exposed to situations of danger and is the individual least likely to be killed by predators or accident; this is typical also of eusocial insects. *Heterocephalus* resembles the termites more closely than it resembles the Hymenoptera in that the mole rats are diploid (*11*), have male and female members of the working castes, the young contribute to colony labor, some working individuals are able to become fully reproductive when the breeding female is removed, pheromones may be involved in caste determination, and the young obtain food from the adults by coprophagy. These features, together with the overlap of generations that allows several generations of offspring to assist the parent in the care of the young and the finding of food, parallel the eusociality of insects and seem to qualify *Heterocephalus* as a eusocial animal—the only vertebrate for which this can be suggested at present. Furthermore, as with the termites, these findings demonstrate that, despite its importance in the Hymenoptera (*12*) haplodiploidy is not a necessary prerequisite for the evolution of eusociality.

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2. Most of the completely subterranean mammals are solitary and aggressive, and the young disperse after weaning. In the family Bathergidae, three genera (*Bathyergus, Georychus,* and *Heliophobius*) are solitary and aggressive. A fourth genus (*Cryptomys*) may occur in small family groups (*C. hottentotus hottentotus*) [G. Hickman, *Z. Saeugetierkd.* **44**, 153 (1978); A. Wakefield, personal communication] or in colonies of over 20 individuals (*C. hottentotus damarensis*) (personal observations). *Heterocephalus*, the fifth genus, evidently has the largest colonies.
3. Individuals in five mixed colonies came from several burrow systems. In time, each established a caste system similar to that described in this report.
4. Blocked portions of the burrows left open by us were evidence that not all the colony had been caught. Two females of the 40 individuals captured were injured and destroyed, and three others (two males and one female) died during the first year.
5. Few breeding females have ever been caught in the field, suggesting that these are the individuals least likely to be caught by predators.
6. The artificial burrow system, consisting of small Perspex chambers linked by 10 m of transparent tubing, was maintained in a heated room (27°C) with a high humidity. Lamps placed against portions of the burrow system provided additional warmth. Damp soil and food (a variety of root crops, greens, and fruit) were placed in some chambers. Dried grass was provided for nesting.
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10. Pheromones in urine are known to influence reproductive behavior in other rodents [J. F. Eisenberg and D. G. Kleiman, *Annu. Rev. Ecol. Syst.* **3**, 1 (1972)]. W. H. Tam (*Symp. Zool. Soc. London* **34**, 363 (1974)] has shown that ovarian interstitial tissue can be a source of progesterone and estrogen in some hystricomorphs; the abundant ovarian interstitial gland tissue in the breeding *Heterocephalus* may be of importance in this respect.
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13. I thank R. D. Alexander, G. M. Branch, G. N. Louw, and P. W. Sherman for reading this manuscript; P. Bally and H. Bally for hospitality in Nairobi and for help in procuring mole rats; P. Withers and P. Withers, N. Kilai, and Subu for helping me dig out the colony of mole rats; the Ministry of Environment and Natural Resources, Kenya, for providing the permits to collect and export *Heterocephalus*; and the National Geographic Society for a research grant.

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Male Vole Urine Changes Luteinizing Hormone–Releasing Hormone and Norepinephrine in Female Olfactory Bulb

Abstract. *Female prairie voles (*Microtus ochrogaster*) exposed to a single drop of male urine on the upper lip showed changes in concentrations of luteinizing hormone–releasing hormone (LHRH) and norepinephrine in olfactory bulb tissue; no such changes occurred in dopamine concentration. The changes were measured in the posterior but not the anterior olfactory bulb tissue of females within 1 hour after they were exposed to urine. These females also showed rapid increases in serum concentrations of luteinizing hormone. Females exposed to water on the upper lip showed none of these changes. These results suggest that in this species LHRH and norepinephrine in the olfactory bulb may mediate luteinizing hormone release in response to external (pheromonal) chemical cues.*

