

Supplementary Information

Logistic Model of Prolactin by Reproductive Status (118 samples from 66 individuals)							Linear Model of Prolactin by Reproductive Status (101 samples from 60 individuals)					
Variable	Null (Intercept Only)			Reproductive Status			Null (Intercept Only)			Reproductive Status		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p
Intercept	10.728	2.769	<0.001	3.332	1.022	0.001	2.058	0.182	<0.001	1.928	0.262	<0.001
Reproductive Status				-0.950	1.016	0.349				0.253	0.290	0.384
Model AICc		101.50			102.50			639.74			641.44	
AICc versus Null model		-			+1.00			-			+1.70	
R ²		-			-			0.06			0.01	

Table SI 1. Results of mixed-effects models in the format:

Urinary Prolactin ~ *Colony* + *Individual* + *Reproductive Status*

Where *Urinary Prolactin* is whether prolactin was detected or the urinary prolactin concentration and *Colony* and *Individual* are random effects. The null model contained just the random effects and other models included *Reproductive Status* as a categorical fixed effect with two levels (breeder and non-breeder). The logistic model was Bayesian and contained weak priors to account for prolactin being detected in every sample from a breeder (see Methods). β = regression coefficient. SE = standard error. Bold text indicates a p-values less than 0.05.

Logistic regression models of prolactin by individual characteristics (99 samples from 55 non-breeders)

Variable	Null			Sex			Age			Mass			Rank		
	β	SE	P	β	SE	P	β	SE	P	β	SE	P	β	SE	p
Intercept	1.910	0.558	0.001	1.445	0.647	0.025	2.047	0.819	0.012	1.372	1.487	0.356	1.795	0.794	0.024
Sex (Base=F)				0.621	0.620	0.317									
Age							-0.004	0.079	0.963						
Mass										0.015	0.040	0.705			
Rank													0.273	1.367	0.842
Model AICc		96.02			97.28			94.50			98.05			98.15	
AICc versus Null model		0.00			+1.26			-1.52			+2.03			+2.13	
R ²		0.01			0.02			0.05			0.01			0.01	

Table SI 2. Results of logistic mixed-effects models in the format:

Urinary Prolactin ~ *Colony* + *Individual* + *Fixed Effect*

Where *Urinary Prolactin* is whether urinary prolactin was detected (binary) and *Colony* and *Individual* are random effects. The null model contained only random effects while other models included one of sex (categorical, male or female), age (years, continuous), mass (grams, continuous) and rank (0-1, scaled, continuous) as fixed effects. β = regression coefficient. SE = standard error. Bold text indicates a p-values less than 0.05.

Logistic Models for Initiation of Aggression by Prolactin and Individual Characteristics

(12 breeders, 60 non-breeders)

Variable	Null			Reproductive Status			Prolactin			Work			Sex			Age			Mass			Rank		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p
Intercept	-1.174	.277	<.001	.703	.640	.272	-1.640	.738	.026	-.567	.516	.271	-.821	.362	.023	-1.353	.766	.077	-2.986	1.221	.014	-.251	.548	.646
Reproductive Status				-2.533	.806	.002																		
Prolactin							.034	.025	.171															
Work										-.003	.002	.187												
Sex													-.788	.575	.170									
Age																.035	.098	.724						
Mass																			.046	.027	.091			
Rank																						-2.201	1.252	.079
Model AICc		85.06			74.49			83.74			85.50			85.35			87.05			83.98			83.56	
AICc versus Null model		0			-10.57			-1.32			0.44			0.29			1.99			-1.08			-1.50	
pseudo-R2		0			0.16			0.06			0.02			0.02			0.00			0.04			0.05	

Table SI 3. Results of logistic mixed-effects models in the format:

Initiated aggression ~ *Colony* + *Individual* + *Fixed Effect*

Where *Initiated Aggression* is whether the individual was observed initiating aggression (binary) and *Colony* and *Individual* are random effects. Fixed Effect was omitted in the null model and one of reproductive status (categorical, breeder or non-breeder), mean prolactin concentration (ng/mg creatinine, continuous), mean work duration (continuous), sex (categorical, male or females), age (years, continuous), body mass (grams, continuous), and rank (0-1, scaled, continuous) was included in the corresponding models. β = regression coefficient. Bold text indicates a p-values less than 0.05.

