

that died during the initial 1-hour feeding period were discarded from the data set, on the assumption that this was due to non-treatment-related manipulation of the insects.

16. Cox regression allows the comparison of entire survival curves against an assigned reference survival function for different variables simultaneously. The best statistical model was found by a backward stepwise procedure.

Treatment, colony (i.e., where the worker came from), and the interactions between these factors were entered into the full model. The treatments were coded as categorical variables (0, 1, 2) for LPS absent, low-LPS, and high-LPS, respectively, and with a second variable (0, 1) for beads being absent or present, respectively.

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Promiscuity and the Primate Immune System

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The behavioral and ecological factors involved in immune system evolution remain poorly explored. We present a phylogenetic analysis of white blood cell counts in primates to test three hypotheses related to disease risk: increases in risk are expected with group size or population density, exposure to soil-borne pathogens, and mating promiscuity. White blood cell counts were significantly greater in species where females have more mating partners, indicating that the risk of sexually transmitted disease is likely to be a major factor leading to systematic differences in the primate immune system.

Basal levels of white blood cells (WBC) are one of the first lines of defense against infectious disease (1). In mammals, disease risk is likely to vary with social, ecological, and sexual factors, providing predictions for differences in WBC counts across species. Social factors, such as group size and population density, are hypothesized to correlate with disease risk through increased transmission opportunities (2–5). Substrate use, an ecological factor, is another potential predictor of disease risk across species in that terrestrial species may be at greater risk of acquiring parasites through fecal contamination of the soil (6). Finally, greater frequency of sexual contact may lead to an increased risk of acquiring sexually transmitted disease (7, 8). Sexual contact frequency is quite variable across primates (9, 10). In gibbons (*Hyllobates* spp.), for example, females are generally monogamous (9), whereas Barbary macaque (*Macaca sylvanus*) females mate with up to 10 males per day during estrus (11).

We used standard phylogenetic comparative methods to test whether evolutionary increases in the above-mentioned factors of disease risk are associated with evolutionary increases in WBC counts (12). We obtained mean WBC counts from adult females, mainly in zoos, with the use of the International Species Information System (13). These data are from healthy animals, with information available on 41 species representing all the major primate radiations. For each species, the mean number of samples was 112 (range, 11 to 357), and information was obtained

from an average of 16 different institutions (range, 1 to 43). The advantage of using data from animals in captivity, as compared to those the wild, is that the health of individual animals is better ascertained, which is critical for estimating baseline WBC counts.

Higher WBC counts were found in species where females mate with more males (Fig. 1). However, bivariate regression analyses of independent contrasts found no support for group size, population density, percentage of time terrestrial, or body mass as predictors of overall WBC across primates (Table 1). In a multiple regression analysis with WBC as the dependent variable, only the number of mating partners was statistically significant (14).

We also tested the predictions using specific WBC types, including neutrophils, monocytes, and lymphocytes. Neutrophils and monocytes function in nonspecific phagocytosis, whereas lymphocytes are involved in adaptive immunity and in recognition of antigens. All of these WBC types contribute to protection against infectious disease (1) and are therefore predicted to increase with social, ecological, and sexual parameters. The effects of group size and population density remained unsupported in regression tests of particular WBC types. However, evolutionary increases in mating partner number were associated with increases in lymphocytes and monocytes, while a mean increase in neutrophils approached significance (Fig. 1). In addition, evolutionary increases in the percentage of time that primates spend on the ground were associated with significant increases in neutrophils [$b = 0.069$, $F(1,15) = 5.65$, $P = 0.02$]. It is well known that larger-bodied primates are more terrestrial (15). Further analysis revealed that

body mass contributes significantly to neutrophil counts, but it was not possible to separate this effect from terrestriality (16).

We repeated analyses using a surrogate measure of female mating promiscuity that is quantitative rather than categorical and is based on estrous duration and testes mass. Longer estrous periods enable females to mate with multiple males (10). Testes mass, after correcting for body size, is a measure of the degree of sperm competition and therefore is a useful surrogate variable, in females, for the number of mating partners that a female is likely to have (17, 18). To assess how these associated traits [$F(1,13) = 6.77$, $P = 0.02$] relate to disease risk, we used principal components analysis (PCA) of contrasts to capture in one variable the effects of estrous duration and residual testes mass. As our measure of mating propensity, we used the first principal component score, which explained 79.5% of the variation and had positive loadings for both variables; thus, a higher PCA score corresponds to increases in mating period and relative testes mass. The regression of WBC contrasts on first principal component scores was significantly positive (Fig. 2) [$F(1,13) = 20.8$, $P = 0.0003$]. In a multiple regression using this measure of mating promiscuity, group size, and percent time terrestrial on WBC contrasts, only promiscuity was statistically significant [$b = 0.012$, $F(1,7) = 5.80$, $P = 0.03$]. We also investigated patterns of male WBC counts and found similar patterns (19). In humans, WBC counts are more consistent with monogamy than promiscuity (20).

