as belonging to a parasite hatched, and the nestlings were raised by the hosts. The parasites increased their expected reproductive output for the year by one to two offspring.

There was an overall increase in parasitism as a function of colony size ($r_s = 0.75$, $P < 0.001$), but there was no correlation between colony size and the percentage of nests parasitized for colonies consisting of more than ten nests ($r_s = 0.17$, $P > 0.05$) (Fig. 1). Thus, the chances of an individual being parasitized in all colonies of more than ten nests may be constant. The cost of intraspecific brood parasitism may therefore influence only whether or not birds choose very small colonies (*24*). The effect of increasing colony size to more than ten nests on the success of parasites is not clear.

Other evidence suggests (i) that intraspecific brood parasitism in cliff swallows involves two related adaptations on the part of the parasites and (ii) that it is even more prevalent than these data indicate. Eggs from presumed parasites occasionally appeared in host nests several days after the hosts had begun incubation, yet the parasites' eggs hatched at the same time as the hosts' eggs ($N = 6$) (*25*). This implies that eggs belonging to parasites may require less incubation time than host eggs, an adaptation reported for interspecific parasites (*1, 3*). Furthermore, cliff swallows tried continually to enter neighboring nests in the colony. When owners were away, intruders tossed out single eggs (*26*). These egg displacements may have been perpetrated by parasites that subsequently replaced the tossed eggs with eggs of their own. If parasites removed eggs and replaced them, the daily nest checks (Fig. 1) underestimated the frequency of brood parasitism. Extensive and continuous observations of marked individuals within a colony, coupled with electrophoretic parental exclusion analyses, are needed to evaluate further the prevalence of this reproductive strategy in the cliff swallow.

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18. Swallows were mist-netted and their white forehead patches colored in distinctive combinations with UniPaint and Decocolor paint marking pens. Marked birds did not appear to behave differently from unmarked birds, nor did other birds seem to react to them in any unusual ways. Sexes of birds were inferred from later copulation attempts and from observing birds parasitizing nests.
19. Nests were examined whenever owners left them unattended and whenever neighbors entered and then exited unattended nests after remaining inside for longer than 10 seconds. In this way any eggs appearing in an unattended nest after a neighbor had entered were verified to have been laid by the neighbor and not the owner. Nest examinations were made with a dental mirror and flashlight within 30 seconds, and disturbances to the colony during the examinations were minimal.
20. One female parasitized a nest 3 days before she began laying eggs in her own nest. Two females parasitized nests on the day after they had completed laying eggs in their own nests. The remaining female parasitized one nest on the day after her own clutch was completed and parasitized a second nest between laying the second and third eggs in her own nest. On that day she did not lay any eggs in her own nest. She also skipped a day between laying her first and second eggs and may have parasitized yet another nest on that day undetected. This same female later lost an egg in her own nest when the owner of the nest immediately adjacent entered her nest and tossed out an egg while she was absent [see (*26*)]. These parasitic females' own clutches did not seem to be parasitized in turn by other birds.
21. In contrast to bank swallows (*Riparia riparia*) [M. D. Beecher and I. M. Beecher, *Science* **205**, 1282 (1979)] and purple martins (*Progne subis*) [C. R. Brown, *Auk* **92**, 602 (1975)], promiscuity and polygyny are apparently rare among male cliff swallows that maintain nests in a colony. Females that mated with or were fertilized by the same male were not observed to lay eggs in the same nest.
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23. If brood parasitism is a significant cost, one might find host defenses that would minimize it, the most obvious defense being intraspecific egg recognition. However, in a series of 12 egg transfer experiments, no egg discrimination abilities were detected in cliff swallows. Eggs transferred from other nests remained in host nests until hatching in all cases. Possibly cliff swallows have evolved other defenses, such as nearly constant nest guarding by one or both members of a pair during the egg-laying period (inasmuch as parasitisms usually occurred only when a nest was left unattended).
24. My ongoing research suggests that benefits associated with social facilitation of foraging and, secondarily, benefits associated with predator avoidance increase with colony size up to sizes of at least 1600 nests. Costs associated with buildups of cimicid bugs also tend to increase with colony size. The benefits and costs attendant on foraging and ectoparasites may be the most important influence on the birds' choice of colony size.
25. C. R. Brown, unpublished data.
26. Swallows were observed destroying neighbors' eggs. Egg destruction increased with colony size ($r_s = 0.81$). In a colony of 1600 nests, 13 percent suffered losses attributed to conspecifics. However, incidents of an egg destruction and a parasitism in the same nest by the same individual at the same time were not seen.
27. I thank Mary Bomberger, Karen Brown, Laura Jackson, and Todd Scarlett for field assistance. Supported by an NSF Predoctoral Fellowship, Princeton University, Bache Fund of the National Academy of Sciences, Frank M. Chapman Fund of the American Museum of Natural History, Sigma Xi, Alpha Chi, Raymond and Kathryn Brown, and the University of Nebraska's Cedar Point Biological Station. I thank the director of the station, John Janovy, Jr., for assistance and permission to work there and E. Bitterbaum, M. Bomberger, E. Greene, J. Hoogland, H. Horn, J. Loye, R. May, T. Root, D. Rubenstein, J. Seger, P. Sherman, and D. Wilcove for comments on the manuscript.

