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1 **Beyond aggression: androgen-receptor blockade modulates social interaction in**
2 **wild meerkats**

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ABSTRACT

26

27

28 In male vertebrates, androgens are inextricably linked to reproduction, social
29 dominance, and aggression, often at the cost of paternal investment or prosociality.
30 Testosterone is invoked to explain rank-related reproductive differences, but its role
31 within a status class, particularly among subordinates, is underappreciated. Recent
32 evidence, especially for monogamous and cooperatively breeding species, suggests
33 broader androgenic mediation of adult social interaction. We explored the actions of
34 androgens in subordinate, male members of a cooperatively breeding species, the
35 meerkat (*Suricata suricatta*). Although male meerkats show no rank-related testosterone
36 differences, subordinate helpers rarely reproduce. We blocked androgen receptors, in
37 the field, by treating subordinate males with the antiandrogen, flutamide. We monitored
38 androgen concentrations (via baseline serum and time-sequential fecal sampling) and
39 recorded behavior (via focal observation). Relative to controls, flutamide-treated
40 animals initiated less and received more high-intensity aggression (biting, threatening,
41 feeding competition), engaged in more prosocial behavior (social sniffing, grooming,
42 huddling), and less frequently initiated play or assumed a dominant role during play,
43 revealing significant androgenic effects across a broad range of social behavior. By
44 contrast, guarding or vigilance and measures of olfactory and vocal communication
45 were unaffected by flutamide treatment. Thus, rather than regulating cooperative or
46 communicative behavior, androgens in adult meerkats are aligned with the traditional
47 trade-off between promoting reproductive and aggressive behavior at a cost to
48 affiliation. Our findings, based on rare endocrine manipulation in wild mammals, show
49 a more pervasive role for androgens in adult social behavior than is traditionally
50 recognized, with possible relevance for understanding tradeoffs in cooperative systems.

51 **Keywords:** antiandrogen, flutamide, testosterone, aggression, communication, prosocial
52 behavior, behavioral neuroendocrinology, subordinate male, field experiment,
53 cooperative breeder

54 INTRODUCTION

55

56 Cooperative breeding, by which dominant individuals monopolize a group's
57 breeding efforts, is rare among vertebrates, although several theories can be invoked to
58 explain why subordinate helpers might delay their own reproduction to care for the
59 offspring of others (Arnold and Owens, 1998; Lukas and Clutton-Brock, 2012). The
60 mechanisms involved in ensuring differential reproduction can differ rather dramatically
61 across species: In some, helpers are hormonally suppressed, such that they are
62 physiologically unable to reproduce (Arnold and Dittami, 1997; Bales et al., 2006;
63 Schoech et al., 1991), whereas in others, helpers are behaviorally suppressed, but retain
64 the physiological capacity to reproduce (Bennett et al., 1993; Creel et al., 1992; Khan et
65 al., 2001; Oliveira et al., 2003). Among the latter, the role of reproductive hormones,
66 such as testosterone (T), which might not vary substantially between breeders and
67 helpers, remains poorly understood. Within social species, reproductive hormones often
68 regulate (or are regulated by) the within-group interactions that are necessary to
69 maintain stable relationships (Albers et al., 2002; Monaghan and Glickman, 1992). In
70 males, androgen function is best understood in the context of mediating reproductive
71 and aggressive behavior – activities that often come at the cost of paternal investment
72 (Hegner and Wingfield, 1987; Ketterson and Nolan, 1994). Androgen function is also
73 invoked to explain rank-related differences in courtship and competition (Wingfield et
74 al., 1987). Nevertheless, there is recent evidence to suggest an even broader role for T in
75 mediating adult social interaction, particularly in monogamous or cooperatively
76 breeding species (Eisenegger et al., 2011; Gleason and Marler, 2010; Storey et al.,
77 2006; van der Meij et al., 2012; Wang and De Vries, 1993). Here, using a wild

78 population of the cooperatively breeding meerkat (*Suricata suricatta*), we investigated
79 these issues by blocking the androgen-receptor system of adult, subordinate males.

80 Meerkats are social mongooses that live in relatively stable clans or structured
81 groups, typically comprising a dominant breeding pair and various subordinate relatives
82 or offspring of both sexes that contribute to pup rearing (Clutton-Brock et al., 2001).
83 Among males, breeders and helpers express similar concentrations of T and luteinizing
84 hormone (LH), and show comparable LH spikes in response to a GnRH challenge
85 (Carlson et al., 2004; O'Riain et al., 2000). Thus, although the dominant male
86 monopolizes most of a group's breeding (Griffin et al., 2003), subordinate males are not
87 reproductively suppressed (Carlson et al., 2004) and may gain some breeding success,
88 as well as experience raised T concentrations, during extraterritorial prospecting forays
89 (Spong et al., 2008; Young et al., 2005, 2007). T does not correlate with aggression or
90 dominance between male social ranks (Carlson et al., 2004) and there is no evidence
91 that T relates to rates of pup provisioning (Carlson et al., 2006a). Yet, because
92 behavioral endocrinologists tend to focus on understanding dominance or the
93 differences between social ranks, little is known about the role of T in regulating
94 subordinate male interaction in this or other species (although see: Virgin and Sapolsky,
95 1997). Given that dominant and subordinate animals may respond differently to the
96 same T treatment (Fuxjager et al., 2015) or that T-associated variation in behavioral
97 'style' may exist within the same class (Virgin and Sapolsky, 1997), it is increasingly
98 relevant to understand how the different social classes respond to endocrine challenges.

99 Meerkats are an appropriate model in which to test the proposition that androgens
100 may regulate social behavior beyond aggression: Firstly, subordinates are far more
101 numerous than are dominant animals and necessarily account for a large proportion of
102 social interaction; secondly, these 'helper' males rarely reproduce, but curiously

103 maintain androgen concentrations commensurate with those of dominant males; thirdly,
104 access to an exceptional wild population allows us to consider social and ecological
105 relevance, while overcoming logistical challenges that typically preclude field
106 neuroendocrine studies (see Fusani et al., 2005).

107 With relatively few exceptions, typically involving avian species (e.g., Hegner and
108 Wingfield, 1987; Schwabl and Kriner, 1991), hormones or their actions are rarely
109 experimentally manipulated in the field (see Fusani et al., 2005), particularly to explore
110 their relationship to the broad social repertoire. Instead, androgen-manipulation studies
111 in laboratory animals, particularly rodents and birds, aim to improve our mechanistic
112 understanding of isolated traits (either e.g., reproduction: Södersten et al., 1975;
113 aggression: Searcy and Wingfield, 1980; play: Meaney et al., 1983; scent marking:
114 Fuxjager et al., 2015; or song: Grisham et al., 2007). This historical focus can occur at
115 the expense of gaining comparative, ecological, and evolutionary understanding of
116 hormone action: detecting tradeoffs and constraints, for instance, requires an integrated
117 approach (Wingfield et al., 2009).

118 To test the role of androgens in subordinate, male meerkats, we administered the
119 nonsteroidal antiandrogen, flutamide, that competitively blocks the binding of
120 androgenic hormones (primarily T) to androgen receptors (Hellman et al., 1977; Peets et
121 al., 1974). Androgens often relate to the initiation of aggression (e.g. Virgin and
122 Sapolsky, 1997) or the outcome of aggressive encounters (e.g. Rose et al., 1972), and
123 androgen-mediated cues can also influence susceptibility to aggressive attacks
124 (Monaghan and Glickman, 1992). Consistent with studies in various species showing
125 that flutamide administration leads to reduced adult aggression (Sperry et al., 2010;
126 Taylor et al., 1984; Vleck and Dobrott, 1993), we expected flutamide-treated meerkats
127 to initiate less, but receive more, aggression than their control counterparts.

