

Supplementary materials:

a) Ethograms and behavioural observations protocol

Scan observations

Instantaneous samples (Altmann, 1974) of the behavioural state of every group members were recorded every 4 minutes following an ethogram including 17 distinct behaviours (Table S1) and following a pre-defined and fixed sequence of individuals. Scan observations lasted 4 or 12 hours, leading respectively to the collection of 60 or 180 instantaneous samples per individual. Twelve-hour scan sessions started between 07:00 and 08:00 and were carried out by at least two observers (PV, RF, RL, RK,) alternating shifts every 2 to 4 hours. Four-hour scan sessions started between 15:30 and 16:30 and were carried out by a single observer (PV).

b) Cortisol quantification

Quality control

To allow for the correction of variation in cortisol quantification within and across batches and assess the reproducibility of sample preparation and analysis, control samples were used. Control samples were created by pooling urine samples from captive male and female Damaraland mole-rats to form a male (MC) and a female control sample (FC) that were split into 1ml aliquots. Aliquots were immediately stored at -20°C until hormone analyses. MC and MF aliquots were independently processed prior to each batch and quantified as any other urine sample.

Radioimmunoassays

The RIAs were conducted using a commercially available kit (Coat a Count, Diagnostic Products Corporation, Los Angeles, CA) validated for Damaraland mole-rats (Clarke et al., 2001). Unextracted urine samples were used to measure native cortisol in the urine with all samples analysed in duplicates following procedures described by the kit supplier. Standard

solutions of known cortisol concentrations provided by the supplier were used to establish a reference standard calibration curve. For each sample duplicate, 25 µl of urine were added into a polypropylene tube coated with anti-cortisol antibodies. One ml of tracer solution containing iodinated (¹²⁵I) cortisol was then added to the tubes to enable the cortisol contained in the urine and the radiolabelled cortisol from the tracer solution to compete for the antibody binding sites. After 45 minutes of incubation at 37 °C, the tubes were decanted, and radioactivity was measured with a gamma counter.

Cortisol concentrations were determined using the standard calibration curve derived from the radioactivity measured in tubes of known CORT concentrations. The limit of detection (LOD) varied across batches and ranged from 0.023 to 0.425 ng/dl. Coefficient of variation (CV) was determined using independent control samples (MC and MF) placed at the beginning and at the end of each batch (minimum of 4 control samples/batch). The intra-assay CV was of 5.4%. while the inter-assay CV was of 7.6%.

Ultra-High Performance Liquid Chromatography – Tandem Mass Spectrometry (UHPLC-MS/MS)

For UHPLC-MS/MS analyses, 100 µl of urine was added to 410 µl of a solution containing 400 µl of sodium phosphate buffer (0.1M, pH7) and 10 µl of methanol containing isotopically labelled internal standards at 80, 40 and 800 ng/ml for cortisol-D4, testosterone-D3 and dehydroepiandrosterone-D5, respectively (Toronto Research Chemicals). Spiking labelled internal standards enabled to accurately account for variations resulting from steroid loss during sample preparation and from matrix effects and sensitivity variation in the mass spectrometer over time (Stokvis et al., 2005). Differing from RIA, the glucuronated forms of steroids excreted in the urine were deconjugated by adding 2.5 µl of beta-glucuronidase from *Escherichia Coli* (Roche chemicals) to each sample and allowing 1hour incubation at 50 °C. A solid phase extraction (SPE) using Isolute C18(EC) cartridges (50 mg/1cc, Biotage, Sweden) was then performed. Briefly, the cartridges were conditioned with 1 ml of methanol 100%, equilibrated with 1 ml of methanol 5%, the samples were passed through the cartridges which were then washed with 1 ml of methanol 5% followed by 1 ml of hexane. Steroids were recovered by eluting the cartridges with 1 ml of ethylacetate which was evaporated in a centrifugal evaporator (Labconco) at 35 °C. The dried extracts were finally reconstituted in 100 µl of methanol 50%.