In many mammalian species estrous cycles and ovulation are viewed as "spontaneous"; that is, females of reproductive age continuously exhibit reproductive cycles (*1*). In some species, however, external stimuli play a prominent role in the control of reproduction. For example, in the prairie vole *Microtus ochrogaster*, both estrus and ovulation depend on stimuli provided by the male. In the vole, female reproductive processes, as measured by behavioral estrus and increases in uterine weight, are activated within 24 to 48 hours by either brief physical contact with the male or with male urine (*2*). The capacity of stimuli from the male, including chemical cues in his urine, to induce estrus in the female requires an intact olfactory system (*3*) and direct physical contact with the urine (*2*). This information as well as evidence from other species (*4*), indicate that the vomeronasal organ–accessory olfactory bulb system may mediate the effects of male urine on female reproductive activation.

A critical component in the timing of events leading to mammalian reproduction is the secretion of luteinizing hormone–releasing hormone (LHRH) (*5*), which regulates pituitary luteinizing hormone (LH) release. Studies of the functional role of LHRH have focused on the preoptic-hypothalamic areas; however, nerve terminals containing LHRH are also found in the olfactory bulb (*6*). Moreover, LHRH positive cell bodies and fibers are localized in the posterior half of the olfactory bulb (*7*).

The catecholamines norepinephrine (NE) and dopamine provide an additional level of regulation over female reproduction (*8*). These neurotransmitters have been found in the olfactory bulb (*9*) and studies have related NE to stimulation and dopamine to inhibition of reproductive processes under olfactory control (*10*). In the experiments described here we examined the relationships among localized (anterior or posterior) olfactory bulb LHRH and catecholamine concentrations as well as serum LH levels in female prairie voles exposed to male prairie vole urine.

Intact, reproductively inactive females (*11*) were exposed to either urine or distilled water. Urine was collected from sexually experienced males, and urine or water was applied to the females by placing a single drop (approximately 200 μl) on the upper lip. Females (seven per group) were decapitated at 1, 15, 30, or 60 minutes after they were stimulated. Blood was collected for LH assay (*12*) and olfactory bulbs were removed and prepared for LHRH (*13*) and NE and dopamine (*14*) assays. Each olfactory bulb was dissected into anterior and posterior tissue samples (*15*).

At all the time intervals after stimulation we found a significantly greater concentration of LHRH in the posterior (Fig. 1A) compared to anterior (Fig. 1B) olfactory bulb extracts in both urine-treated [F (1,48) = 31.8, $P < .001$] and water-treated [F (1,48) = 31.3, $P < .001$] females (*16*). Sixty minutes after urine application, concentrations of LHRH in the posterior olfactory bulb increased 185 percent and were significantly higher

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Table 1. Concentrations of LH in the serum of female prairie voles exposed to male vole urine or to water. The results are expressed as means ± standard error. When the mean LH concentrations were calculated, samples with nondetectable amounts were assigned a value of 50 ng/ml, which is the sensitivity of the assay.

Time after treatment (minutes)	Urine		Water	
	LH (ng/ml)	Percentage of samples with detectable LH	LH (ng/ml)	Percentage of samples with detectable LH
1	156 ± 39	71	53 ± 3	14
15	170 ± 49	57	157 ± 37	71
30	250 ± 57	71	83 ± 23	29
60	58 ± 5	29	50 ± 0	0

(*17*) than concentrations measured 1 minute after urine application ($U = 10$, $P < .05$) (Fig. 1A) and were higher than concentrations in water-treated females at 60 minutes after treatment ($U = 8$, $P < .02$) (Fig. 1A) (*18*). Concentrations of NE were also significantly greater [$F(1,43) = 10.0$, $P < .01$] in posterior compared to anterior olfactory bulb extracts from water-treated females (compare Fig. 1, C and D) (*19*). A significant time-dependent decrease in the concentration of NE at 1 minute compared to 60 minutes ($U = 3$, $P < .01$), reaching 54 percent depletion, was observed in the posterior olfactory bulb of urine-treated females (Fig. 1C). Concentrations of dopamine in females exposed to urine did not differ significantly between anterior (117 ± 11.8 pg/mg, mean ± standard error) and posterior (119 ± 11.0 pg/mg) olfactory bulb sections or as a function of time after exposure to urine (for each of the four intervals, 129 ± 18.7, 123 ± 14.9, 115 ± 14.8, and 106 ± 15.7 pg/mg, respectively). Control females receiving water did not show significant time-dependent changes in LHRH, NE, or dopamine.