128 Beyond the relationship to overt aggression, androgens also may be linked to other
129 more subtly competitive or even prosocial interaction in animals. Rough-and-tumble
130 play, for instance, which can facilitate the establishment of dominance relations among
131 the males of certain species (Panksepp, 1981; Pellegrini, 1995), is often sexually
132 differentiated, with males playing more vigorously than females (Boulton, 1996; Goy
133 and Phoenix, 1971; Meaney et al., 1985). The expression of mammalian social play is
134 masculinized through early androgen exposure (Goy and Phoenix, 1971; Olioff and
135 Stewart, 1978; Wallen, 2005) and can be feminized through reduced prenatal exposure
136 to androgens (Meaney and Stewart, 1981; Meaney et al., 1983). Typically, postnatal
137 androgens do not mediate social play (Meaney et al., 1985), as neither the frequency nor
138 vigor of play are influenced by administration of T to juvenile females (Joslyn, 1973) or
139 by castration of juvenile males (Beatty et al., 1981; Goy, 1970; Pedersen et al., 1990).
140 Nevertheless, few researchers have addressed the potential link between activational
141 androgens and adult social play, largely because playful behavior tends to decrease
142 dramatically in adulthood. Meerkats, however, continue to play as adults (Sharpe,
143 2005), so we might expect flutamide-treated meerkats to play less vigorously than those
144 experiencing normal androgen action.

145 With regard to the role of androgens in more purely prosocial, affiliative, or even
146 cooperative behavior, the nature of the correlations can vary considerably. Paternal care
147 (including huddling and grooming), for instance, is generally thought to be inhibited by
148 T (Hegner and Wingfield, 1987; Ketterson et al., 1992), but can increase with androgens
149 in the males of various species (Desjardins et al., 2008; Gleason and Marler, 2010; Neff
150 and Knapp, 2009; Rodgers et al., 2006; Storey et al., 2000; Trainor and Marler, 2001;
151 Wang and De Vries, 1993). Moreover, depending on prenatal androgen exposure
152 (Millet and Dewitte, 2006; van Honk et al., 2012), T in men can increase affiliative

153 behavior (van der Meij et al., 2012), reduce deceit (Wibral et al., 2012), promote
154 reciprocity (Boksem et al., 2013) and increase cooperation (Huoviala and Rantala,
155 2013). Meerkats show a range of prosocial behavior (including grooming, social
156 sniffing, and huddling) and cooperative behavior (including babysitting and
157 provisioning pups, as well as vigilance and guarding against predators: Clutton-Brock et
158 al., 1999, 2000, 2001). If androgens in meerkats implicate the traditional tradeoff
159 between aggression and affiliation, we might expect rates of prosocial interaction to
160 increase with flutamide treatment. If androgens in meerkats function to increase
161 cooperation, to the benefit of the entire group, we might expect flutamide treatment to
162 reduce pup care or antipredator activities.

163 Lastly, androgens also may be involved in aspects of olfactory and vocal
164 communication (Dryden and Conaway, 1967; Ulibarri and Yahr, 1988; Wingfield et al.,
165 1987). In this regard, scent marking is often linked to territorial defense (Hediger, 1949;
166 Johnson, 1973) and reproductive advertisement (Brown and Macdonald, 1985; Drea,
167 2015; Eisenberg and Kleiman, 1972) with dominant individuals generally marking more
168 than subordinates (Johnson, 1973; Ralls, 1971). Scent marking increases following
169 early exposure to androgens and decreases if such exposure is inhibited (Epple, 1981;
170 Turner, 1975; Ulibarri and Yahr, 1988). Postnatal T similarly mediates the frequency of
171 scent marking (Johnston, 1981) and can also influence the chemical composition of
172 odorants (Novotny et al., 1984). Castration causes retardation or atrophy of scent
173 glands, with accompanying effects on odorant production (Dryden and Conaway, 1967;
174 Epple, 1981), whereas hormone replacement restores these attributes (Dryden and
175 Conaway, 1967). Within adult male meerkats, there is no strong evidence of rank-
176 related differences in scent marking at latrines (Jordan, 2007), although we suspect that
177 they might emerge in other contexts. Despite equivalence in circulating T between male

178 ranks, anal gland secretions appear to be more pronounced in dominant males than in
179 subordinate males (see Figure 1 in Leclaire et al., 2014) and preliminary analyses of
180 these secretions reveal rank-related differences in chemical composition (Drea,
181 unpublished data). Moreover, the bacterial communities associated with anal pouch
182 secretions vary with social status (Leclaire et al., 2014). Overall, therefore, we expect
183 that androgens might regulate certain aspects of olfactory communication in adult
184 meerkats, such that flutamide treatment would reduce rates of scent marking.

185 Vocalizations likewise function in territorial defense (Bates, 1970; Peek, 1972;
186 Hall, 2009; Shonfield et al., 2012) and reproductive advertisement (Robertson, 1986;
187 Waas, 1988). Vocal cues are often studied in relation to T, providing evidence that the
188 frequency or structure of vocal signals correlate with androgens (Barelli et al., 2013;
189 Charlton et al., 2011; Evans et al., 2008; Solís and Penna, 1997; Wingfield et al., 1987).
190 Manipulation of T prenatally, neonatally or in adulthood shows that vocalizations are
191 regulated by androgens. Early androgen exposure masculinizes calls (Holman et al.,
192 1995; Tomaszycski et al., 2001, 2005), whereas prenatal exposure to antiandrogens
193 feminizes calls (Tomaszycski et al., 2001). In adulthood, increased T concentrations have
194 been linked to increased call rate, duration or quality (Ball et al., 2003; Charlton et al.,
195 2011; Cynx et al., 2005; Gyger et al., 1998; Ketterson et al., 1992). Conversely,
196 castration has been shown to negatively influence call rate or signal structure (Pasch et
197 al., 2011). As shown with androgen-receptor blockade in other species (Behrends et al.,
198 2010), we expect flutamide treatment in meerkats to influence vocalization, potentially
199 reducing calling rate, decreasing call duration or raising call pitch.

200

201 **METHODS**

202

203 *Field site, study population, and research cohorts*

204

205 Our subjects were members of a well-studied and habituated population of
206 meerkats, comprising 15-20 groups that inhabit the Kuruman River Reserve and
207 surrounding farms in the Kalahari region of South Africa (26°58'S, 21°49'E).
208 Information about the climate, landscape, and vegetation for this region have been
209 provided elsewhere (Clutton-Brock et al., 1998; Russell et al., 2002). All habituated
210 members of the population are microchipped and easily identifiable from unique dye
211 marks applied to their fur and routinely renewed without the need for capture (Clutton-
212 Brock et al., 2008). Minimally one animal per group (typically, the dominant female) is
213 fitted with a radio collar (Sirtrack, Havelock North, New Zealand) to facilitate locating
214 the group when necessary.

215 Our main subjects, deriving from five different groups, were 24 subordinate
216 males, 12 of which received flutamide treatment and 12 of which served as controls (see
217 research design, below). These animals were aged 11-18 months at the start of
218 treatment. Because meerkats of both sexes typically reach adulthood at 1 year of age
219 (Clutton-Brock et al., 2008), but can reproduce successfully at younger ages (Young et
220 al., 2006), we considered our subjects to be sexually mature.

221 Starting in 2011, we studied these animals in two cohorts. Cohort 1 included nine
222 animals (5 flutamide, 4 controls) followed from February to March 2011, at the end of
223 the breeding season. Cohort 1 served in a pilot study to establish our endocrine,
224 behavioral, and surgical procedures, including treatment dosage (see Electronic
225 Supplementary Material, ESM, §a) and to supply preliminary data (Fig. S1). Cohort 2
226 included 15 animals (7 flutamide, 8 controls; ESM, §b and Table S1) followed from
227 December 2011 to January 2012, at the beginning of the following breeding season, and

228 served in the experimental study described in detail herein. These latter subjects were
229 closely age-matched (mean age \pm standard error: 1.04 ± 0.04 years) and derived from 3
230 large groups totalling 96 animals (KungFu: $n = 36$; Lazuli: $n = 30$; Whiskers: $n = 30$).

