Supporting information (SI)

Materials and Methods.

Voice recording equipment

All voices were detected by using a condenser microphone (PRO35, Audio-Technica, Tokyo, Japan) designed for sound pressure level (SPL) measurements between 50 Hz and 18,000 Hz. The microphone was connected to an amplifier (AR501, Fostex, Tokyo, Japan) and acoustic signals were transmitted to a Digital Audio Interface (DUO-CAPTURE EX, Roland, Shizuoka, Japan) with functions such as analog-to-digital converter, frequency filters, and signal input–output terminals.

Voice recording

We recorded pup's voice from 3- to 7-day-old pups. A pup was separated from the queen and placed in an acrylic case that had air cap on its inner side. The acrylic case was located in a box with a lid, which was filled with cushioning. The microphone was hung 4 cm above the bottom of the acrylic case and aligned with its center. Subordinate's voice was recorded from 24-week-old subordinates. One subordinate was placed into an acrylic case and then another subordinate was placed into the same case. The microphone was held manually 10 cm from the bottom of the acrylic case. The microphone noise floor was around 18 dB, high-pass filter processing was used with a corner frequency of 18 kHz, and the sampling frequency rate was 44.1 kHz.

Creating playback sound

We analyzed the recording of each voice by Avisoft-SASLab Pro (Avisoft Bio-acoustics, Berlin, Germany) and characterized approximate property of each voice, such as duration, interval length, peak frequency, and bout patterns (Table S2). We used the voice properties as a reference and connected the bouts to create playback sounds using audio generator software (Adobe Audition, version 2.0). Furthermore, we created a recording of pup's voice in which the speed of the voice was reduced by one-fourth without altering the frequency.

Pup voice preference test

We used female subordinates that had not experienced the queen giving birth. Each subject was individually removed from the colony to the preference test box and allowed to adapt to the test box for 15 min. One of the acrylic boxes used for housing of the animals was modified to a preference test box (15 × 15 × 20 cm). The modified test box had two tubes (5 cm inner diameter, 16 cm long), with ends covered with a wire mesh. One of the two tubes contained a speaker playing the subject's voice. We played pup's voice during the gestation and non-lactation periods. During the postpartum, we played pup's voice followed by subordinate's voice and one-fourth pup's voice. Subordinate's voice and one-fourth pup's voice were randomly played on day 2 and day 4 after playing pup's voice. We observed the searching behavior of the subordinate while playing these sounds for 5 min, which consisted of partially entering into the tubes, staying in the tubes, and searching the wire meshes.

Pup voice preference test with feeding feces

In this preference test, we used female subordinates that had experienced the queen giving birth and

belong to the colony whose queen was in the non-lactation period. We conducted the aforementioned pup voice preference test before, during, and after feeding the subordinates with pellets containing queen's feces. The feces of pregnant queens and non-pregnant queens were collected for 40–70 d after the start of their pregnancy and 80–90 d after their parturition, respectively. The pellets, which were prepared by mixing bananas (0.84 g/g) and oatmeal (0.16 g/g) in a bowl, were fed to the animals for 9 days. When needed, the dry feces of pregnant queens (0.01 g/g), dry feces of non-pregnant queens (0.01 g/g), and estradiol dissolved in corn oil (0.1 mL/g) were added to the pellet. Estradiol (180 ng/mL) was melted by stirring it in cone oil heated to 65℃. These pellets were offered to the colony *ad libitum* and exchanged with new pellets during the feeding period. The daily feeding amount of 3 g per individual was provided for all individuals in the colony who were more than 3 weeks old. We visually verified that more than half of individuals in the colony containing the subject female subordinates ate the pellets.