Our analyses demonstrate that basal immune system parameters vary among primates. The surprising result is that this variation appears to be driven by risk of acquiring sexually transmitted disease rather than disease that is transmitted as a function of social group size (4) or terrestrial locomotion (6). The precise reason for this result requires further study. It might be that sexually transmitted diseases are simply more common in nature than previously thought (21), or that behavioral mechanisms to avoid infectious disease (22, 23) are less effective against sexually transmitted pathogens. Different components of the immune system may also be used to combat different types of disease (1). Thus, sexually transmitted diseases tend to be persistent and immuno-evasive (21), in the sense that they have mechanisms to avoid or combat induced responses. On the con-

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trary, many general contact diseases have a "hit-and-run" strategy, where infection and transmission occur before the inducible im-

mune system can be brought into action (1, 24). Our study, therefore, raises larger issues about the relative roles of the different blood

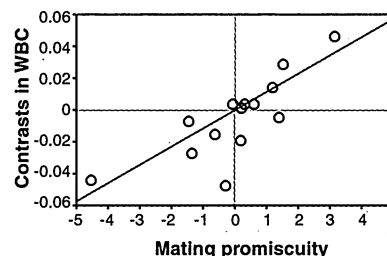
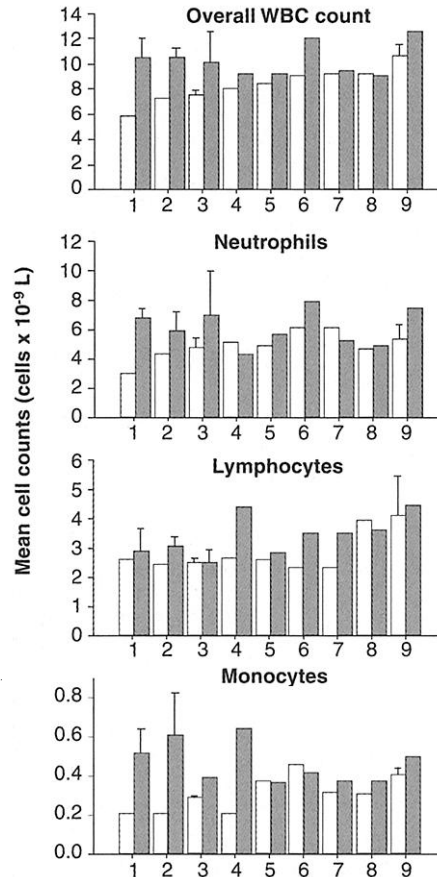
cell types in combating different types of diseases, the role of inducible versus noninducible defense systems in mammals, and the degree to which trade offs exist between behavioral and cellular defense mechanisms.

Fig. 1. WBC counts used in phylogenetic analysis of mating partner number. Bars represent mean blood cell counts for comparisons of less promiscuous taxa (open bars) to those that are relatively more promiscuous (closed bars) for nine pairs of taxa. Standard errors are provided for bars representing averaged values of two species (i.e., a contrast involving a higher node). Contrasts used in the analyses were differences in bar height corrected for branch length (27). The sum of the nine contrasts was tested versus the null hypothesis of no change using a t test (27) (overall white blood cell counts, $t = 3.26$, $P = 0.006$; neutrophils, $t = 1.83$, $P = 0.052$; lymphocytes, $t = 2.45$, $P = 0.02$; monocytes, $t = 2.48$, $P = 0.02$). Comparisons were based on an independent source (10) that used a three-part classification of female mating patterns: one mating partner (1 mate), most copulations with one male, but also regularly with other males (1+ mates), and multiple mating partners (many mates). Taxa used in the comparisons were: 1, *Callicebus molli* vs. *Saguinus oedipus* and *Leontopithecus rosalia*; 2, *Macaca silenus* vs. *M. nigra* and *M. fuscata*; 3, *Cercopithecus mitis* and *Erythrocebus patas* vs. *Papio sp.* and *Cercocebus torquatus*; 4, *Callithrix jacchus* vs. *Cebuella pygmaea*; 5, *Saimiri sciureus* vs. *Cebus apella*; 6, *Gorilla gorilla* vs. *Pan troglodytes*; 7, *Hylobates lar* vs. *Pongo pygmaeus*; 8, *Varecia variegata* vs. *Lemur catta*; and 9, *Aotus trivirgatus* and *Callicebus donacophilus* vs. *Alouatta caraya*. Callitrichids are known to have mating systems that are extremely flexible behaviorally (36), but exclusion of these contrasts (1 and 4) also produced significant results for overall WBC, neutrophils and lymphocytes ($P < 0.04$ in all cases).