Logistic Models for Receipt of Aggression by Prolactin and Individual Characteristics

(12 breeders, 60 non-breeders)

Variable	Null			Reproductive Status			Prolactin			Work			Sex			Age			Mass			Rank		
	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p	β	SE	p
Intercept	-0.571	.245	.020	-0.336	.586	.566	-0.655	.351	.062	-0.192	.477	.688	-0.823	.397	.038	-1.018	.442	.021	-1.537	.809	.058	-0.543	.493	.271
Reproductive Status				-0.283	.645	.661																		
Prolactin							.007	.017	.691															
Work										-0.002	.002	.363												
Sex													.486	.508	.338									
Age																.089	.062	.149						
Mass																			.027	.021	.205			
Rank																						-0.060	.921	.948
Model AICc	10.54			102.59			102.69			101.94			101.81			100.46			101.15			102.78		
AICc versus Null model	0			+2.05			+2.08			+1.40			+1.27			-0.08			+0.61			+2.24		
pseudo-R ²	0			0			0			.01			.01			.02			.02			0.00		

Table SI 4. Results of logistic mixed-effects models in the format:

Received aggression ~ *Colony* + *Individual* + *Fixed Effect*

Where *Received aggression* is whether the individual was observed receiving aggression (binary) and *Colony* and *Individual* are random effects. *Fixed Effect* was omitted in the null model and one of reproductive status (categorical breeder or non-breeder), mean prolactin concentration (ng/mg creatinine, continuous), mean work duration (seconds, continuous), sex (categorical, male or females), age (years, continuous), Body mass (grams, continuous), and rank (0-1, scaled, continuous) was included in the corresponding models. Bold text indicates a p-values less than 0.05.

Behaviour and Physiology During Cabergoline Administration

We observed behaviour before, during and after administering the prolactin-suppressant drug Cabergoline (a dopamine agonist used to treat infertility due to elevated prolactin) to a group of animals. In total, we observed 79 naked mole-rats for 10 minutes, twice a week for 16 weeks. In weeks five to 13, eight individuals received the prolactin-suppressing treatment, eight received a blank (vehicle) control injection, and 63 other colony members received neither (19 breeders, 44 non-breeders). Here, we report comparisons between the treatment groups on four measures: the duration of working behaviour observed, the proportion of focal periods that contained working behaviour, the proportion of focal periods in which the focal animal received aggression, and urinary progesterone levels.