Samples were injected in an Acquity UPLC™ coupled to a Xevo TQ-S triple quadrupole (Waters, Milford, MA, USA) with all aspects of the system optimized for steroid analyses (Binning et al., 2017). Calibration solutions containing cortisol, at 0.1, 1, 20, 100 and 250 ng/ml as well as internal standards were prepared in methanol 50%. The mass spectrometer peaks were integrated using the program Quanlynx™ and normalized to those of the internal standards following an automated method developed at the NPAC. The peak integration was visually controlled for each sample. Calibration equations were separately applied to each batch of samples by selecting the most appropriate model (linear, quadratic or cubic) and weighting factor (in most cases 1/x).

All cortisol concentrations measured in urine samples fell well above the LOQ of the method which was set at a signal to noise ratio of 8 corresponding to 0.7 ng/ml of cortisol. The inter-batch CV, calculated over 36 batches (2494 samples) split in 4 distinct analyses periods spread over 3 years (September 2015 to August 2018), was of 8.36% for FC and 9.12% for MC. Most of the variation between control samples occurred across distinct analyses period rather than within analyses period. We therefore corrected the raw cortisol concentrations for the variation in concentrations that could be explained by variation across analyses period by multiplying the raw cortisol concentration by a correction factor obtained as follow:

Correction Factor = mean [control samples] over all batches / mean [control samples] of specific period of analyses during which sample of interest was analysed.

Applying this correction reduced the inter-day coefficient of variation to 4.8% for FC and 5.3% for MC.

Determination of specific gravity and corrections of raw hormone concentrations

All raw cortisol concentrations were corrected for variation in urine dilution by the determination of urine specific gravity (SG) using a digital hand-held pen refractometer (Atago Ltd). Correction of hormone concentration with SG has been shown to be reliable and arguably more accurate than creatinine correction (Miller et al., 2004). For each sample, triplicate SG values were determined with 10 µl of urine each, at the few exceptions of insufficient urine volume available where only one value was measured. For each urine sample, SG values were averaged and hormone concentrations were obtained following Miller and colleagues (Miller et al., 2004) formula:

$$[\text{Corrected Hormone}] = [\text{Raw Hormone}] \times (\text{SG}_{\text{Population}} - 1) / (\text{SG}_{\text{Target Sample}} - 1),$$

where $\text{SG}_{\text{Population}}$ represents the population average of SG values and $\text{SG}_{\text{Target Sample}}$ represents the SG value of the sample which hormone concentration is to be corrected.

Table S1 – Instantaneous sampling ethogram of scan observation. The left side of the table shows how behaviours were grouped to form variables used in the statistical analyses. Pup carry was only observed once during Experiment 1 and once during Experiment 2 and was thus excluded from analyses.

Variables	Behaviour	Description	
Activity	Food Carrying	Food carrying	Transporting of food pieces
	Nest Building	Nest building	Preparing nest material for transport and transporting nest material
	Burrowing	Digging	Excavating sand using incisors and front paws
		Sweeping	Moving sand backwards using hind legs
		Kicking	Compating sand against tunnel using nose or hind legs
		Locomotion while working	Moving between bouts of the above behaviours
	Non-Cooperation	Locomotion	Moving unrelated with cooperative behaviours
		Sniffing	Investigating objects with the nose
		Eating	Ingesting food
		Self grooming	Hygiene maintenance behaviours directed to the actor's body
		Social interaction	Any interaction with another individual
		Pumping	Repetitive up and down movement of the body
		Other	Any behaviour that cannot be assigned to the described behaviours
		Gnawing	Chewing the plastic tunnels with incisors
	Rest	Resting	Sleeping in the nest or tunnels
Huddling		Resting in the tunnels in physical contact with at least one individual	
Excluded	Carrying pup	Grabbing and/or moving a pup using incisors	

Model outputs of Experiment 1: Cortisol manipulation of female helpers

Table S2 – Validation of cortisol treatment step 1: Effect of treatment on urinary cortisol concentrations.