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Behavioral Sensitivity to Purinergic Drugs Parallels Ethanol Sensitivity in Selectively Bred Mice

Abstract. *Behavioral responses to an adenosine receptor agonist and antagonist were examined in mice genetically selected for differential sensitivity to the soporific effects of ethanol. Both ethanol and the adenosine receptor agonist L-phenylisopropyladenosine had greater sedative and hypothermic effects in ethanol-sensitive "long-sleep" mice than in ethanol-insensitive "short-sleep" mice. Long-sleep mice were also more sensitive to the excitatory behavioral effects of theophylline, an adenosine receptor antagonist. These data suggest that adenosine may be an endogenous mediator of responses to ethanol.*

The mechanism by which ethanol produces its effects on the brain remains obscure. One of the most promising techniques for studying the effects of this drug has been the selective breeding of two mouse lines (SS, short-sleep mice, and LS, long-sleep mice), which differ in central nervous system sensitivity to the depressant effects of ethanol (*1*). Characterizing the differences between these two lines of mice may help to establish which neuronal systems are specifically

involved in behavioral responses to ethanol. Relatively few differences have been found between the two lines of mice in neurotransmitter concentrations, receptor numbers, and other neurochemical indices (*2*). In contrast, we report that these two lines of mice differ markedly in their behavioral responses to both an adenosine receptor agonist and an adenosine receptor antagonist.

Adenosine analogs have potent depressant effects on the central nervous system in a number of mammalian species (*3–6*). Theophylline and caffeine, which competitively displace adenosine ligands from putative adenosine receptors in brain membranes (*7*), antagonize the behavioral as well as physiological responses to adenosine (*5, 6, 8–10*). In addition, these antagonists have excitatory actions on the central nervous system that reflect antagonism of the effects of endogenous adenosine (*8, 10, 11*). To investigate a possible link between central adenosine systems and ethanol, we examined the behavioral responses of LS and SS mice to a metabolically stable adenosine analog, L-phenylisopropyladenosine (L-PIA), which binds with high affinity to adenosine receptor sites in the brain (*12*) and which has potent effects on electrophysiological and behavioral responses in rats and mice (*4–6*). We also characterized the behavioral responses of the two lines of mice to the adenosine receptor antagonist theophylline (1,3-dimethylxanthine).

The behavioral effects of purinergic drugs were investigated by several methods. For one set of observations, we used a simple behavioral test that is sensitive to both depressant and excitatory actions of these drugs. The animal was given an injection of the drug (*13*) and 30 minutes later was placed on a 4.5-cm-diameter platform 18 cm above a tabletop. An observer unaware of the drug condition recorded escape attempts (that is, the number of times the animal lowered its head below the platform) during a 2-minute test period. Animals given theophylline were scored by the time between placement on the platform and successful escape. A separate group of animals was tested with the light-dark box developed by Crawley and Goodwin (*14*) as a test for the action of benzodiazepines in mice. Animals were placed in a box divided into light and dark compartments, and total time in the light area and the number of crossings between the compartments were recorded for a 5-minute test period. Body temperature was measured at the end of behavioral testing (*15*).