Metabolism of ingested steroid

We fed pellets to female subordinates for 4 days and collected their urine and feces before, during, and after pellets were consumed. The pellets consisted of banana, oatmeal, and estradiol, and progesterone dissolved in corn oil (0.1 mL/g). Both of estradiol (180 ng/mL) and progesterone (120 ng/mL) were melted by stirring them in cone oil heated to 65 ℃. Female subordinates were removed from the colony to a new test cage individually, where they were kept for 3 hours and offered the pellets. We measured the amount of pellets ate by each subordinate. Subordinates had constantly eaten about 1.0 g of the pellets on each feeding. The urine and feces of subordinates were collected within 12 h and 3 h after feeding, respectively, to measure estradiol

concentration in collected urine and feces.

Fecal steroid extraction

Collected urine was stored at −20°C prior to assays, and collected feces was extracted followed the protocol described previously (1). The feces were dried in an incubator at 49.5°C ± 1.0°C overnight. Dried samples were crushed in a mill and 0.05 g of each sample was placed individually in capped tubes. Each sample was dissolved with 1.5 mL ultrapure water and vortexed for 10 min, and then further diluted by adding 5 mL of diethyl ether and vortexed for additional 10 min. After 30 min of incubation, the samples were quickly cooled in a methanol cooler for 15 min. Cooled samples consisted of two layers—the frozen layer and a liquid ether layer. The liquid ether layer was isolated, evaporated, and used as a fecal sample.

Progesterone assays

We followed a previously described protocol (2) for progesterone assays, which has a range from 0.98–1,000 ng/ml. Fecal samples was diluted 10-fold with the assay buffer. Briefly, we coated each well of a microplate (ELIZA Plates 9018; Corning, New York, NY, USA) with 100 mL of secondary antibody solution (anti-rabbit g-globulin serum raised in goats, 5 mg/200 mL; Seikagaku Co., Tokyo, Japan) and incubated the plate overnight at room temperature. Non-bound antibodies were removed from the wells by inverting the plate several times and washing the wells three times using a plate washer (IMMUNOWASH MODEL 1575; Bio-Rad, Hercules, CA, USA). Standard progesterone (Wako Chemicals, Osaka, Japan) was diluted in the assay buffer (pH 7.2 sodium phosphate buffer containing bovine serum albumin at 1 g/L). The anti-progesterone serum (the first antibody was

raised in a rabbit, FKA-302E; COSMO Bio, Tokyo, Japan) was diluted 200,000-fold, and horseradish-peroxidase (HRP)-labeled progesterone (FKA-301; COSMO Bio, Tokyo, Japan) was diluted 1,000,000-fold with assay buffer. We sequentially added 15 μL aliquots of the standard or sample, 100 μL aliquots of anti-serum solution, and 100 μL aliquots of labeled steroid hormones into each well. The plates were incubated overnight at 4°C. Non-bound ligands were removed from the plates, and 150 μL aliquot of the substrate solution with HRP was added to each well. Then, the plates were incubated for 15 min at room temperature and 50 μL 4 N H_2SO_4 was added to stop the reaction. The absorbance at 405 nm of each sample was recorded with an automatic microplate reader. The results of the assay were calculated by the software Microplate Manager III (Bio-Rad).

Estradiol assays

Estradiol concentrations were measured using ELISA kits (Estradiol EIA Kit 582251, Cayman Chemical, Ann Arbor, MI, USA) following a protocol described previously (3). This kit has an assay range from 6.6–4,000 pg/ml. Urine or fecal samples were each diluted 3- or 10-fold with the assay buffer. When the estradiol concentration of fecal samples exceeded the assay range, the samples were diluted 50-fold with the assay buffer. Each microplate contained a sample, standard, blank, non-specific binding, and total activity well. The absorbance was measured at 405 nm for each sample in an automatic microplate reader. Estradiol concentration of each sample was determined from the standard curve calculated by using the standard and urinary estradiol concentration was expressed as estradiol/creatinine ratio.