Two outliers from the cortisol treatments, returning outstandingly high cortisol values by [treatment day 1 \(x on day 3 of treatment, 1x on day 6 of treatment\)](#), were removed from the dataset prior to analysis (n=28 control treatment, n=26 cortisol treatment). Cortisol level was specified as response variable in Gamma GLMM with a log link. Treatment (cortisol and control), subjects' body mass at the beginning of treatment and treatment day (day 3 and day 6) and all possible interactions were specified as model covariates. Interactions were included because we anticipated that the release of cortisol from the implant may not be constant across time and that the degree to which cortisol concentrations were affected by the cortisol treatment may depend on how heavy subjects were. Both aspects were relevant as they may be reflected on the influence of the cortisol treatment on behaviours. The delay between placement in the urine chamber and urination was also included as sampling procedure could represent a stressful event and cause an elevation in cortisol levels. All variables shown in bold were retained in the minimal model. The intercept of the model refers to the predicted cortisol level of a female in the control treatment. Treatment refers to the cortisol treatment and Day to day 6 of treatment. ^a indicates centred variables.

Predictors of CORT - Treatment validation step 1

Covariates	Estimate	SE	test statistic	p-value
Intercept	1.094	0.132	8.301	
Treatment	1.063	0.162	6.542	<0.001
Day	-0.288	0.157	-1.835	0.070
Weight ^a	0.001	0.005	0.227	0.820
Urination delay	0.000	0.003	0.003	0.971
Treatment x Day	-0.518	0.311	-1.670	0.100
Treatment x Weight ^a	-0.002	0.008	-0.270	0.789
Weight ^a x Day	-0.002	0.008	-0.210	0.835
Treatment x Weight ^a x Day	0.015	0.016	0.900	0.371

Table S3 – Validation of cortisol treatment step 2: Comparison of cortisol levels between experimental and non-experimental female helpers. Cortisol condition refers to cortisol levels measured in subjects from experiment 1 that had received a 5mg cortisol implant. Baseline condition refers to cortisol levels that were measured in non-experimental female helpers living in a socially stable environment in which no social conflict was apparent and whose cortisol levels were expected to be low. Eviction condition refers to cortisol levels that were measured in non-experimental female helpers within 2 days after they been evicted from their group by the dominant female and which cortisol levels were expected to be high. One outlier from the cortisol treatment, with an outstandingly high level of cortisol, was removed from the dataset prior to analysis (n=27 cortisol treatment, n=29 baseline n=12 eviction). Cortisol level was specified as response variable in Gamma GLMM with a log link. Urination delay was included in the model as placement in a urine chamber could represent a stressful stimulus and increase cortisol level. Body mass was not included in the model as only non-experimental females of similar body mass than experimental females were included in the dataset. Generalized linear hypotheses testing were performed on all pairwise comparisons between the cortisol treatment, baseline and eviction using the glht function from the multcomp package and p-value adjusted for multiple comparisons using the single method (Hothorn et al., 2008). All variables shown in bold were retained in the minimal model. ^a indicates centred variables.

Deleted: The cortisol treatment was set as a reference in the model to allow direct comparisons of cortisol levels with baseline and eviction conditions

Predictors of CORT - Treatment validation step 2

Covariates	Estimate	SE	test statistic	p-value
Intercept	4.598	0.123	37.440	
Urination delay ^a	0.005	0.002	2.280	0.023
Cortisol Condition				< 0.001
<i>Multiple comparisons of means - Generalized linear hypotheses</i>				
Cortisol treatment - Baseline	0.948	0.181	5.250	<0.001
Cortisol treatment - Eviction	0.407	0.212	1.922	0.132
Eviction - Baseline	0.542	0.213	2.539	0.030

Table S4 – Effect of cortisol treatment on the total expression of cooperative and non-cooperative behaviours in female helpers: Comparisons of the proportion of count of instantaneous samples during 12-hour scan observations between the cortisol and the control treatment for each of the 3 categories of cooperative behaviours and non-cooperative behaviours (n= 14 datapoints per treatment and per day of treatment, leading to a total of 56 datapoints for each category of helping). The count of instantaneous samples during which the subject was recorded performing the activity under investigation was recorded as the response variable in beta-binomial GLMMs with a logit link. The total count of instantaneous samples of the scan observation session was specified as the binomial total. Beta-binomial GLMMs were preferred over conventional binomial GLMMs because of overdispersion. For a-b), d) and f) Treatment day and subjects' body mass, as well as their interactions with treatment, were specified as model covariates as it was expected that the effect of treatment could vary throughout the treatment week and depend on how heavy subjects were. No interactions were specified in b) because the model aims to test whether lower cortisol levels induced by the cortisol treatment had a different effect on burrowing than higher cortisol levels and in e) because of model convergence issues. All variables shown in bold were retained in the minimal model. ^a indicates centered variables.