231

232 *Research design*

233

234 We tested each focal subject of cohort 2 over a four-week period (with a one-week
235 maximum offset between subjects). Each subject's first week served to provide baseline
236 endocrine values and was followed by a capture day, to administer treatment, and
237 another day of post-capture monitoring. We randomly assigned these animals to one of
238 three treatment conditions, including flutamide ($n = 7$), placebo ($n = 4$), and no
239 treatment or 'no-pellet' ($n = 4$), with the constraint that littermates be assigned to
240 different treatments and that flutamide-treated animals be evenly distributed between
241 the three groups (see ESM, §b and Table S1). Treatment was followed by another three
242 weeks of data collection to evaluate endocrine and behavioral effects (see below). One
243 of the flutamide-treated individuals was struck by a vehicle (along with two other non-
244 intervention animals) and died early in the study. This animal contributed to baseline
245 fecal and serum values only, reducing our sample for examining the behavioral effects
246 of flutamide to $n = 6$ (2 per group).

247 All protocols were approved by Duke University's Institutional Animal Care and
248 Use Committee (Protocol Registry Numbers A171-09-06 and A143-12-05) and the
249 University of Pretoria's Animal Use and Care Committee (Ethical Approval Number
250 #C074-11, to CMD). The Northern Cape Conservation Authority in South Africa
251 provided permission for the project.

252

253 *Sampling, capture, and treatment administration*

254

255 We visited our focal groups 3-5 days per week, during both a morning (0600-1100
256 h) and evening (1600-2000 h) session. We obtained ad lib fecal samples prior to
257 treatment (to establish baseline) and across the 3-week treatment period. Whenever a
258 subject was observed defecating, we collected the fresh sample into a plastic bag and
259 placed it immediately on ice (in a cooler box or thermos). We stored all of the fecal
260 samples at -20 °C within 4 hours of collection.

261 We performed all of the captures over the course of five consecutive days in mid
262 December, with 1-2 capture mornings (0600-0800 h) per group. We processed
263 maximally four subjects, in succession, per day. Shortly after emergence from their den
264 or 'sleeping burrow,' we captured our subjects by gently picking them up by the base of
265 the tail, placing them into a cloth bag, and anesthetizing them with isoflurane (Isofor;
266 Safe Line Pharmaceuticals, Johannesburg, South Africa), administered in oxygen via
267 face mask. We first obtained a blood sample (~ 2 mL) from the jugular vein of each
268 individual, using a 25 G needle and 2-mL syringe. We immediately transferred blood
269 samples to serum separator tubes (BD Vacutainer; BD Franklin Lakes, NJ, USA) and
270 allowed them to clot at ambient temperature. Following a morning's captures, we
271 centrifuged the blood samples at 3000 rpm for 10 min and pipetted the serum layer into
272 a clean Eppendorf tube. We stored serum samples on site at -20 °C until transport, on
273 ice, along with all fecal samples (see above), to Duke University in Durham, North
274 Carolina, where we stored samples at -80 °C until further processing or analysis.

275 The animals that received flutamide, at roughly 15 mg/kg/day (Table S1), or
276 placebo underwent a minor surgical procedure performed by JD, a veterinarian licensed
277 in South Africa. Using sterile procedures, we implanted one 21-day release pellet (either

278 150 mg flutamide (treatment) or carrier only (placebo), Innovative Research of
279 America, Sarasota, FL) subcutaneously between the subject's shoulder blades. Briefly, a
280 dorsal skin incision of 1-2 cm was made using a scalpel, a small subcutaneous pocket
281 was created using blunt dissection, and the pellet was inserted using forceps. Incisions
282 were sutured using dissolvable material (Vicryl). These subjects also received a
283 subcutaneous injection of a non-steroidal, anti-inflammatory painkiller (0.2-0.3mg/kg
284 meloxicam: Metacam, Boehringer) at the time of capture. The animals that served as
285 no-pellet controls underwent captures and blood sampling only. After recovery from
286 anesthetic, all of the subjects were immediately returned to their groups (20-30 min
287 postcapture) and closely monitored throughout that and the following day. One male
288 developed a minor infection at the implant site, for which he received a 3-day course of
289 antibiotics (5-10 mg/kg enrofloxacin: Baytril, Bayer), injected subcutaneously, by
290 gently lifting the skin, once per day. Animals in this population are sufficiently well-
291 habituated that injections can be administered to conscious animals, typically while they
292 are foraging. We suspended data collection from this animal during his period of
293 medication.

294

295 *Behavioral data collection*

296

297 We began data collection two days following surgery. We conducted focal
298 observations (Altmann, 1974) of our subjects roughly 3 days per week (average = $3.1 \pm$
299 0.35 days) across the 3-week treatment period. Morning sessions began as soon as about
300 half of the group had emerged from the sleeping burrow. Because most prosocial
301 interaction occurs while meerkats are clustered and sedentary, including during the brief
302 periods spent at the burrow, we conducted a series of short (~ 5 min) 'burrow focals' (in

303 random order) to ensure that we obtained some data from all focal subjects in a given
304 group before the meerkats began to forage and disperse. Thereafter, we conducted
305 longer, 30-min ‘foraging focals’ (rotating through our subjects in random order) until
306 the group settled into its mid-day siesta. After a break of several hours, we used
307 radiotelemetry to relocate the group, which had typically recommenced foraging.
308 Evening sessions thus began with foraging focals and ended with burrow focals that
309 were terminated once about half of the group had entered its sleeping burrow. Using this
310 regimen, we collected 524 focals, representing over 130 hours of behavioral data.

311 We collected behavioral data in real time using the CyberTracker software
312 package (version 3.263, CyberTracker Conservation) on handheld palm pilots (Palm
313 T|X, Palm, Inc.). We established our data recording protocol (see ESM, §a) and
314 ethogram (Table 1) for use both during burrow and foraging focals. For all social
315 interaction, we included the partners and the directionality of behavior. We paused
316 observation whenever the focal subject was out of view (e.g. if it entered a ‘bolt hole’
317 following a predator alarm call) and resumed observation once the focal subject was
318 back in sight. We recorded the frequency and, in some cases, duration of behavior,
319 which fell into the following seven categories: (1) aggression, (2) submission, (3) play
320 (Fig. 1), (4) other prosociality, (5) vigilance, (6) olfactory communication and (7) vocal
321 communication (see Table 1). Because occurrences of submission were so rare, we
322 dropped this category from our analyses. Also, owing to a drought-induced shortage of
323 pups at the time of our study, there were no opportunities to observe babysitting or pup
324 provisioning; therefore, the only cooperative behavior included in our study were
325 various forms of vigilance. For details about the vocal analyses, see below. In assessing
326 intra- and inter-observer reliability for the remaining five behavioral categories, we
327 obtained indices of concordance that were minimally 87.0% (see ESM, §c).

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- Insert Table 1 and Fig 1 -

Vocal recordings and sound analysis

We assessed any potential treatment effects on vocalizations by examining the rate and acoustic structure of meerkat close calls, which are thought to be important in the maintenance of group cohesion (Manser, 1998). We conducted 5-15 min sound focals on each individual every third day during the treatment period, resulting in 5-7 recording sessions per male (12 hours of sound recordings in total). We recorded close calls during the mornings, after groups had left the sleeping burrow and the focal males had started foraging. We recorded individuals from a distance of 0.5-1.0 m with a directional Sennheiser microphone (ME66 with a K6 power module and a MZW66 pro windscreen, Old Lyme, CO, U.S.A) connected to a Marantz Professional PMD661 solid-state recorder (16bit, 44.1kHz, Marantz Japan Inc.).