Working Behaviour Proportion

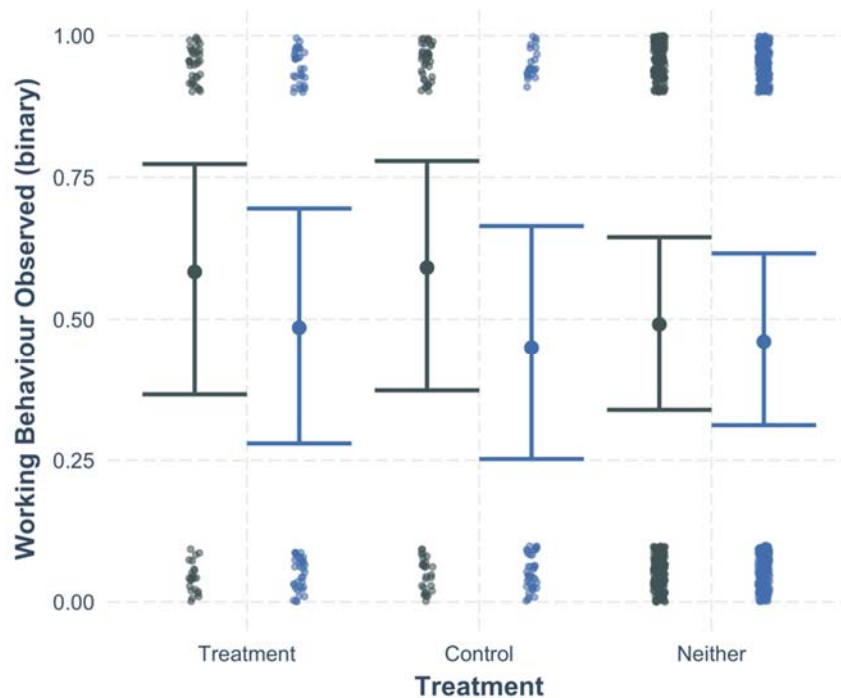


Figure SI 1. There was a small decrease in the proportion of focal observations that recorded working behaviour during the treatment period (blue) compared with outside the treatment phase (black). Treatment group did not predict whether an individual was observed working. Large, solid points are group means for the proportion of observations that recorded working behaviour, whiskers are 95% confidence intervals, and data are plotted as smaller points. Data were generated in the model: *Presence of working behaviour* ~ *Colony + Individual + Treatment Group * Phase*, where *Presence of working behaviour* is whether the focal animal was observed working, colony and individual are categorical random effects, and *Treatment Group * Phase* is the interaction between treatment group (treatment, control, neither) and treatment phase (during treatment, not during treatment).

49% of the focal sampling periods recorded working behaviour and this was relatively stable throughout the observation period. Working behaviour occurred slightly less often during the treatment period, with the differences being similar across the groups. Treatment did not predict whether an individual was observed working.

Logistic Model of Working Behaviour by Treatment						
(1,920 observations of 60 individuals)						
Variable	Null			Treatment x Phase		
	β	SE	p	β	SE	p
Intercept	-0.040	0.307	0.896	0.339	0.452	0.453
Control vs Treatment				0.031	0.496	0.951
Neither vs Treatment				-0.374	0.382	0.327
During Treatment vs Not During Treatment				-0.398	0.282	0.158
Treatment Outside Treatment Phase vs Control During Treatment				-0.173	0.402	0.668
Treatment Outside Treatment Phase vs Neither During Treatment				0.275	0.306	0.369
Model AICc		2,327.85			2,330.27	
AICc versus Null model		-			+2.42	
R ²		0.13			0.13	

Table SI 5. Results of logistic mixed-effects models in the format: *Presence of working behaviour* ~ *Colony* + *Individual* + *Fixed Effect*. Where *Presence of working behaviour* is whether working behaviour was observed per ten-minute focal period (binary), *Colony* and *Individual* are random effects. Fixed Effect was omitted in the null model and the interaction between treatment group (treatment, control, neither) and whether the behaviour was observed while treatment was being administered (during treatment phase, not during treatment phase) was included in the full model. β =regression coefficient. Bold text indicates a p-values less than 0.05.

