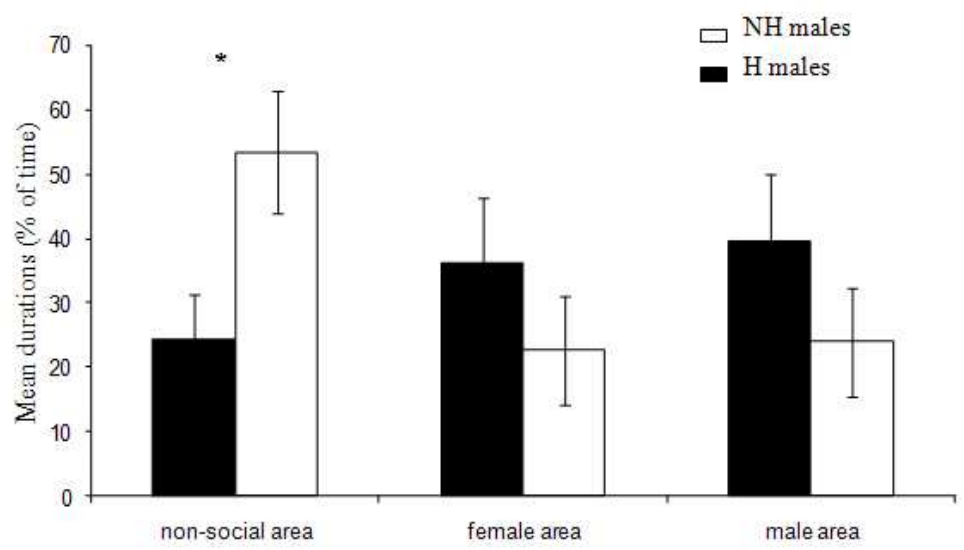
Tests	Parameters measured	NH young	H young	U	p
Test of separation	Latency of distress calling (s)	24.3 ± 11.22	6.07 ± 0.72	298.5	0.08
	Number of distress calls	142.66 ± 11.33	137.69 ± 8.66	407	0.39
Runway test	Time spent in the non-social area (s)	64.23 ± 22.28	30.31 ± 11.17	43.5	0.03
	Time spent in the middle area (s)	35.46 ± 8.98	51.54 ± 16.94	83	0.93
	Time spent in social area (s)	201.23 ± 21.73	218.15 ± 19.17	69	0.42
	Latency of first step (s)	6 ± 1.52	2.84 ± 0.78	55.5	0.12
	Latency of distress calling (s)	68.31 ± 26.91	36.23 ± 23.04	75	0.62
	Number of distress calls	49.69 ± 11.46	41.30 ± 9.18	78.5	0.76

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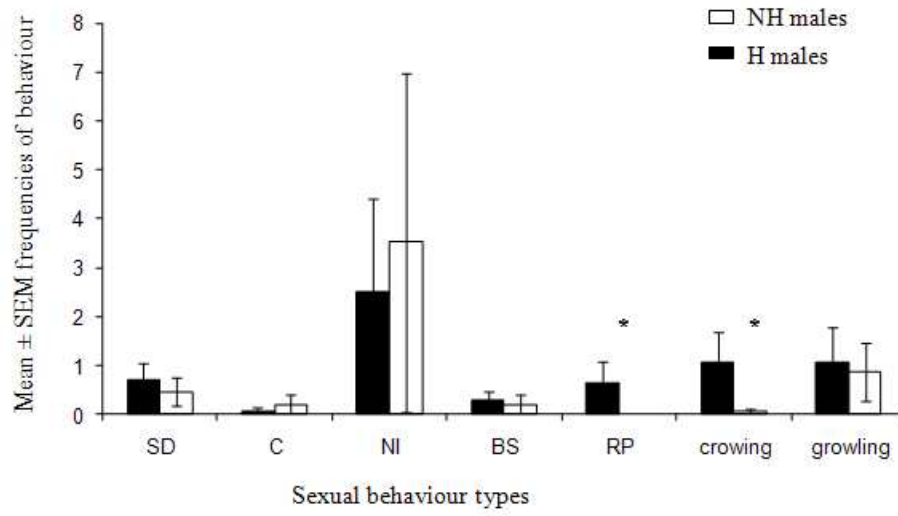
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595 **Note:** Significant p values are in boldface type, near-significant p values (0.05 < p < 0.1) are in boldface-italic type.

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