a) Predictors of total activity (all behavioural states but rest)

Covariates	Estimate	SE	test statistic	p-value
Intercept	-0.649	0.179	-3.628	
Treatment (Cortisol)	0.433	0.126	-3.438	0.001
Day (Day 6)	-0.110	0.124	-0.889	0.376
Body mass ^a	-0.006	0.008	-0.721	0.475
Treatment x Body mass ^a	0.009	0.007	1.372	0.174
Treatment x Day	-0.085	0.245	-0.348	0.728

b) Predictors of burrowing

Covariates	Estimate	SE	test statistic	p-value
Intercept	-2.410	0.191	-12.649	
Treatment (Cortisol)	0.568	0.182	3.126	0.001
Day (Day 6)	-0.213	0.174	-0.898	0.226
Body mass ^a	-0.007	0.008	-0.898	0.382
Treatment x Body mass ^a	0.015	0.010	1.474	0.137
Treatment x Day	0.185	0.350	0.527	0.599

c) Predictors of burrowing, separating cortisol treatment into a low and high cortisol level groups

Covariates	Estimate	SE	test statistic	p-value
Cortisol Low	-1.949	0.223	-8.726	
Control	-0.465	0.224	-2.076	0.004
Cortisol High	0.218	0.255	0.793	0.428

Table S4 (continued)

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d) Predictors of food carrying

Covariates	Estimate	SE	test statistic	p-value
Intercept	-4.571	0.252	18.137	
Treatment (Cortisol)	0.404	0.266	1.517	0.132
Day (Day 6)	-0.218	0.262	-0.830	0.406
Body mass ^a	0.004	0.009	0.456	0.647
Treatment x Body mass ^a	0.010	0.014	0.697	0.487
Treatment x Day	0.031	0.528	0.059	0.953

e) Predictors of nest building

Covariates	Estimate	SE	test statistic	p-value
Intercept	-4.980	0.285	-17.491	
Treatment (Cortisol)	0.120	0.343	0.351	0.725

f) Predictors of active non-cooperative behaviours

Covariates	Estimate	SE	test statistic	p-value
Intercept	-1.177	0.138	-8.541	
Treatment (Cortisol)	0.211	0.095	2.211	0.032
Body mass ^a	-0.005	0.006	-0.814	0.415
Day (Day 6)	-0.026	0.096	-0.266	0.790
Treatment x Day	-0.221	0.188	-1.176	0.243
Treatment x Body mass ^a	0.006	0.005	1.138	0.258

Table S5 – Effect of cortisol treatment on the proportion of burrowing and non-cooperative behaviours during activity period in female helpers: Comparisons of the count proportion of burrowing and during activity period of 12-hour scan observation sessions between the cortisol and the control treatment (n= 14 datapoints per treatment and per day of treatment for each category of cooperative behaviours leading to a total of 56 datapoints). The count of instantaneous samples during which the subject was scored burrowing was specified as the response variable in beta-binomial GLMMs with a logit link. The total count of instantaneous samples for which individuals were recorded as active during the scan observation session was used as the binomial total. Beta-binomial GLMMs were preferred over conventional binomial GLMMs because of overdispersion. Treatment day and subject's body mass, as well as their interactions with treatment, were specified as model covariates as it was expected that the effect of treatment could vary throughout the treatment week and depend on how heavy subjects were. All variables shown in bold were retained in the minimal model. ^a indicates centred variables.

a) Predictors of burrowing during activity bouts

Covariates	Estimate	SE	test statistic	p-value
Intercept	-1.204	0.143	-8.451	
Treatment (Cortisol)	0.391	0.157	2.484	0.014
Day (Day 6)	-0.198	0.152	-1.299	0.200
Body mass ^a	-0.007	0.006	-1.166	0.258
Treatment x Body mass ^a	0.009	0.008	1.022	0.302
Treatment x Day	0.295	0.305	0.967	0.339

Table S6 – Effect of cortisol treatment on changes in body mass of female helpers between the end and the beginning of experimental treatments. Individual differences in body mass were computed by subtracting the body mass measured in the morning following the end of treatment (day 8) and the body mass measured immediately after implantation procedure on the first day of treatment (day 1) (n= 14 datapoints per treatment leading to a total of 28 datapoints). Individual difference in body mass was specified as response variable in a gaussian LMM. To account for the fact that heavier animals may experience larger variation in body mass (regression to the mean effect), the body mass of subjects at the beginning of treatment was specified as a model covariate. An interaction between treatment and body mass at the beginning of treatment was also specified to determine whether the effect of the cortisol treatment on changes in body mass varied as a function of subjects' body mass. ^a indicates centred variables.