Fig. 2. Evolutionary change in WBC versus a derived measure of female mating promiscuity using independent contrasts. Female mating promiscuity was measured using the first principal component of contrasts in the duration of estrus (10) and testes mass (18) after controlling for body mass by taking residuals. We controlled for phylogeny by calculating PCA scores from contrasts rather than species data, following methods from (37), including those for forcing the PCA through the origin. Species without well-defined periods of female mating activity were excluded from the analysis.

Table 1. Analyses of female WBC counts using independent contrasts. All results were nonsignificant in one-tailed tests based on a priori predictions. Data on group size, population density, and percentage of time terrestrial were compiled from the published literature. Female body mass was taken from (34). We predicted an increase in WBC with female body mass because mass is correlated with life history parameters in primates (35) and longer lived animals may require greater investment in immune defense. We also performed tests treating categorical values of substrate use as a quantitative variable (14). Although this increased the sample size substantially, substrate use remained nonsignificant [$b = 0.048$, $F(1,36) = 2.02$, $P = 0.08$].

Test	No. of contrasts	Slope	F statistic
Group size	36	0.001	1.05
Population density	32	-0.037	3.04
Percent time terrestrial	16	0.034	2.52
Female body mass	39	0.031	0.48



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12. Analyses were based on independent contrasts (25, 26), with the exact method depending on the types of variables examined (27). For bivariate analysis of mixed categorical and quantitative variables (e.g., Fig. 1), we examined specific evolutionary transitions in the dependent variable using the BRUNCH algorithm in the CAIC computer program (27). For two quantitative variables (e.g., Table 1) and for all multivariate analyses [see (14)], we used the CRUNCH algorithm (27). The primate phylogeny was taken from (28). We found that logarithmic transformation of the data and equal branch lengths best met the assumptions of independent contrasts (29). We checked for and removed outliers in contrasts plots, as such outliers often have high leverage and may represent the influence of confounding variables (30). Because specific directional predictions were tested, we used one-tailed statistical tests, but the primary results are provided such that two-tailed probabilities can be calculated. The unpublished data sets and links to programs used to conduct the analyses are available at <http://faculty.virginia.edu/charliennun>
13. International Species Information System, Physiological Reference Values [CD-ROM] (Minnesota Zoological Garden, Apple Valley, MN, 1999).
14. Substrate use: $b = 0.04$, $F(1,20) = 0.86$, $P = 0.18$; group size: $b = -0.04$, $F(1,20) = 0.41$, $P = 0.26$; number of mating partners: $b = 0.06$, $F(1,20) = 4.21$, $P = 0.03$. In multivariate analysis of substrate use and number of mating partners, we used a three-part ranked categorization of substrate use to match variation in the three-level mating partner categories. Substrate categories included: arboreal, semi-terrestrial in a wooded environment as intermediate, and maximally terrestrial in an open environment [updated from (31)]. Because no established methods exist for examining multiple categorical independent variables in contrasts analysis, and to increase sample sizes for these tests, we treated both variables as continuous in the CAIC computer program (27).
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16. We investigated the relative roles of substrate use, mating partner number, and body mass as predictors of neutrophil counts. First, in a multivariate regression analysis of contrasts, both body mass and categorical measures of substrate use were statistically significant predictors of neutrophil counts [$b = 0.17$, $F(1,35) = 9.69$, $P = 0.002$ and $b = 0.08$, $F(1,35) = 3.51$, $P = 0.03$, respectively]. Second, to determine if substrate use impacted the analysis of mating promiscuity, we excluded one contrast with a co-occurring shift in substrate use and mating partner num-