We assessed the calls for quality using Cool Edit 2000 (Syntllillium Software Corporation, Phoenix, AZ, USA), selected for analyses 16-68 calls per individual, and carried out quantitative acoustic analyses in Praat v.5.3.84 (www.Praat.org). From each call, we selected four acoustic parameters, including the number of pulses, call duration (s), average pulse duration (s), and mean fundamental frequency (F0, Hz), as these have been shown to be affected by androgen concentrations in other species (Bass and Ramage-Healey, 2008; Fusani et al., 1994; Pasch et al., 2011; Rek et al., 2011). We based final analyses on 554 calls for all acoustic parameters, except average pulse duration, which was based on 324 calls because the duration of all pulses in the calls could not always be reliably calculated.

353 *Enzyme immunoassays*

354

355 To prepare fecal samples for analysis, we lyophilized, pulverized, and sifted fecal
356 samples into a fine powder within six months of collection, and stored the powder in
357 vials at -80 °C until extraction. We extracted steroid metabolites from fecal samples
358 following a protocol described elsewhere (Starling et al., 2010; Wasser et al., 2000).
359 Briefly, we weighed 0.2 g of dry fecal powder and mixed it with 2 mL of 90%
360 methanol. We placed the mixture on a rotating shaker for 30 min and centrifuged it
361 twice, discarding the sediment each time. We stored the methanol-extracts at -80 °C
362 until analysis.

363 We analysed serum and fecal extracts for circulating T and androgen metabolites
364 (hereafter fecal T or fT), respectively, via enzyme immunoassay (EIA). We used an
365 anti-T antibody raised in mice (Fitzgerald Industries International) that cross reacts
366 100% with T, 9% with dihydrotestosterone, < 1% with androstenediol, and < 0.1% with
367 androstenedione, estriol, estradiol, and progesterone. We paired this antibody with a
368 matched T 3-CMO-HRP conjugate (Fitzgerald Industries International). Plate
369 sensitivity was 0.2-12.5 ng/mL. Our EIA protocol is detailed in the ESM (§d).

370 To assess intra-assay reliability we assayed low, medium, and high controls in 10
371 wells on each of two plates. The average coefficient of variation (CV) between the two
372 plates was 9.8% (low control), 7.7% (medium control), and 5.7% (high control). Inter-
373 plate reliability was assessed by assaying low, medium, and high controls in duplicate
374 on each of 10 plates. The average interplate CV was 5.5% (low control), 6.4% (medium
375 control), and 6.8% (high control). Serial dilutions of serum and fecal extracts pooled
376 from multiple individuals produced linear displacement curves that were parallel to the
377 T standard curve. We also combined serum and fecal pools with low, medium, and high

378 concentrations of T prior to analysis. Recovery percentages for serum spikes were
379 90.2% (low control), 108.1% (medium control), 95.4% (high control), and for fecal
380 spikes were 93.6% (low control), 110.5% (medium control), and 104.3% (high control).
381 To assess our extraction efficiency for fecal samples, dried feces from multiple subjects
382 were pooled and spiked with T prior to extraction. Extraction efficiency was 85.4%.

383

384 *Physiological validation*

385

386 One means of biological validation of fecal hormone metabolites is to show that
387 the metabolites reveal a physiologically relevant difference across groups, detectable
388 from varying circulating hormone concentrations (Brown et al., 2005). We performed a
389 biological validation of fT in wild meerkats and obtained the expected age-related
390 change in fT characteristic of male puberty (Beehner and Whitten, 2004) (see ESM, §e
391 and Fig. S2). Another means of validation, particularly for showing a cause-and-effect
392 relationship, is to administer a drug known to stimulate hormonal production. In this
393 case, our administration of flutamide might also serve as a biological validation of our
394 assay, because in sufficient doses, flutamide is known to impair the negative feedback
395 loop, somewhat paradoxically raising T concentrations (Hellman et al., 1977).

396 Accordingly, flutamide-treated animals might reveal an initial increase in fT relative to
397 control animals.

398

399 *Statistical analyses*

400

401 We conducted our statistical analyses using R, version 2.15.2 (R Core Team,
402 2012), and SPSS 22.0. We set significance at $P < 0.05$. After log transformation, our

403 endocrine data, which derived both from fecal and serum samples, were normally
404 distributed. To determine if serum T concentrations between our experimental
405 conditions (flutamide, placebo, no-pellet) differed prior to treatment, we ran a single
406 ANOVA using the aov function in R. Once we determined that the placebo and no-
407 pellet conditions did not differ (see results), we combined these two conditions and ran
408 a single student's *t*-test to compare serum concentrations of all control subjects against
409 those of flutamide-treated individuals.

410 We tested the influence of flutamide treatment on fecal T metabolites by
411 implementing a series of generalized linear mixed models (GLMMs) using the
412 glmmADMB package, version 0.7.4 (Skaug et al., 2013) in R, using Gaussian
413 distributions. The log of fT (ng/g) was entered as the response variable in each model.
414 The fixed effects in the full model were treatment (three levels: flutamide, placebo or
415 no-pellet), treatment period (two levels: pre-treatment or treatment), and time of
416 deposition (two levels: AM or PM). We included the individual nested within its social
417 group as a random effect. Following Crawley (2002), we included all probable
418 independent terms and interactions in the full model and excluded terms sequentially
419 until the model contained only statistically significant terms.

420 If fT concentrations did not differ significantly between the placebo and no-pellet
421 treatments, we pooled these two conditions in a single 'control' treatment and reran
422 models with only two levels for the treatment factor (flutamide and control). Because
423 treatment period (i.e. pre-treatment or treatment) influenced fT concentration, we
424 subsequently re-ran the model within the two treatment periods. Moreover, as the
425 response to flutamide treatment may have been different across weeks, we ran a model
426 within each week of treatment. For all of the models, we included all probable

427 independent terms and interactions in the full model and excluded terms sequentially
428 until the model contained only statistically significant terms (Crawley, 2002).

429 For the behavioral data, we also used the glmmADMB package, version 0.7.4
430 (Skaug et al., 2013) to implement GLMMs with zero-inflation. Each behavioral
431 category was entered as the response variable. The fixed effects in the full model were
432 treatment (three levels: flutamide, placebo or no-pellet), days on treatment (continuous
433 variable), location (two levels: burrow or forage), time of day (two levels: AM or PM),
434 and group size (continuous variable). Individual and group identities were entered as a
435 nested random effect in the models. The duration of each observation was accounted for
436 as an offset in the model. If a behavior did not significantly differ between the placebo
437 and no-pellet conditions, we pooled these two treatments in a single control treatment
438 and reran the model with only two levels for the treatment factor (flutamide and
439 control). As in our endocrine analyses, we included all probable independent terms and
440 interactions in the full model and excluded terms sequentially until the model contained
441 only statistically significant terms. For each model we used the Poisson and negative
442 binomial distributions and selected the model with the lowest AIC value.

443 We analysed call rates and vocal parameters using linear mixed effects models
444 (procedure lmer from package lme4 in R, version 1.1-7), except for number of pulses,
445 which we analysed using general linear mixed effects models with specified poisson
446 distribution (glmer procedure, nlme package version 3.1-118). We calculated call rates
447 for each recording session. We used treatment (three levels: flutamide, placebo or no-
448 pellet) as a between-subjects factor and individual identity in all models to account for
449 multiple observations per individual. Call rates and average pulse duration were natural-
450 log transformed to conform with linearity assumptions. Call rate analyses are based on
451 100 sound recordings.