Predictors of changes in body mass throughout treatment week				
Covariates	Estimate	SE	test statistic	p-value
Intercept	0.107	0.820	0.131	
Treatment (Cortisol)	-1.214	0.905	-1.342	0.189
Start body mass ^a	-0.028	0.036	-0.768	0.450
Treatment x Start Body mass ^a	-0.015	0.047	-0.324	0.746

Table S7 – Relationship between individual changes in contributions to burrowing and cortisol levels across treatments in female helpers. Differences in the proportion of burrowing were computed by subtracting the proportion of burrowing measured in the 12-hour scan session of the control treatment to the ones measured during the cortisol treatment for each individual and treatment day. Absolute differences in cortisol levels were obtained by subtracting the cortisol levels measured during the control treatment to the ones measured during the cortisol treatment for each individual and treatment day (day 3 and day 6 of treatment). Relative differences in cortisol levels were obtained by dividing the cortisol levels measured during the cortisol treatment to the ones measured during the control treatment for each individual and treatment day (day 3 and day 6 of treatment). Individual differences in the proportion of contribution to burrowing were specified as the response variable in LMMs. Although they were not collinear, absolute and relative differences in cortisol levels had to be specified as explanatory variables in two distinct models because of model convergence issues when they were included in the same model. The proportion of burrowing during the control treatment was specified as a model covariate to account for the possibility that individuals that burrowed more during the control treatment had less scope to increase their burrowing contributions during the cortisol treatment. a) Effects of absolute differences in cortisol levels on changes in burrowing contributions (n=14 individual differences per day of treatment, leading to a total of 27 data points after the exclusion of one data point associated with an exceptionally large difference in cortisol levels). b) Effects of relative differences in cortisol levels on changes in burrowing contributions (n=14 individual differences per day of treatment, leading to a total of 27 data points after the exclusion of one data point associated with an exceptionally large relative difference in cortisol levels).

a) Predictors of changes in proportion of burrowing

Covariates	Estimate	SE	test statistic	p-value
Intercept	0.099	0.028	3.476	
Burrowing proportion control treatment	-0.510	0.190	-2.686	0.013
Absolute cortisol difference (cortisol - control)	-9.675E-05	2.022E-04	-0.479	0.632

b) Predictors of changes in proportion of burrowing

Covariates	Estimate	SE	test statistic	p-value
Intercept	0.093	0.028	3.300	
Burrowing proportion control treatment	-0.465	0.185	-2.514	0.019
Relative cortisol difference (cortisol /control)	5.887E-05	0.002	-0.024	0.981

Model outputs of Experiment 2: Manipulation of sand provisioning of entire captive groups

Table S8—Effect of cortisol treatment on the total expression of cooperative and non-cooperative behaviours in female and male helpers: Comparisons of the individual proportion of count of instantaneous samples during 12 and 4-hour scan observations between the sand and the control treatment for each of the 3 categories of cooperative and non-cooperative activities. The count of instantaneous samples during which helpers were recorded performing the activity under investigation was recorded as the response variable in beta-binomial GLMMs with a logit link. The total count of instantaneous samples of the scan observation session was specified as the binomial total. Beta-binomial GLMMs were preferred over conventional binomial GLMMs because of overdispersion in the scan data. For analyses of burrowing (a) and non-cooperative activities (d), we specified a three-way interaction between treatment, sex and body mass because we anticipated that the response to increased sand provisioning could differ between males and females and that such effect may depend on how heavy subjects were. No interaction between treatment and body mass was specified in the models where food carrying (b) and nest building (c) were specified as response variables because of convergence issues. To determine the effect of increased sand provisioning on the behaviours of helpers, we only included in the dataset, the behavioural data of helpers that had their urinary cortisol concentration determined from a sample collected after the scan session (control treatment: n=61 female helper, n=50 male helper ; sand treatment: n=60 female helper, n=51 male helper). All variables shown in bold were retained in the minimal model. ^a body mass data were centred within sex.