Serum LH concentrations (Table 1) for urine-stimulated female voles were elevated in comparison to water-exposed controls at 1 minute ($U = 8$, $P < .02$) and 30 minutes ($U = 12$, $P < .06$) after stimulation and returned to nondetectable levels at 60 minutes. In general, serum LH concentrations for water-exposed females were low except for an apparent surge at 15 minutes after exposure when LH concentrations in water- and urine-stimulated females were equivalent.

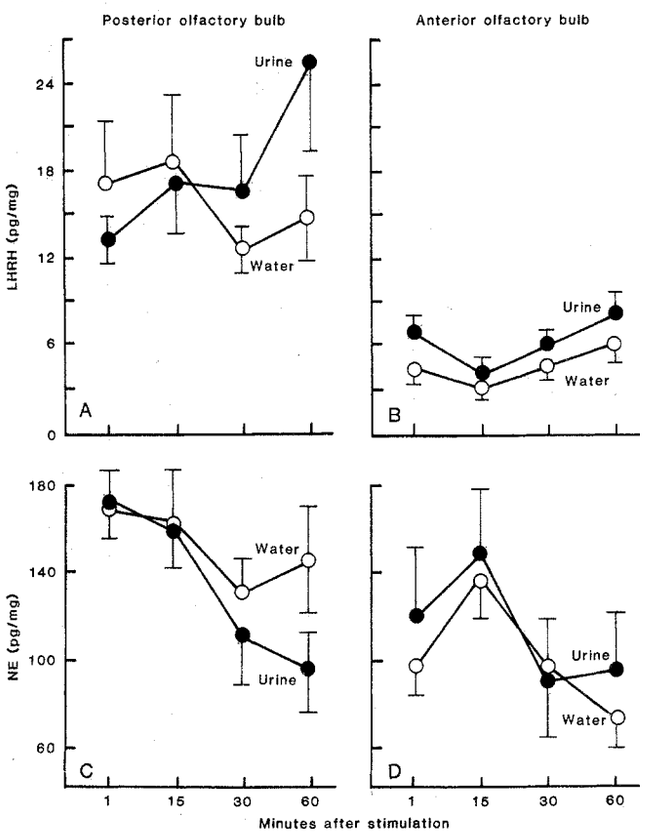

Fig. 1. Mean (± standard error) concentrations (picograms per milligram) of LHRH and NE in posterior and anterior olfactory bulb extracts. Female prairie voles were exposed to either male urine or to water and killed at 1, 15, 30, or 60 minutes later ($N = 7$ per group).

As in the rat (*7*), LHRH was preferentially localized in the posterior half of the olfactory bulb. An area within the posterior half of the olfactory bulb that has been implicated in LH release (*20*) and is believed to influence reproduction in general, is the accessory olfactory bulb (*4*). Although we cannot document a causal relationship between the observed changes in olfactory bulb LHRH and LH in the vole, the localization and changes of LHRH concentration within the posterior olfactory bulb indicate that a specific neuroendocrine pathway may link the external chemical environment to LH release and reproductive activation. We suggest that olfactory and gustatory information contained in male urine [which is known to stimulate vomeronasal organ receptors (*21*)], is transmitted to the accessory olfactory bulb (*22*) where LHRH neurons may then participate in the release of LH from the pituitary by a yet unidentified neural pathway (*23*).