452

453 **RESULTS**

454

455 *Baseline androgen patterns*

456

457 During the baseline week of fecal endocrine monitoring, prior to treatment
458 administration, subordinate male meerkats that were to receive placebo, no pellet, or
459 flutamide did not differ in their fT concentrations (ANOVA: $F_{2,11} = 0.65$, $P = 0.53$).
460 These pre-treatment placebo and no-pellet conditions did not differ from each other (t -
461 test: $t_{10,903} = 0.29$, $P = 0.78$), nor did males in the single collapsed, control group differ
462 in their baseline fT values from males that were assigned to the flutamide condition (t -
463 test: $t_{11,161} = 1.10$, $P = 0.29$). Likewise, serum T concentrations from blood samples
464 collected at the time of capture (representing a more immediate pre-treatment baseline)
465 did not vary by the three eventual experimental conditions (ANOVA: $F_{2,11} = 0.62$, $P =$
466 0.55). There were also no differences in circulating T when the males assigned to the
467 two comparable control conditions (t -test: $t_{4,25} = 0.54$, $P = 0.62$) were collapsed and
468 compared against the males assigned to the flutamide condition (t -test: $t_{11,87} = -1.01$, $P =$
469 0.33). Thus, there were no baseline differences in the androgen profiles of our subjects.

470

471 *Effect of flutamide on fecal androgens*

472

473 During treatment, no-pellet and placebo males also did not differ in their fT
474 concentrations, either across all weeks of treatment (z value = -0.52 , $P = 0.60$) or when
475 considering the first (z value = -1.14 , $P = 0.25$) and second (z value = -0.4 , $P = 0.69$)
476 weeks separately. We had too few fecal samples from these males in week three to

477 compare these two conditions in the last week. Given the lack of differences, we
478 collapsed the two control categories in subsequent analyses.

479 Despite the absence of an overall difference in fT concentrations between
480 flutamide and control males across the entire 3-week treatment period (z value = 1.12, P
481 = 0.26), there was a clear time course in the effect of antiandrogen treatment on fT (Fig.
482 2). Notably, in the first week of treatment, flutamide-treated males showed the expected
483 effect of this form of antiandrogen treatment and had significantly greater fT
484 concentrations than did control males (z value = 3.71, $P < 0.001$; Fig. 2). Thereafter,
485 this difference disappeared: Flutamide and control males no longer differed in fT in
486 either the second (z value = -0.8, $P = 0.42$) or third (z value = 0.39, $P = 0.70$) weeks of
487 treatment.

488 - Insert Fig 2 -

489

490 *Behavioral equivalence between placebo and no-pellet conditions*

491

492 Consistent with their equivalent androgen values (and intact androgen function),
493 males in the no-pellet and placebo conditions did not differ in any of their behavioral
494 patterns. This equivalence was true for week 1 only (see ESM, §f and Table S2),
495 confirming that, after a 48-hour recovery period, the minor surgery for pellet implants
496 had no effects on behavior. Moreover, the same pattern of behavioral equivalence
497 maintained across all weeks of the study, as evidenced, for instance, by initiating (z
498 value = -0.02, $P = 0.98$) and receiving (z value = -1.62, $P = 0.11$) high-intensity
499 aggression (HIA; see Table 1) or initiating (z value = -1.64, $P = 0.10$) and receiving (z
500 value = -0.09, $P = 0.93$) prosocial interaction (see ESM, §f and Fig. S3). Therefore, we

501 collapsed the two control categories in subsequent behavioral comparisons against
502 flutamide-treated males.

503

504 *Effects of flutamide on behavior and vocal parameters*

505

506 As expected, compared to all control males, flutamide-treated males initiated
507 significantly less (z value = -2.93, $P = 0.003$; Fig. 3a) and received significantly more (z
508 value = 2.10, $P = 0.036$; Fig. 3a) HIA (Table 2). The most frequent aggressive behavior
509 within the HIA category was food competition, which we examined independently.
510 Compared to all control males, flutamide-treated males initiated significantly fewer
511 foraging competitions (z value = -2.91, $P = 0.004$). The rates of receiving foraging
512 competition, however, were not affected by treatment (z value = 1.07, $P = 0.29$).

513 Flutamide treatment also altered certain aspects of social play. Compared to all
514 control males, flutamide-treated subjects were significantly less likely to initiate play
515 using the play-face invitation (z value = -4.32, $P < 0.0001$; Fig. 1a and Table 2). As
516 anticipated, flutamide treatment also decreased the expression of 'dominant' types of
517 play, such as pinning during wrestling (Fig. 1b). Whereas control and flutamide males
518 were equally likely to play in a 'subordinate' (e.g. pinned) position (z value = -0.97, $P =$
519 0.33), flutamide-treated males played significantly less in the dominant position than
520 did control males (z value = -2.09, $P = 0.036$; Fig. 3b).

521 Compared to all control males, flutamide-treated males also initiated significantly
522 more prosocial behavior at the burrow after foraging (z value = 1.99, $P = 0.046$; Table 2
523 and Fig. 3c). We could detect no effect of receiving other prosocial interaction relative
524 to an individual's treatment (z value = 1.4, $P = 0.16$). Interestingly, when considering
525 the identity of the focal subjects' partners in all of these aggressive, playful, and

526 prosocial interactions, the vast majority (82.3%) occurred with non-focal group
527 members (see ESM, §f and Table S3). The minimal involvement of the dominant male
528 and other flutamide-treated subjects suggests, respectively, that the effects of treatment
529 were unlikely to have been biased by the dominant, male breeder in each group or
530 confounded by having flutamide-treated animals as both the actor and recipient in given
531 interactions.

532 Unlike the patterns we observed for direct social interaction, flutamide males did
533 not differ from control males in their more solitary expression of vigilance (z value =
534 0.23, $P = 0.82$) or scent-marking (z value = -0.27, $P = 0.79$) behavior (Table 2, although
535 see Fig. S1b). Also contrary to expectations, call rate was not affected by treatment
536 (LMM: all $t < 0.19$, all $P \geq 0.8$). Instead, individual identity explained a large proportion
537 of the variation in all models, revealing high individual variability in all of the measured
538 acoustic parameters of close calls (see ESM, §g and Table S4).

539 - Insert Fig 3 and Table 2 -

540

541 *Time course of behavioral treatment effects*

542

543 The number of days that subjects spent on treatment explained little to none of
544 the overall variance in our GLMM models. Owing to limited sample sizes, non-normal
545 distribution, and zero-inflation, we lacked the statistical power to further test for time-
546 course effects in our data. Nevertheless, for comparison with the endocrine effects (Fig.
547 2), similar graphical representation of various types of behavior across weeks of
548 treatment shows consistency in the relationship between flutamide-treated and control
549 animals and, if anything, that treatment effects became stronger (rather than weaker)
550 with time (Fig. 4).

551

552

- Insert Fig 4 -

553

554 **DISCUSSION**

555

556 In this first-ever, experimental manipulation of androgen action in wild meerkats,
557 we found that androgens were involved in regulating a range of social behavior among
558 subordinate, male helpers. Specifically, based on the effects of the antiandrogen,
559 flutamide, we deduce that androgens facilitate various forms of aggressive and
560 dominance interaction, influence aspects of social play, and dampen prosociality or
561 affiliative behavior. By contrast, androgens have potentially no effect on cooperative
562 antipredator behavior, scent marking or various parameters of close-call vocalizations.
563 Given that T concentrations do not differ between the social classes of adult male
564 meerkats (Carlson et al., 2004), androgens may not fully explain the social stratification
565 and behavioral roles of breeders and helpers; nevertheless, based on present results,
566 circulating androgens clearly play an important part in the daily, social lives of
567 subordinate males, perhaps maintaining their reproductive potential and roaming
568 proclivities to overcome the limited, unpredictable, and fleeting nature of their breeding
569 opportunities.