a) Predictors of burrowing

Covariates	Estimate	SE	test statistic	p-value
Intercept	-3.041	0.117	-25.921	
Treatment (Sand)	1.297	0.117	11.052	<0.001
Body mass ^a	-0.006	0.002	-2.559	0.009
Sex (Male)	0.146	0.139	1.050	0.296
Treatment x Sex	0.115	0.228	0.505	0.614
Treatment x Body mass ^a	0.002	0.004	0.507	0.612
Sex x Body mass ^a	0.001	0.004	0.224	0.823
Treatment x Sex x Body mass ^a	-0.003	0.008	-0.381	0.703

b) Predictors of food carrying

Covariates	Estimate	SE	test statistic	p-value
Intercept	-6.128	0.231	-26.570	
Sex (Male)	-0.081	0.313	-0.258	0.796
Body mass ^a	0.001	0.005	0.125	0.901
Treatment (Sand)	-0.012	0.235	-0.053	0.958
Treatment x Sex	-0.192	0.481	-0.399	0.688

Table S8 (continued)

c) Predictors of nest building

Covariates	Estimate	SE	test statistic	p-value
Intercept	-6.124	0.244	-25.112	
Body mass^a	-0.013	0.006	-2.231	0.017
Treatment (Sand)	-0.430	0.277	-1.553	0.119
Sex (Male)	0.382	0.289	1.323	0.194
Treatment x Sex	-0.013	0.549	-0.023	0.982

d) Predictors of active non-cooperative behaviours (excluding rest)

Covariates	Estimate	SE	test statistic	p-value
Intercept	-1.328	0.063	-21.004	
Body mass^a	-0.004	0.001	-2.857	0.004
Sex (Male)	0.080	0.072	1.104	0.270
Treatment (Sand)	0.016	0.056	0.282	0.778
Treatment x Body mass ^a	-0.002	0.002	-1.080	0.279
Treatment x Sex	0.038	0.113	0.334	0.739
Sex x Body mass ^a	0.000	0.002	-0.209	0.834
Treatment x Sex x Body mass ^a	-0.001	0.004	-0.194	0.846

Table S9 – Effect of increased sand provisioning on cortisol levels of female and male helpers: Urinary cortisol concentration (control treatment: n=61 female helper, n=50 male helper; sand treatment: n=60 female helper, n=51 male helper) was specified as response variable in a Gamma GLMM with a log link. Treatment (control and sand), the sex of helper and the sampling period of the urine samples used for cortisol measurements (in the morning the day after a 12-hour scan session or in the evening immediately after a 4-hour scan session) were specified as model covariates. All possible interactions between these covariates were also included because we anticipated that the effect of increased sand provisioning on cortisol levels could differ between males and females and depend on whether cortisol levels were determined from samples collected in the evening immediately after the end of treatment or the next morning. The delay between placement in the urine chamber and urination and the quadratic polynomial of this delay were also included in the model since sampling procedure could represent a stressful event and cause an elevation in cortisol levels. Body mass its interactions with treatment and urine sampling period were not included in the full models as it caused convergence issues. All variables shown in bold were retained in the minimal model.

Predictors of urinary cortisol concentrations

Covariates	Estimate	SE	test statistic	p-value
Intercept	2.809	0.069	40.950	
Urination delay (linear effect)	5.768	0.709	8.140	<0.001
Sex (Male)	-0.243	0.101	-2.390	0.017
Urine sampling period (evening, immediately after 4hours scan session)	-0.173	0.094	-1.829	0.067
Treatment (Sand)	0.068	0.086	0.787	0.444
Urination delay (quadratic effect)	-0.276	0.689	-0.401	0.689
Sex x Urine sampling period	-0.020	0.190	-1.056	0.291
Sex x Treatment	-0.156	0.173	-0.901	0.368
Treatment x Urine sampling period	-0.100	0.174	-0.516	0.565
Sex x Treatment x Urine sampling period	-0.190	0.345	-0.550	0.582