References

1. Wasser, S. K., Monfort, S. L., Southers, J., and Wildt, D. E. (1994). Excretion rates and metabolites of oestradiol and progesterone in baboon (*Papio cynocephalus cynocephalus*) faeces. J. Reprod. Fertil. *101*, 213–220.

2. Kikusui, T., Nakamura, K., Kakuma, Y., and Mori, Y. (2006). Early weaning augments neuroendocrine stress responses in mice. Behav. Brain Res. *175*, 96–103.

3. Munro, C. J., Stabenfeldt, G. H., Cragun, J. R., Addiego, L. A., Overstreet, J. W., and Lasley, B. L. (1991). Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. Clin. Chem. *37*, 838–844.

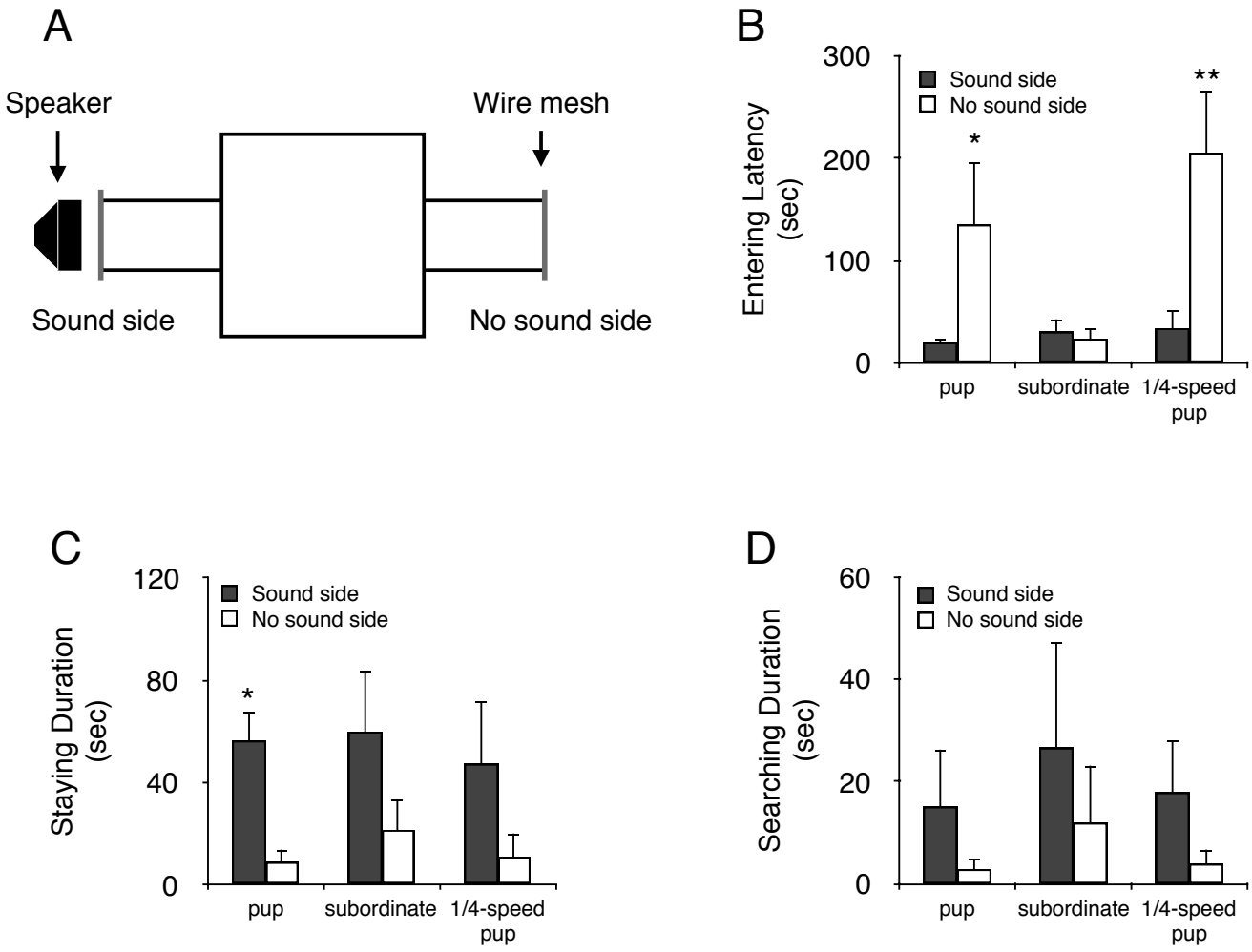

Figure S1. Subordinates did not enhance their response to reproduced subordinate voice. Related to Figure 1

(A) Schematic diagram of test box used in the two-way choice test. (B–D) The approach response of subordinates to the sound (black) and no-sound (white) side of the tube during the postpartum period: pup's voice (n = 5), subordinate's voice (n = 5), and 1/4-speed pup's voice (n = 5). (B) Latency to enter a tube [Linear mixed model (LMM), F_{voice} (1, 15) = 9.231, P = 0. 