570 We found no inherent bias in circulating or fecal androgen concentrations between
571 our control and treated subjects, but we observed a significant, short-term (i.e., week-
572 long) rise in fT concentrations as a result of blocking a subordinate male's androgen
573 receptors with flutamide. This seemingly paradoxical result is consistent with effects of
574 flutamide treatment observed in other studies (e.g. Stone and Clejan, 1991) and likely
575 owes to a decrease in androgen negative feedback causing a compensatory increase in

576 androgen production (Södersten et al., 1975). Beyond indicating that our early flutamide
577 treatment was successful and that we had achieved an effective dosage, this result
578 represents a second physiological validation of our assay of fecal androgen metabolites
579 (the first being detection of pubertal endocrine changes). Nevertheless, there is great
580 variation across studies in the impact of flutamide on circulating androgen
581 concentrations: In some cases, significant behavioral impacts of flutamide treatment
582 occur without any increase in T (Searcy and Wingfield, 1980) or occur even with a
583 decrease in T (Hegner and Wingfield, 1987); in other cases, increases in T remain in
584 effect long-term and until cessation of treatment (Stone and Clejan, 1991) or, as in our
585 study, over only a short time span (e.g. from day 5 to 7 of a week-long treatment,
586 despite daily injections: Södersten et al., 1975; see also Fusani, 2008). This range of
587 physiological responses to flutamide treatment across studies could owe to the varying
588 dosages achieved, the mode of administration used, the social context or the species
589 tested.

590 The possibility exists that the decrease in fT we observed after week 1 might have
591 indicated that, rather than lasting the full 21-day period, the pellets were exhausted after
592 only one week. This interpretation is contradicted by the persistent behavioral effects of
593 treatment across weeks, suggesting instead that androgen-receptor blockade remained in
594 effect, but that feedback mechanisms may have stabilized, effectively ‘resetting
595 homeostasis’, or that initial receptor blockade had lasting behavioral consequences,
596 perhaps via altered receptor sensitivity (Fusani, 2008). Although we cannot distinguish
597 between these alternative mechanistic explanations, it is clear that behavioral effects of
598 antiandrogen treatment persisted minimally throughout the three-week study period.

599 These behavioral effects of flutamide administration, as expected, were manifest
600 in meerkat aggressive behavior, with treated males initiating less, but receiving more,

601 aggression than controls. The reduced initiation of aggression by treated males provides
602 strong evidence for a direct effect of androgens on agonism. That treated males also
603 received more aggression from conspecifics implicates additional indirect effects of
604 androgens on behavior. Perhaps group members perceived a difference or ‘weakness’ in
605 flutamide-treated males, which may have prompted an increase in the frequency with
606 which treated subordinates were targeted. Alternately, the stability of social relations
607 among subordinate males may be partially maintained by balanced interactions, such
608 that a mismatch in the aggressive performance between flutamide-treated males and
609 controls may have led to an escalation in the aggression against treated animals.

610 We also found that androgen-receptor blockade mediated certain aspects of social
611 play in adult meerkats. Notably, flutamide-treated males initiated less play and were
612 less dominant in their expression of social play than were control males. Thus, in the
613 absence of androgenic influence, male meerkats were less bold, assertive, or
614 competitive in their play. Although the directionality in these patterns is not unexpected,
615 these findings provide rare evidence of activational effects of androgens on adult social
616 play. Across mammalian taxa, prenatal, neonatal or prepubertal androgens have been
617 shown to influence rough-and-tumble play, specifically, during infancy or juvenility
618 (Meaney et al., 1985; Panksepp, 1981; Pedersen et al., 1990; Pellegrini, 1995). Those
619 studies established that organizational, rather than activational, T is important for
620 modulating social play (Meaney et al., 1985) – a generalization that is called into
621 question by our present findings.

622 Flutamide administration also affected other prosocial interaction, although in the
623 opposite, enhancing direction. Flutamide-treated males were more likely to initiate
624 affiliative behavior, such as grooming, huddling, and social sniffing. Combined with the
625 depressive effects of flutamide on the initiation of aggressive or dominance behavior,

626 these results are consistent with the hypothesis that there is an androgen-mediated trade-
627 off between aggression and affiliation (Albers et al., 2002; Hegner and Wingfield, 1987;
628 Ketterson and Nolan, 1994). Nonetheless, it must be noted that in some monogamous or
629 cooperatively breeding mammals, T (either directly or following conversion to
630 estrogen) can promote, rather than inhibit, paternal or affiliative care (Storey et al.,
631 2006; Trainor and Marler, 2001, 2002). We might therefore have expected androgen-
632 receptor blockade to influence various facets of meerkat cooperation, but based on
633 vigilance behavior only, we found no such evidence. These results are in accord with a
634 previous study that found no relation between another form of cooperation – pup
635 provisioning – and T in subordinate males (Carlson et al., 2006a). As indicated by the
636 relation between prolactin and babysitting (Carlson et al., 2006b), other neuroendocrine
637 circuits may be involved in promoting pup care.

638 Conservatively, we might interpret that androgen function does not play a pivotal
639 role in regulating cooperative behavior in adult meerkats; however, it is important to
640 note that we lack information about any role androgens may play in prenatally priming
641 meerkats for their adult behavioral repertoire. In humans, for instance, there is evidence
642 to suggest that T's action in promoting prosociality or cooperation may stem from
643 prenatal androgen exposure. Specifically, experimentally increasing circulating T in
644 humans leads to an increase in cooperative behavior, but only in those individuals who
645 had low prenatal exposure to androgens (van Honk et al., 2012).

646 Antiandrogen treatment also did not appear to influence scent-marking behavior,
647 including anal marking, body rubbing, chewing, and chinning vegetation. Nonetheless,
648 although expressed evenly among the treatment groups, scent marking occurred in only
649 22 focal observations (4.2%). It may be that these null results reflect a floor effect of
650 low scent-marking frequencies by subordinate males, rather than any lack of androgenic

651 involvement in olfactory behavior or odorant quality. Although Jordan (2007) reports
652 no rank-related difference in male marking patterns at latrines, we suspect that the
653 marking behavior of male meerkats may be strongly rank related in other contexts (see
654 Leclaire et al., 2014).

655 Although androgens have been shown to affect vocalizations in various species,
656 including humans (e.g. Gyger et al., 1988, Charlton et al., 2011; Baker, 1999, Damrose,
657 2009), we did not detect any significant effects of flutamide treatment on meerkat close
658 calls. These null results, albeit consistent with the findings of some antiandrogen studies
659 in avian species (Grisham et al., 2007; Schwabl and Kriner, 1991), may owe, in part, to
660 the significant individual variability we observed: This variability confirms previous
661 findings of individual-specific close calls in meerkats (Townsend et al., 2010), but it
662 may have overridden any potential treatment effects. Alternately, it may be that close
663 calls produced during foraging are particularly insensitive to the actions of androgens.
664 Indeed, previous findings of significant androgenic or antiandrogenic effects on
665 vocalizations have involved calls produced in the contexts of reproductive
666 advertisement and antipredator behavior (Ball et al., 2003, Behrends et al., 2010,
667 Charlton et al., 2011, Gyger et al., 1988). In the future, it may be worth exploring if
668 meerkat vocalizations produced in more directed social interaction relate to circulating
669 androgen concentrations.