Since serum LH concentrations were increased in urine-exposed females at 1 minute after treatment, a rapid activation of posterior olfactory bulb neurons may have preceded this interval. The LHRH concentrations measured after this period were presumably the net result of changes in LHRH synthesis and release. The time-dependent decrease in NE concentration, also localized to the posterior olfactory bulb, may implicate NE in the synthesis or release of LHRH. The highest concentration of posterior olfactory bulb LHRH in urine-stimulated females appeared at a time when serum LH was lowest (60 minutes). This high concentration of LHRH may represent a relatively greater increase in synthesis than release. It is possible that high concentrations of serum LH specifically produced by olfactory and gustatory stimuli contained in male urine feed back to the posterior olfactory bulb and inhibit the release process.

It is also possible that a stress factor (handling of the animals) contributed, in part, to the activation of the hypothalamic-pituitary axis causing the release of LH. A moderate increase in serum LH in water-stimulated females 15 minutes after the application of the stimulus might reflect general handling stress. Some changes, albeit not significant, were observed in LHRH (Fig. 1B) and NE (Fig. 1D) concentrations within the anterior olfactory bulb of both urine- and water-

treated females 15 minutes after application.

Our results indicate that exposure to male urine may produce measurable endocrine and neurohormonal changes in LHRH and NE and that these changes are measurable in posterior, but not anterior, olfactory bulb tissue. These changes implicate olfactory bulb LHRH and NE as possible mediators for LH secretion and may offer support for a possible role of the vomeronasal system in the activation of female reproduction in this species.