008; Bonferroni, P = 0.046, p = 0.006); (C) Time spent in a tube [LMM, F_{voice} (1, 15) = 13884, P = 0. 008; Bonferroni, P = 0.024]; (D) Time subordinates searched the mesh. Subordinates responded to the pup's voice, but not to the subordinate's voice, during the postpartum period. In addition, their response to the 1/4-speed pup's voice was partially preserved, suggesting that not only the speed but other factors in pup's voice attract the subordinates. *P < 0.05, **P < 0.01. Data are represented as mean ± SEM.

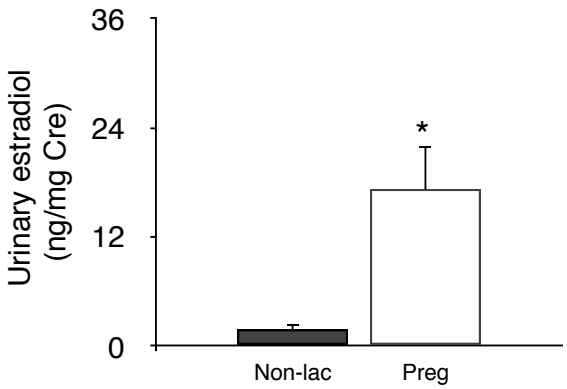

Figure S2. Urinary estradiol concentration at different stages of queen's reproduction. Related to Figure 3

Female subordinates showed higher urinary estradiol concentrations during the gestation period (white, $n = 5$) than during the non-lactation period (black, $n = 5$) [t test, $t(9.815) = 4.011$, $P = 0.036$]. *$P < 0.05$. Urinary estradiol concentrations (ng/mg of creatinine [Cre]) are represented as mean ± SEM.

Table S1. Subject animal list.