Working Behaviour Duration

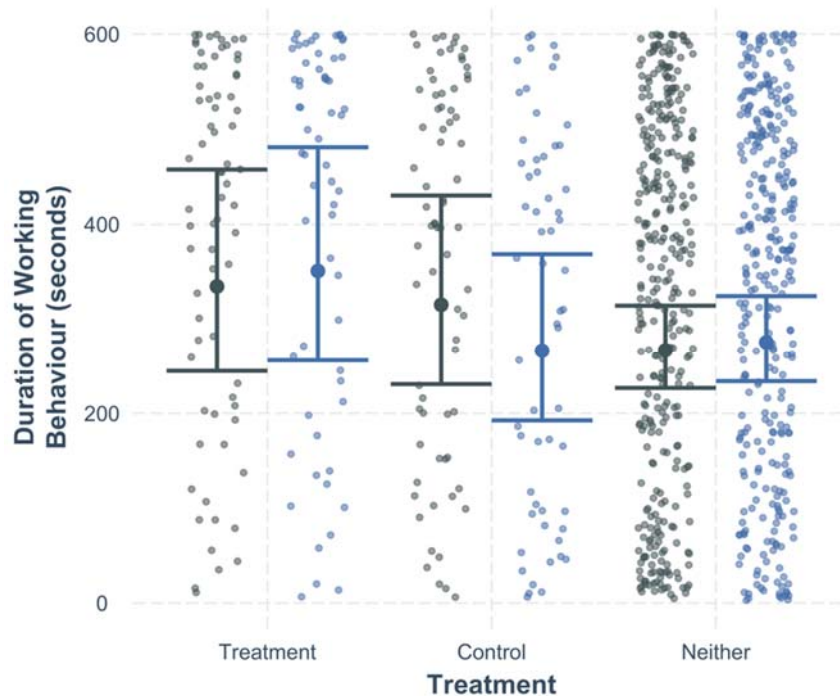


Figure SI 2. There was a small decrease in the duration of working behaviour observed during the treatment period (blue) compared with outside the treatment phase (black). Treatment group did not predict whether an individual was observed working. Large, solid points are group means for the duration of working behaviour, whiskers are 95% confidence intervals, and data are plotted as smaller points. Data were generated in the model: $Duration\ of\ working\ behaviour \sim Colony + Individual + Treatment\ Group * Phase$, where $Duration\ of\ working\ behaviour$ is the duration of working behaviour recorded in the focal observation, colony and individual are categorical random effects, and $Treatment\ Group * Phase$ is the interaction between treatment group (treatment, control, neither) and treatment phase (during treatment, not during treatment).

The average duration of working behaviour per 10-minute observation was 167.7 seconds (range 0 – 600, SE 4.9 seconds) in non-breeders, with the mean duration for all animals lowest during the treatment phase. Animals receiving the treatment worked slightly longer during the treatment phase, while animals receiving the control worked slightly shorter. Overall, the mean durations of animals in all treatment groups were similar throughout the experiment and treatment did not predict variation in the duration of working behaviour.

Linear Model of Duration by Treatment						
(943 observations from 60 individuals)						
Variable	Null			Treatment x Phase		
	β	SE	p	β	SE	p
Intercept	5.645	0.073	<0.001	5.813	0.159	<0.001
Control vs Treatment				-0.060	0.213	0.779
Neither vs Treatment				-0.226	0.165	0.170
During Treatment vs Not During Treatment				0.047	0.071	0.506
Treatment Outside Treatment Phase vs Control During Treatment				-0.216	0.110	0.050
Treatment Outside Treatment Phase vs Neither During Treatment				-0.016	0.081	0.843
Model AICc	13,089.23			13,091.95		
AICc versus Null model	-			+2.72		
R ²	0.17			0.16		

Table SI 6. Results of linear mixed-effects models in the format:

Work duration ~ *Colony* + *Individual* + *Fixed Effect*

Where *Work duration* is the length of working behaviour observed per ten-minute focal period (seconds, continuous), *Colony* and *Individual* are random effects. Fixed Effect was omitted in the null model and the interaction between treatment group (treatment, control, neither) and whether the behaviour was observed while treatment was being administered (during treatment phase, not during treatment phase) was included in the full model.

β =regression coefficient. Bold text indicates a p-values less than 0.05.