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11. The female prairie vole, an induced ovulator, will remain reproductively suppressed (with regard to the induction of ovarian function and onset of estrus) if housed with male or female family members or other females, and requires stimuli from an unfamiliar male to activate reproductive processes (*2*, *3*). Uteri from females in the present experiment were weighed to further verify reproductive suppression. Uterine weights of females in this experiment were less than 20 mg, which indicates an absence of prior activation.
12. For the radioimmunoassay for LH we used the NIAMDD rat LH system obtained from the Rat Pituitary Program of the NIAMDD, NIH. Sensitivity for the LH assay at 80 percent binding is 5 ng. Samples (100 μl) were run in duplicate. This LH assay for vole serum has been used and validated [V. D. Ramirez, J. E. Hauffe, L. L. Getz, V. D. Ramirez, *Trans. Ill. Acad. Sci.* **72**, 42 (1979)].
13. For the radioimmunoassay for LHRH we used a procedure previously described in which free and bound hormone are separated with ethanol. The antibody was provided by T. M. Nett and used at a final dilution of 1:200,000. The sensitivity of this assay at 80 percent binding is between 0.5 and 1.0 pg of a synthetic LHRH standard (Beckman) [D. E. Hartter and V. D. Ramirez, *Endocrinology* **107**, 375 (1980)].
14. Catecholamine concentrations were determined by a radioenzymatic assay previously described, with the exception that tetraphenylborate was added for the extraction of catecholamines. The sensitivity for both NE and dopamine is 32 pg (twice the blank value) [J. Becker and V. D. Ramirez, *Neuroendocrinology* **31**, 18 (1980); L. L. Zschaeck and V. D. Ramirez, *J. Neural Transm.* **39**, 29 (1976)].
15. The olfactory bulbs were placed on ice and perpendicularly bisected along the longitudinal axis into bilateral anterior and posterior halves. Each half was further divided into right and left halves. The tissue samples were weighed, and samples from anterior and posterior olfactory bulbs were placed in separate vials containing either 100 μl of 0.1*N* $HClO_4$ for catecholamine extraction, 100 μl of 0.1*N* HCl, for extraction of LHRH. Observations from our laboratory indicate no lateralization of catecholamines or LHRH in olfactory bulb tissue, and sample extracts from right and left olfactory bulbs were equally represented in the two assays. Tissue extracts were homogenized and either brought to 10 percent volume by weight with 0.1*N* $HClO_4$ for catecholamine determination or to a final volume of 1.0 ml with 0.1*N* HCl for LHRH assay. Both catecholamine and LHRH extracts were centrifuged for 20 minutes at 5000 rev/min (Beckman J-21B, J.S.7.5 head) and duplicate samples from the supernatant were taken for the respective assays.
16. In an experiment with unstimulated female voles ($N = 5$), a significant ($U = 1$, $P < .008$) LHRH concentration was localized in posterior (15.2 ± 0.94 pg/mg) compared to anterior (4.5 ± 0.99 pg/mg) olfactory bulb tissue. Varying volumes of olfactory bulb samples used in the assay resulted in a dose response parallel to the LHRH standard curve.
17. Nonparametric Mann-Whitney *U* tests were used for pairwise comparisons.
18. We measured LHRH concentrations from additional groups of females (five per group) stimulated with urine from either males castrated 21 days previously or intact males and decapitated at 30, 60, or 120 minutes later. The combined results (mean ± standard error) for each time interval for the two groups were: anterior tissue = 5.2 ± 1.3, 4.6 ± 0.5, 4.8 ± 0.5 pg/mg; posterior tissue = 17.0 ± 2.7, 21.3 ± 3.4, 25.6 ± 2.8 pg/mg). Urine from castrated males, although less effective than that of intact males in increasing uterine weight, does significantly increase uterine weight in comparison to nonstimulated females (*2*). The elevated LHRH concentrations at 60 minutes in urine-stimulated females are apparently maintained 120 minutes after urine stimulation.
19. In another experiment with unstimulated female voles ($N = 5$) a significant ($U = 4$, $P < .05$) localization of NE was obtained from posterior (250 ± 24 pg/mg) compared to anterior (192 ± 27 pg/mg) olfactory bulb extracts.
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23. An anatomical pathway for the olfactory-stimulated LH release in male rats exposed to odors from estrous females may involve accessory olfactory bulb projections to the medial preoptic area where LHRH neurons or other projections of the medial preoptic area to the medial basal hypothalamus may stimulate LH release [F. Scalia and S. S. Winans, *J. Comp. Neurol.* **161**, 31 (1975); L. A. C. Conrad and D. W. Pfaff, *Science* **190**, 1112 (1975)]. However, olfactory-dependent LH release also occurs after lesions are made in the medial preoptic area [F. Kamel and A. I. Frankel, *Horm. Behav.* **10**, 10 (1978); E. L. Ryan and A. I. Frankel, *Biol. Reprod.* **19**, 971 (1978)]. Thus there may be other pathways or mechanisms of action through which olfactory or gustatory stimuli mediate LH release.
24. We thank N. Ramirez for performing the catecholamine assays and G. Jackson and E. Roy for their comments on this manuscript. This work was supported by NSF BNS 79-25713 (C.S.C.), NSF PCM 77-04656 (V.D.R.) and NIH HDO9328 and NSF DFB 78-25864 (L.L.G.).

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Age Determination for the Shanidar 3 Neanderthal

Abstract. *Close agreement between the age at death estimated by macroscopic and microscopic methods was obtained for the Shanidar 3 Neanderthal. This suggests the possibility of obtaining age at death estimates by microscopic methods in other fossil hominids where the skeletal remains are highly fragmentary and macroscopic methods are not applicable.*

Age assessment for fossil hominids has relied primarily on macroscopic indicators. These include morphological changes of the pubic symphysis and sacroiliac joints, cranial suture closure, the degree of dental occlusal attrition, proximal femoral trabecular bone loss, and a variety of osteoarthritic changes (*1*). These indicators have been shown to provide ages of death from recent skeletal samples that are reliable primarily for individuals under 40 years of age. The age at death determinations for the fossils are based on the assumption that the rates of skeletal growth and degeneration among Pleistocene human populations were similar to those of preindustrial modern populations, although some processes, such as dental attrition, may have occurred at a faster rate.

Analysis of the degree of remodeling of cortical bone and the development of

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