Figure		Experiment	Colony	ID	Class	Reproductive period of Queen	Age (weeks)
Figure 1	B-D	Experiment 1	G	G15	Subordinate	Gestation, Postpartum, Non-lactation	53
				G17	Subordinate	Gestation, Postpartum	53
				G18	Subordinate	Gestation, Postpartum, Non-lactation	53
			M	M3	Subordinate	Gestation, Postpartum	55
				M9	Subordinate	Gestation, Postpartum, Non-lactation	55
				M14	Subordinate	Postpartum, Non-lactation	29
				M15	Subordinate	Gestation, Postpartum, Non-lactation	29
Figure 2	B-D	Experiment 2	M	M3	Subordinate	Non-lactation	72
				M6	Subordinate	Non-lactation	72
				M9	Subordinate	Non-lactation	72
				M13	Subordinate	Non-lactation	46
				M14	Subordinate	Non-lactation	46
Figure 3	A&C	Experiment 1	G	G14	Subordinate	Gestation, Postpartum, Non-lactation	52
				G15	Subordinate	Gestation, Postpartum, Non-lactation	52
				G17	Subordinate	Gestation, Postpartum, Non-lactation	52
				G18	Subordinate	Gestation, Postpartum, Non-lactation	52
			H	H13	Subordinate	Gestation, Postpartum, Non-lactation	85
				H14	Subordinate	Gestation, Postpartum, Non-lactation	86
				H15	Subordinate	Gestation, Postpartum, Non-lactation	86
				H24	Subordinate	Gestation, Postpartum, Non-lactation	74
				H27	Subordinate	Gestation, Postpartum, Non-lactation	74
			M	M3	Subordinate	Gestation, Postpartum, Non-lactation	39
				M6	Subordinate	Gestation, Postpartum, Non-lactation	39
				M9	Subordinate	Gestation, Postpartum, Non-lactation	39
				M13	Subordinate	Gestation, Postpartum, Non-lactation	28
				M14	Subordinate	Gestation, Postpartum, Non-lactation	28
				M15	Subordinate	Gestation, Postpartum, Non-lactation	28
Figure 3	B&D	Experiment 1	G	G7	Queen	Gestation, Non-lactation	235
			H	H1	Queen	Gestation, Non-lactation	> 300
			M	M1	Queen	Gestation, Non-lactation	332
Figure 3	F-I	Experiment 3	M	M3	Subordinate	Gestation, Non-lactation	90
				M6	Subordinate	Gestation, Non-lactation	90
				M15	Subordinate	Gestation, Non-lactation	64
			O	O7	Subordinate	Gestation, Non-lactation	94
				O10	Subordinate	Gestation, Non-lactation	94
Figure 4	A-C&G	Experiment 4	G	G14	Subordinate	Non-lactation	120
				G15	Subordinate	Non-lactation	120
			M	M3	Subordinate	Non-lactation	122
				M6	Subordinate	Non-lactation	122
				M9	Subordinate	Non-lactation	122
Figure 4	D-G	Experiment 4	L	L16	Subordinate	Non-lactation	133
				L28	Subordinate	Non-lactation	52
				L30	Subordinate	Non-lactation	52
			M	M26	Subordinate	Non-lactation	27
				M27	Subordinate	Non-lactation	27
Figure S1	B-D	Experiment 1	G	G15	Subordinate	Postpartum	53
				G18	Subordinate	Postpartum	53
			M	M3	Subordinate	Postpartum	55
				M9	Subordinate	Postpartum	55
				M15	Subordinate	Postpartum	29
Figure S2		Experiment 1	G	G14	Subordinate	Non-lactation	92
				G15	Subordinate	Gestation	92
				G21	Subordinate	Gestation	38
			L	L3	Subordinate	Gestation	37
				L5	Subordinate	Non-lactation	37
			M	M3	Subordinate	Gestation	93
				M6	Subordinate	Non-lactation	93
				M9	Subordinate	Non-lactation	93
			O	O7	Subordinate	Gestation	98
				O13	Subordinate	Non-lactation	98

Table S2. Sound properties of subject voices.

Type of voice	Number of bouts (times/min)	Duration of bout (msec)	Interva length (sec)	Peak freqency (kHz)
Sampling pup voice (n=6)	17 ± 6	126.64 ± 12.39	1.56 ± 0.32	3.55 ± 0.21
Reproduced pup voice	27	172.67	1.68	3.64
Reproduced 1/4-speed pup voice	21	697.29	1.61	3.64
Sampling subordinate voice (n=3)	18 ± 2	156.57 ± 6.19	1.17 ± 0.30	2.75 ± 0.11
Reproduced subordinate voice	33	187.67	1.33	2.65

Data are represented as mean ± SEM.

Movie S1. Subordinates' response to reproduced pup voice

Although subordinates did not respond to reproduced pup voice during the queen's non-lactating period, they quickly responded to it during the queen's postpartum period.