Receipt of Aggression

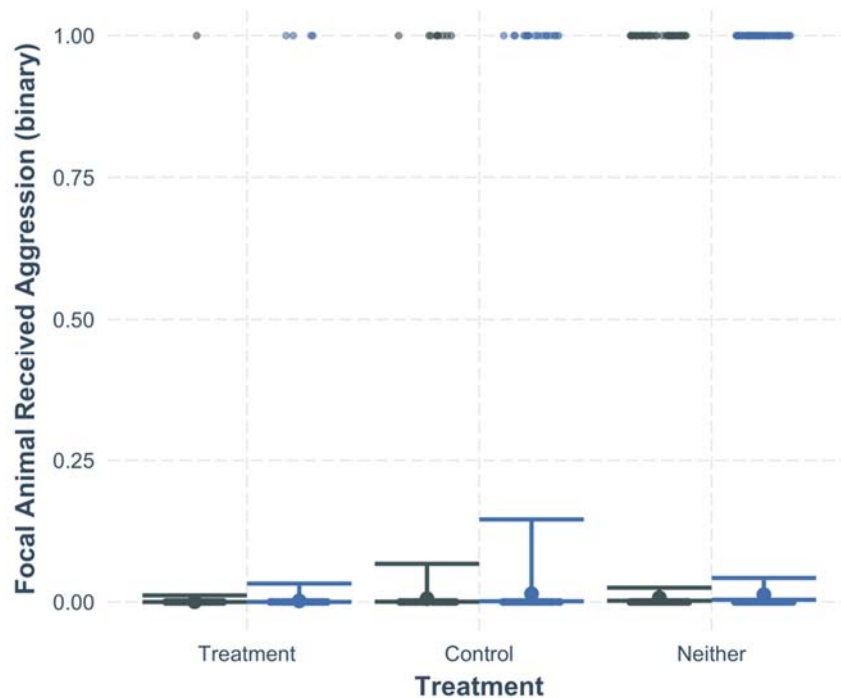


Figure SI 3. There was an increase in the proportion of focal animals that received aggression during the treatment period (blue) compared with outside the treatment phase (black). The difference was greatest in control animals but the interaction between treatment group and treatment phase did not improve the performance of the model. Large, solid points are group means for the proportion of observations in which the focal animal received aggression, whiskers are 95% confidence intervals, and data are plotted as smaller points. Data were generated in the model: $Received\ aggression \sim Colony + Individual + Treatment\ Group * Phase$, where *Received aggression* is whether the focal animal received aggression, colony and individual are categorical random effects, and $Treatment\ Group * Phase$ is the interaction between treatment group (treatment, control, neither) and treatment phase (during treatment, not during treatment).

Focal animals received aggression in 7% of sampling periods, which was highest during the treatment phase. Animals receiving the treatment received less aggression across all phases and aggression was highest in the treatment phase for all groups (Figure SI 3). Table SI 7 confirms that more aggression was observed in the treatment period than when we were not administering treatments. However, adding the interaction between treatment and

phase does not improve the fit of the model and the effect sizes of the interactions are relatively small. The animals in the treatment group received less aggression but this does not appear to be related to the treatment.

Logistic model of received aggression by treatment

(1,920 samples from 60 individuals)

Variable	Null			Treatment + Phase			Treatment x Phase		
	β	SE	p	β	SE	p	β	SE	p
Intercept	-4.939	0.001	<0.001	-7.183	1.464	<0.001	-7.864	1.743	<0.001
Control vs Treatment				2.196	1.762	0.213	2.703	2.031	0.183
Neither vs Treatment				2.220	1.436	0.122	2.961	1.730	0.087
During Treatment vs Not During Treatment				0.668	0.215	0.002	1.585	1.176	0.178
Treatment Outside Treatment Phase vs Control During Treatment							-0.648	1.290	0.616
Treatment Outside Treatment Phase vs Neither During Treatment							-1.019	1.200	0.396
Model AICc		713.82			707.24			710.14	
AICc versus Null model		-			-6.58			-3.68	
McFadden's pseudo-R ²		0.27			0.28			0.29	

Table SI 7. Results of logistic mixed-effects models in the format:

Received aggression ~ *Colony* + *Individual* + *Fixed Effect*

Where *Received Aggression* is whether the focal animal received aggression in the ten-minute focal period (binary) and *Colony* and *Individual* are random effects. Fixed Effect was omitted in the null model and the interaction between treatment group (treatment, control neither) and whether the behaviour was observed while treatment was being administered (during treatment phase, not during treatment phase) was included in the full model.

β =regression coefficient. Bold text indicates a p-values less than 0.05.