670 In summary, we found that androgen receptor blockade had important effects in
671 wild, subordinate male meerkats beyond modulating aggression: antiandrogens affected
672 a broad range of social interaction, from competitive to affiliative behavior. Continued
673 studies of equally ranked individuals are thus likely to reveal new insights into the
674 hormonal regulation of behavioral interaction. Whereas androgens are increasingly
675 recognized for their role in mediating social behavior, estrogens have received

676 considerably less attention, particularly in males. Because androgens can be readily
677 converted to estrogens, depending on local enzyme activity, addressing the role of
678 estrogens in monogamous and cooperatively breeding species will be an important next
679 step. In future studies, researchers should also examine the role of prenatal androgens in
680 establishing receptor distribution that might help explain how differential activational
681 responses may arise from animals showing roughly equivalent endocrine profiles. That
682 influencing the action of activational androgens could have such wide-ranging effects
683 within members of the same social class leads us to expect even more dramatic
684 influences of organizational androgens. It is noteworthy that all of the effects we
685 observed became evident in a relatively short time span. With longer-term endocrine
686 manipulation, even greater effects may be revealed. In sum, experimental endocrine
687 manipulation in the field, albeit challenging, is key to revealing the mechanisms
688 supporting social relationships, within and between classes.

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690

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705

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1046 **Table 1**

1047 Ethogram for codifying meerkat (*Suricata suricatta*) behavior. The definitions for
 1048 behavior, grouped by category (i.e., aggression, submission, play, prosociality,
 1049 vigilance, olfactory, and vocal) and subcategory (e.g. high- vs. low-intensity
 1050 aggression), are adopted primarily from meerkat studies, but also from studies of other
 1051 carnivores.

1052

Behavior by category	Definition	References
<i>Aggression</i>		
Bite ^a	Grabbing, with one's teeth, any part of another individual's body, ranging from quick forceful nips to prolonged or intense contact.	Clutton-Brock et al., 2006
Chin rub ^a	Touching or wiping another with one's chin, often accompanied by head shaking.	Kutsakake and Clutton-Brock, 2008
Food competition ^a	Approaching another's food item or hole, prompting a defensive response via growling, blocking approach, pushing, threatening, and biting.	Ewer, 1963; Madden et al., 2009
Hip slam ^a	Using one's hindquarters to push intensely against the side of another individual.	Clutton-Brock et al., 2006
Push ^a	Slamming one's hindquarters against another's in an interaction that can be resolved immediately or can persist for a measurable duration.	Madden et al., 2009
Threat ^a	Lunging at another individual, often accompanied by growling.	Drea et al., 1996
Block approach	Shifting one's body to prevent another's access to a resource.	Ewer, 1963; Madden et al., 2009
Chatter ^b	Breathy, repetitive clucking vocalization.	Ewer, 1963
Growl ^b	Emitting a low, rumbling vocalization.	Clutton-Brock et al., 2006
<i>Submission</i>		
Grovel	Adopting a crouched body posture, often while peeping.	Clutton-Brock et al., 2006
Peep ^b	High-pitched vocalization, often occurring in rapid repetition.	Clutton-Brock et al., 2006
<i>Play</i>		
Play bite ^c	Short nips that are not forceful; commonly expressed during wrestling and grappling, but only scored when independent of	Ewer, 1963; Wemmer and Fleming, 1974

	wrestling or grappling.	
Play bite shake ^c	Non-harmful, open-mouth contact of another individual's body using a slow, side-to-side motion of one's head.	Drea et al., 1996
Play chase ^c	Pursuit with a bouncy gate.	Ewer, 1963; Wemmer and Fleming, 1974
Play mount ^c	Clasping another individual's ribcage or groin while participants are a ventro-dorsal position.	Wemmer and Fleming, 1974
Stand on ^c	Simultaneously placing both forelimbs on the torso of an individual that is either sitting or prone.	Wemmer and Fleming, 1974
Wrestle-top or wrestle-bottom ^c	Vigorous, mutual rolling around or pushing, often coupled with play biting, shaking, pawing, and clasping.	Wemmer and Fleming, 1974
Play face	Type of play initiation involving an exaggerated open mouth, often shown while in a prone body position with the tail pointing upward.	Drea et al., 1996
<i>Other prosociality</i>		
Groom	Moving the mouth/teeth through another's fur; recorded as a dyadic interaction for each pair of individuals; considered as a new bout after switching to a new partner or after 1 min of inactivity.	Ewer, 1963; Madden et al., 2009
Social sniff	Olfactory investigation of another individual.	Drea et al., 2002
Sniff genitals	Olfactory investigation of individual's genital region.	Drea et al., 2002
Huddle	Body contact with another individual; recorded as one event regardless of how many individuals are involved.	Madden et al., 2009
<i>Vigilance</i>		
Guard ^d	Standing on the ground, on hind legs, while scanning the environment.	Clutton-Brock et al., 1999
Raised guard ^d	Standing on a raised (>10 cm) perch, on hind legs, while scanning the environment.	Clutton-Brock et al., 1999
Other vigilance	Quick scans of the environment from a quadrupedal position.	English, 2009
<i>Olfactory</i>		
Anal mark environment	Everting the anal pouch and rubbing it across a vertical or horizontal substrate.	Ewer, 1963; Moran and Sorensen, 1986
Chin rub environment	Wiping of the face or cheek region across a substrate.	Moran and Sorensen, 1986
Chew marking	Biting vegetation, usually accompanied by rapid head shaking.	Jordan, 2007

<i>Vocal</i>		
Contact or	Short pulsed vocalization made during	Townsend et al.,
close call	foraging, but not during a direct social	2010
	encounter.	

1053

1054 ^a Included in the collapsed subcategory of high-intensity aggression.

1055 ^b Vocalization that is clearly associated with aggressive/dominance or submissive
 1056 interaction, but that we did not record acoustically.

1057 ^c Included in determining ‘dominant’ vs. ‘subordinate’ role assumed during play.

1058 ^d Indicates behavior recorded as a state (all other behavior recorded as an event).

1059

1060 **Table 2**

1061 Effect of flutamide treatment on the behavior of subordinate male meerkats. A 95%
 1062 confidence interval (CI) excluding 0 indicates a statistically significant relationship. The
 1063 *P* values and CI that indicate statistical significance are shown in bold.

Dependent variable	Treatment	<i>P</i> value	95% CI
	coefficient¹		
Initiate aggression (all types)	-0.15	0.37	(-0.47) – 0.17
Receive aggression (all types)	0.21	0.17	(-0.09) – 0.51
Initiate high-intensity aggression	-0.75	0.003	(-1.26) – (-0.25)
Receive high-intensity aggression	0.43	0.036	0.03 – 0.83
Initiate food competition	-1.05	0.004	(-1.75) – (-0.34)
Receive food competition	0.40	0.29	(-0.34) – 1.14
Play face	-4.32	<0.0001	(-2.65) – (-1.00)
Dominant play	-1.18	0.036	(-2.28) – (-0.08)
Subordinate play	-0.50	0.33	(-1.51) – 0.51
Initiate prosocial behavior ²	0.47	0.046	0.01 – 0.92
Receive prosocial behavior ²	0.28	0.16	(-0.16) – 0.95
Vigilance	0.03	0.82	(-0.20) – 0.25
Scent marking	-0.20	0.79	(-1.67) – 1.27

1064

1065 ¹Positive or negative values indicate that the behavior values were higher or lower in
 1066 response to the flutamide treatment than to the control treatment (including both the
 1067 no-pellet and placebo conditions), respectively.

1068 ²Indicates prosocial behavior that occurred around the burrow system after foraging.

Figure 1: Adult, subordinate male meerkats playing. (A) The individual in the top-left corner is inviting play by showing a 'play face'. (B) Two individuals involved in play wrestling can either occupy a dominant position (shown by the standing animal) or a subordinate position (shown by the pinned animal).