Table S10 – Effect of increased sand provisioning on individual changes in body mass of helpers between the end and the beginning of experimental treatments. Individual differences in body mass were computed by subtracting the body mass measured within one day after the end of treatment and the body mass measured within one day preceding the start of treatment (n=169 individual body mass differences). Individual difference in body mass was specified as a response variable in a gaussian LMM. Treatment (control and sand), the time of body mass measurements at the end of treatment (in the morning the day after a 12-hour scan session or in the evening immediately after a 4-hour scan session), the body mass measured before the treatment (to account for regression to the mean, or the fact that that heavier animals may be experience larger variation in body mass) were specified as model covariates. All possible interactions between these covariates were also included because we anticipated that the effect of increased sand provisioning on changes in body mass could differ depending on whether body mass was measured immediately after the end of treatment or the next morning, after a night of non-experimental condition. We did not differentiate individual differences in body mass between males and females since we observed no sex difference in the behavioural response to the increase in sand provisioning (see Table S8). ^a indicates centred variables.

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Predictors of changes in body mass throughout treatment week				
Covariates	Estimate	SE	test statistic	p-value
Intercept	-2.042	0.586	-3.499	
Treatment (Sand)	1.871	0.545	3.433	<0.001
Body mass start^a	-0.033	0.012	-2.832	0.040
Body mass measurement period (evening, immediately after 4hours scan session)	0.586	0.655	0.895	0.374
Treatment x Body mass start ^a	0.025	0.017	1.457	0.149
Treatment x Body mass measurement period	-0.671	1.094	-0.614	0.541
Body mass start ^a x Body mass measurement period	7.31E-06	2.12E-02	0.000	1.000
Treatment x Body mass start ^a x Body mass measurement period	0.001	0.035	0.038	0.970

Statistical analyses: additional remarks on model specifications

Effect of treatment order on behaviours:

It is possible that the order in which treatments were performed could have affected our response variables. For example, female helpers that underwent the cortisol treatment first may burrow less in the control treatment than female helpers that underwent the control treatment first, because they may be recovering from the increased level of activity and burrowing contributions caused by the cortisol treatment.

Although carry-over effect from the cortisol treatment may have been possible, we did not specify the interaction between treatment and treatment order that would have allowed us to test this possibility. The reasons for omitting such interaction are twofold:

i) The aim of our study was to determine the overall effect of increases in GCc and sand provisioning on GCc and cooperative behaviour, not to investigate the existence and direction of carry-over effects of elevated GCc and burrowing contributions. Our experiments were designed to fulfill this aim and different designs would have been used for the investigation of carry-over effects. For example, we separated our treatments by a period that we anticipated would be largely sufficient for individuals to “recover” from the experimental treatment and return to their baseline GCc and behaviour before the start of the control treatment.

ii) For experiment 1, the number of parameters estimated by the full models specified to investigate the effect of GCc on behaviours was already large for our dataset (8 parameters estimated for 56 datapoints) (Harrison et al., 2018). Thus, we were wary to specify additional covariates in our models, i.e. an interaction between treatment and treatment order would have led to two additional parameters in our full models.

To check that the effect of the cortisol treatment on burrowing had not been magnified by a carry-over causing individuals to burrow less than usual during control treatment that were performed after the cortisol treatment, we specified a few additional beta-binomial GLMM (log link) and found no support for a carry-over effect of the cortisol treatment.

a) When an interaction between treatment and treatment order was added to the model presented in Table S4b, this interaction did not significantly explain the proportion of

burrowing (full model shown), suggesting that the effect of the cortisol treatment did not vary as a function of treatment order.