Progesterone

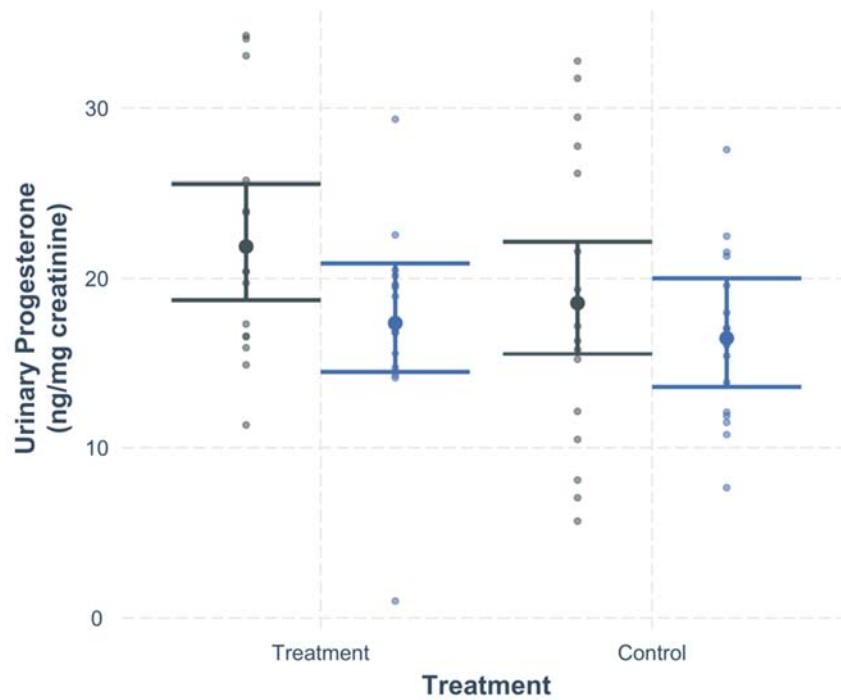


Figure SI 4. Urinary progesterone was similar in samples collected during the treatment phase (blue) and outside the treatment phase (black). Progesterone levels were similar in the treatment and control groups. Large, solid points are group means for the urinary progesterone, whiskers are 95% confidence intervals, and data are plotted as smaller points. Data were generated in the model: $Urinary\ progesterone \sim Colony + Individual + Treatment\ Group * Phase$, where *Urinary progesterone* is the urinary progesterone concentration, colony and individual are categorical random effects, and *Treatment Group * Phase* is the interaction between treatment group (treatment, control, neither) and treatment phase (during treatment, not during treatment).

After excluding one sample that failed to change colour during the creatinine assay, we detected progesterone in 64 out of 65 urine samples. The average urinary progesterone concentration from experimental animals was 17.5 ng / mg creatinine (0 – 33.27, SE 0.86). Mean progesterone levels dipped during the treatment phase, but the differences were relatively small (Figure SI 4). Linear regressions confirmed that progesterone did not change

substantially during the treatment administration (Table SI 8). Administering Cabergoline for eight weeks did not cause an increase in progesterone.

Linear Model of Progesterone by Treatment and Phase						
(65 samples from 12 individuals)						
Variable	Null			Reproductive Status		
	β	SE	p	β	SE	p
Intercept	2.916	0.046	<0.001	2.92	0.089	<0.001
Treatment vs Control				0.165	0.118	0.162
Not During Treatment vs During Treatment				-0.119	0.132	0.367
Treatment vs Control x During Treatment				-0.112	0.178	0.530
Model AICc		446.05			447.58	
AICc versus Null model		-			+1.53	
R ²		0.00			0.00	

Table SI 8. Results of linear mixed-effects models in the format:

Urinary Progesterone ~ *Colony* + *Individual* + *Fixed Effect*

Where *Urinary Progesterone* is the urinary progesterone concentration (continuous) and *Colony* and *Individual* are random effects. Fixed Effect was omitted in the null model and the interaction between treatment group (treatment, control, neither) and whether the behaviour was observed while treatment was being administered (during treatment phase, not during treatment phase) was included in the full model. β =regression coefficient. Bold text indicates a p-values less than 0.05.