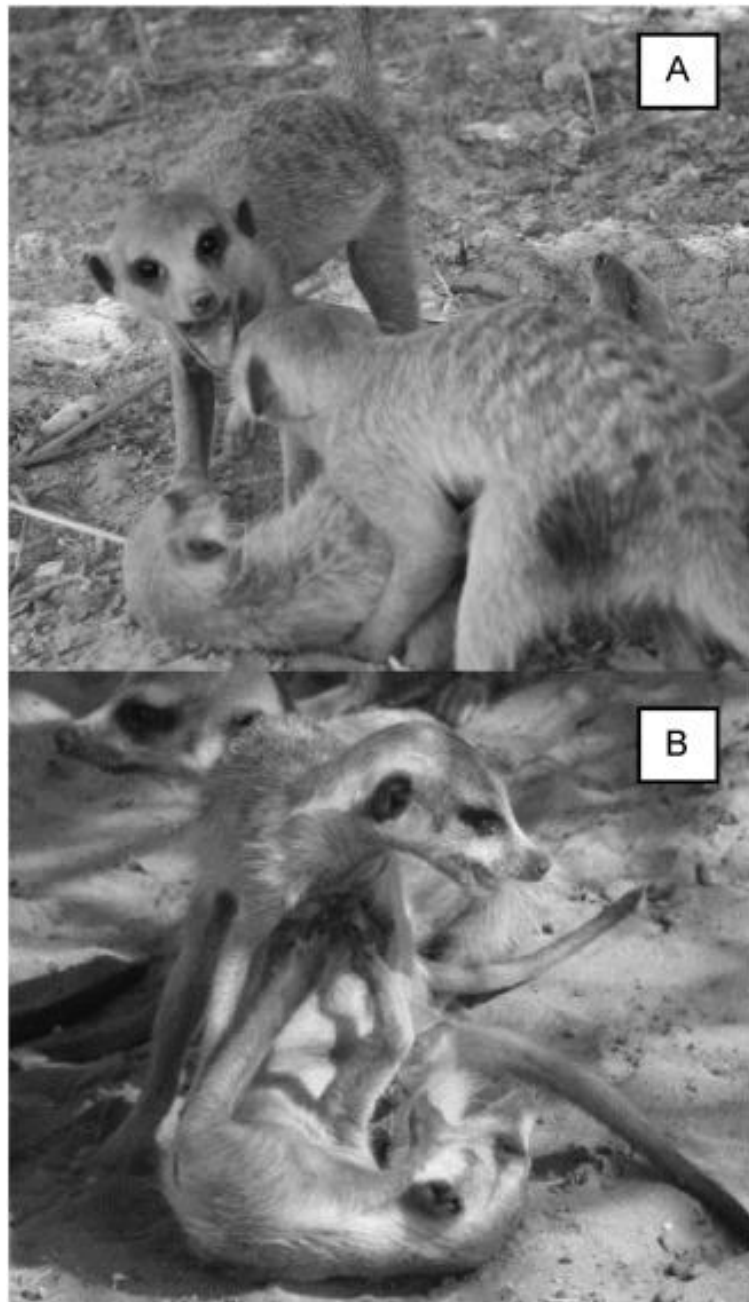


Figure 2: Fecal testosterone in adult, subordinate male meerkats across the three-week treatment period. ***, $P < 0.001$.

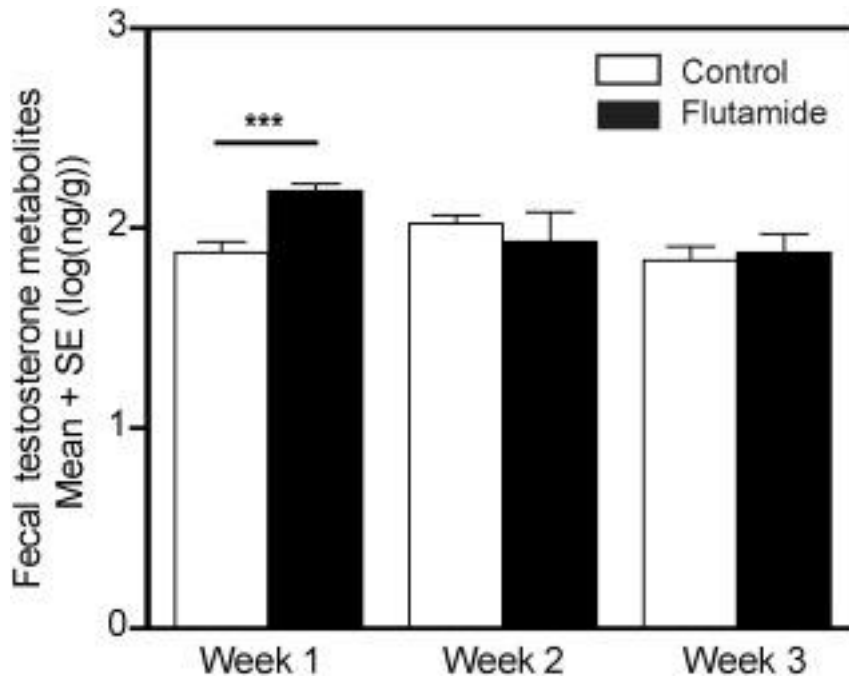


Figure 3: Effect of flutamide treatment on the frequency (per focal) of behavior in subordinate male meerkats: (A) Initiating and receiving high-intensity aggression, (B) playing in the dominant position, and (C) initiating prosocial behavior after foraging around the burrow. *, $P < 0.05$; **, $P < 0.01$.

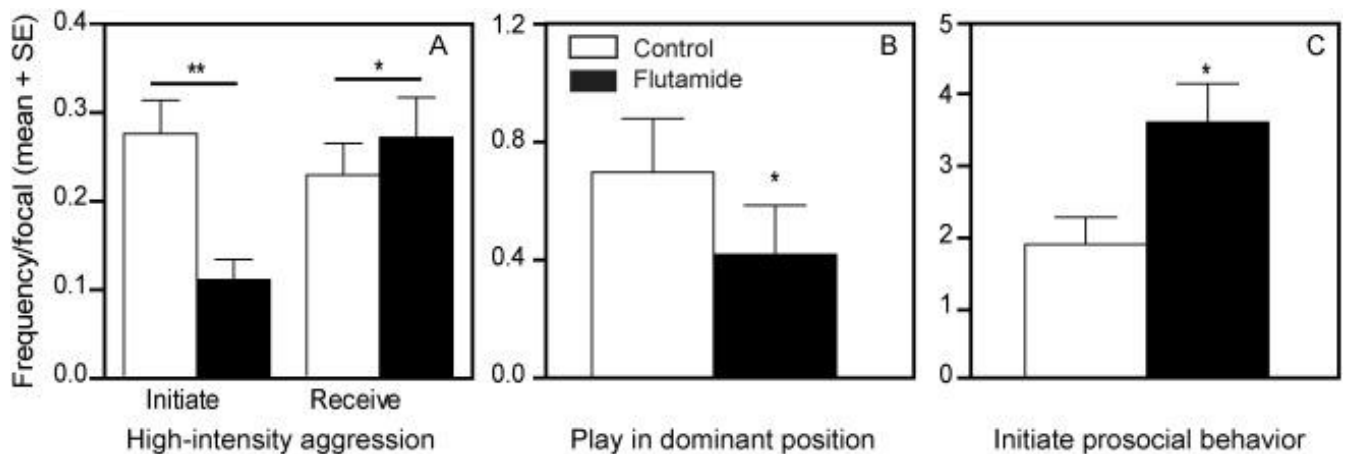


Figure 4: Behavior of adult, subordinate male meerkats across the three-week treatment period: (A) Initiating high-intensity aggression, (B) initiating foraging competition (during foraging focals), (C) rough-and-tumble play, and (D) initiating prosocial behavior.