Predictors of burrowing

<u>Covariates</u>	<u>Estimate</u>	<u>SE</u>	<u>test statistic</u>	<u>p-value</u>
<u>Intercept</u>	<u>-2.166</u>	<u>0.276</u>	<u>-7.843</u>	
<u>Treatment (Cortisol)</u>	<u>0.640</u>	<u>0.293</u>	<u>2.183</u>	<u>0.029</u>
<u>Weight^a</u>	<u>-0.017</u>	<u>0.010</u>	<u>-1.734</u>	<u>0.083</u>
<u>Day (Day 6)</u>	<u>-0.334</u>	<u>0.271</u>	<u>-1.233</u>	<u>0.218</u>
<u>Treatment order (Cortisol then Control)</u>	<u>-0.247</u>	<u>0.347</u>	<u>-0.711</u>	<u>0.477</u>
<u>Treatment x Weight^a</u>	<u>0.015</u>	<u>0.010</u>	<u>1.547</u>	<u>0.122</u>
<u>Treatment x Day</u>	<u>0.180</u>	<u>0.348</u>	<u>0.516</u>	<u>0.606</u>
<u>Treatment x Treatment order</u>	<u>-0.237</u>	<u>0.354</u>	<u>-0.669</u>	<u>0.503</u>

b) When treatment, treatment order and their interaction were the sole covariates specified in the model, similar conclusions as in a) were reached (Treatment x Treatment order: p=0.554).

<u>Covariates</u>	<u>Estimate</u>	<u>SE</u>	<u>test statistic</u>	<u>p-value</u>
<u>Intercept</u>	<u>-2.277</u>	<u>0.251</u>	<u>-9.080</u>	
<u>Treatment (Cortisol)</u>	<u>0.669</u>	<u>0.243</u>	<u>2.753</u>	<u>0.006</u>
<u>Treatment order (Cortisol then Control)</u>	<u>-0.263</u>	<u>0.360</u>	<u>-0.732</u>	<u>0.464</u>
<u>Treatment x Treatment order</u>	<u>-0.215</u>	<u>0.363</u>	<u>-0.591</u>	<u>0.554</u>

c) When only subjects that underwent the control treatment first (i.e. subject not subjected to potential carry-over effects from the cortisol treatment) were retained in the dataset, the cortisol treatment still significantly increases burrowing.

<u>Covariates</u>	<u>Estimate</u>	<u>SE</u>	<u>test statistic</u>	<u>p-value</u>
<u>Intercept</u>	<u>-2.264</u>	<u>0.253</u>	<u>-8.954</u>	
<u>Treatment (Cortisol)</u>	<u>0.668</u>	<u>0.251</u>	<u>2.656</u>	<u>0.008</u>

Temporal autocorrelations of behaviours:

The aim of our behavioural analyses was to determine the effect of treatments (cortisol in experiment 1 and sand provisioning in experiment 2) on how often the behaviours under investigation were expressed during a fixed period of time, not to assess how those behaviours change over time within an observation session. To fulfill this aim, it was not necessary to

[account for the possible temporal autocorrelation of behaviours within scan session and we used behavioural data summarized over entire scan session as a response variable in the relevant statistical models.](#)

References:

- Altmann, J., 1974. Observational Study of Behavior: Sampling Methods. *Behaviour* 49, 227–266. <https://doi.org/10.1163/156853974X00534>
- Binning, S.A., Rey, O., Wismer, S., Triki, Z., Glauser, G., Soares, M.C., Bshary, R., 2017. Reputation management promotes strategic adjustment of service quality in cleaner wrasse. *Sci. Rep.* 7, 1–9. <https://doi.org/10.1038/s41598-017-07128-5>
- Clarke, F.M., Miethe, G.H., Bennett, N.C., 2001. Reproductive suppression in female Damaraland mole-rats *Cryptomys damarensis*: dominant control or self-restraint? *Proceeding R. Soc. London B* 268, 899–909. <https://doi.org/10.1098/rspb.2000.1426>
- Harrison, X.A., Donaldson, L., Correa-cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E.D., Robinson, B.S., Hodgson, D.J., Inger, R., 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* 1–32. <https://doi.org/10.7717/peerj.4794>
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous Inference in General Parametric Models. *Biometrical J.* 50, 346–363.
- Miller, R.C., Brindle, E., Holman, D.J., Shofer, J., Klein, N.A., Soules, M.R., O'Connor, K.A., 2004. Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. *Clin. Chem.* 50, 924–932. <https://doi.org/10.1373/clinchem.2004.032292>
- Stokvis, E., Rosing, H., Beijnen, J.H., 2005. Stable isotopically labeled internal standards in quantitative bioanalysis using liquid chromatography/mass spectrometry: Necessity or not? *Rapid Commun. Mass Spectrom.* 19, 401–407. <https://doi.org/10.1002/rcm.1790>

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