- ber. Overall WBC counts, lymphocytes, and monocytes remained significantly associated with mating partner number ($n = 8$ contrasts, $P = 0.01$ to 0.04), whereas both neutrophils and body mass increased with transitions to greater terrestrial substrate use ($n = 5$ contrasts, $t = 2.87$, $P = 0.02$ and $t = 2.81$, $P = 0.02$, respectively). However, multivariate analysis of these variables using the CRUNCH algorithm (27) provided no significant results ($n = 23$ contrasts, $P = 0.09$ to 0.11). Finally, we tested whether the allometric relationship with neutrophils reflects an underlying life history correlate. In particular, a stronger immune system might be required in species with a longer life-span. However, longevity did not account for neutrophil counts when holding body mass constant in multiple regression [$b = -0.23$, $F(1,34) = 1.82$, $P = 0.19$]. No significant results were found in allometric analysis of other WBC types, although a negative slope for lymphocytes approached significance ($b = -0.11$, $F(1,38) = 3.66$, $P = 0.06$).
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 19. In males, overall WBC counts were highly associated with female counts in contrasts analysis [$b = 0.64$, $F(1,38) = 24.07$, $P < 0.0001$], as were particular WBC types ($P < 0.05$ in all tests). This makes sense in the case of sexually transmitted disease: when one sex experiences increased risk, then the other sex should experience a corresponding increase (32). Male overall WBC ($t = 3.14$, $P = 0.007$), neutrophils ($t = 3.15$, $P = 0.007$), and lymphocytes ($t = 1.87$, $P < 0.05$) increased significantly over evolutionary transitions in female promiscuity (monocytes, $t = 1.61$, $P = 0.07$). Patterns of male promiscuity are difficult to analyze because there is less detailed information on male partner number, and, within species, greater variance in male mating success (i.e., sexual selection) may weaken patterns across species. Thus, analyses of discrete transitions to increased partner number in males produced significant results for neutrophils ($n = 4$ contrasts, $t = 2.66$, $P = 0.04$) when using the same set of species, as in analyses of females. But other analyses were not significant, including those using a wider range of species to give more contrasts. In multivariate contrasts analysis, however, residual testes mass accounted for variation in male WBC in excess of that explained by female WBC counts [$b = 0.08$, $F(1,20) = 4.86$, $P = 0.04$]. Although other explanations are possible, this relation is consistent with male mating promiscuity affecting basal WBC counts.
 20. First, we compared human standard reference WBC counts (33) to values for nonhuman primates among the discrete mating categories. For overall WBC, neutrophils, and lymphocytes, ranges for humans closely matched equivalent ranges in monogamous species, while human monocyte values best matched ranges for the intermediate category of "1+ mates" (see Fig. 1 caption). Second, we used the midpoint of human reference values in a hierarchical cluster analysis of the apes. We found that humans align most closely with the gorilla (*Gorilla gorilla*), a polygynous species with low sperm competition (17), and secondarily with a monogamous gibbon (*Hylobates lar*).
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NMDA Receptor-Dependent Synaptic Reinforcement as a Crucial Process for Memory Consolidation

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The hippocampal CA1 region is crucial for converting new memories into long-term memories, a process believed to continue for week(s) after initial learning. By developing an inducible, reversible, and CA1-specific knockout technique, we could switch *N*-methyl-D-aspartate (NMDA) receptor function off or on in CA1 during the consolidation period. Our data indicate that memory consolidation depends on the reactivation of the NMDA receptor, possibly to reinforce site-specific synaptic modifications to consolidate memory traces. Such a synaptic reinforcement process may also serve as a cellular means by which the new memory is transferred from the hippocampus to the cortex for permanent storage.

The hippocampus is critical for converting short-term memories into long-term memories (1–7). The NMDA receptor in the CA1 region serves as a gating switch for the modification of major forms of synaptic plasticity (8–13) and is required for certain types of learning (14–17). Despite both gain-of-function and loss-of-function genetic evidence linking the NMDA receptor to memory formation (18–20), its role in memory consolidation, which occurs over the days and weeks after initial learning, has not been well studied (5). The lack of coherent effort may be in part due to the general knowledge that activation of the NMDA receptor is required for induction, but not maintenance of synaptic plasticity. This has led to the popular belief that consolidation at the synaptic level is the result of molecular cascades initiated by a single long-term potentiation (LTP)-like event triggered during learning. However, this time scale of a single LTP-like molecular event (e.g., protein synthesis and gene expression) may not be adequate to account for the long-term memory consolidation process

that is known to continue many days and weeks after initial learning experience.

To examine the role of the NMDA receptor in long-term memory consolidation, we used the third-generation knockout technique [see supplementary Web material (21)] and generated the inducible, reversible, and CA1-specific NR1 knockout mice (iCA1-KO) (22, 23). Our overall strategy is to use both tTA (24–26) and Cre/loxP system (27) to achieve CA1-specific, tetracycline-regulated expression of the NR1-GFP transgene (28), thereby restoring the CA1 NMDA receptor function in the CA1-specific NR1 knockout mice (14, 16). However, feeding the iCA1-KO mice with drinking water containing doxycycline (doxy), a tetracycline analog with higher permeability through the blood-brain barrier, will switch off NR1-GFP transgene expression and return the mice to the NR1 knockout state in the CA1 region. Furthermore, removal of doxy from the water restores NR1-GFP expression in the CA1 region. Using a green fluorescent protein (GFP)-specific antibody (29), we found that the level of NR1-GFP protein was mostly restricted to the CA1 region of untreated iCA1-KO mice (Fig. 1, A and B), whereas the doxy treatment (1 mg/ml) suppressed NR1-GFP expression in the CA1

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