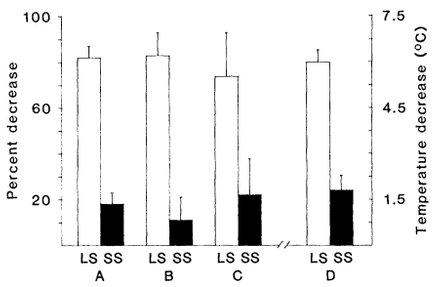

Fig. 1. Effect of L-PIA on behavior and temperature in LS and SS mice. Percent decrease (mean ± S.E.M.) in (A) escape attempts, (B) time spent in the light portion of a light-dark box, and (C) the number of light-dark crossings during a 5-minute test period elicited by intraperitoneally administered L-PIA (0.1 mg/kg). (D) Decrease in rectal temperature produced by this dose of L-PIA at room temperature (21°C). Values (mean ± S.E.M.) for saline-injected control LS and SS mice, respectively, were 102 ± 7 and 107 ± 8 escape attempts per 2-minute test period, 74 ± 25 and 60 ± 17 seconds in the light compartment, 46 ± 12 and 37 ± 9 crossings per 5 minutes, and 38.2° ± 0.2°C and 38.0° ± 0.2°C; none of these differences were statistically significant ($P > 0.10$; t-test). In each group, 6 to 18 animals were tested.

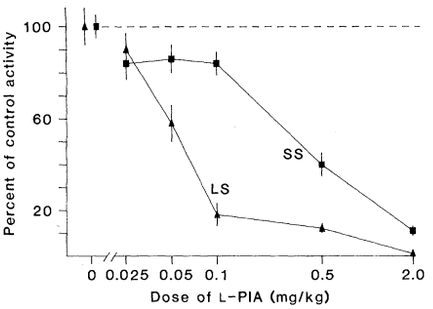

Fig. 2. Effect of L-PIA on escape attempts for LS (triangles) and SS (squares) mice. Escape attempts are shown as a percentage of the control; each point is the mean for 6 to 18 animals. Control values for the two lines (107 ± 8 and 102 ± 7 escape attempts for SS and LS, respectively) were not significantly different.

Long-sleep mice, which are more sensitive than SS mice to the soporific effects of ethanol, showed greater sensitivity to L-PIA than did SS mice on each of the behavioral tests used in these experiments (Fig. 1). The number of escape attempts, time in the light compartment, and light-dark crossings were all decreased by L-PIA (0.1 mg/kg). The average change in these measures was an 80 ± 2.8 percent [mean ± standard error of the mean (S.E.M.)] decrease relative to controls in LS mice, but only a 16 ± 3.2 percent reduction in SS mice ($P < 0.001$, t-test). Dose-response curves for L-PIA (Fig. 2) indicate that this drug is less potent in SS mice throughout the range of doses tested. Although there was no apparent difference in the maximal drug response in the two lines of mice, Hill plot analysis (*16*) indicated that the LS mice were significantly more sensitive to L-PIA EC_{50} (drug concentration required to elicit a half-maximal response) values were 0.07 mg/kg for LS mice and 0.29 mg/kg for SS mice, with 95 percent confidence limits of 0.03 to 0.19 and 0.10 to 0.82 mg/kg, respectively]. The heterogeneous stock (*17*), which is intermediate in ethanol sensitivity to the LS and SS lines, is intermediate in sensitivity to L-PIA (EC_{50}, 0.10 mg/kg). Theophylline completely reversed the behavioral effects of L-PIA in both LS and SS animals (data not shown).

We investigated the effect of ethanol to confirm that a differential sensitivity to ethanol could be observed with our testing protocol. At an ethanol dose of 1.75 g/kg, LS mice showed a marked decrease in escape attempts (75 ± 8.8 percent), whereas SS mice were not significantly affected (3 ± 6 percent increase) (Fig. 3). A higher dose of ethanol (2.3 g/kg) reduced this response measure in SS mice to approximately the same extent (68 ± 11 percent) as the lower dose in LS mice. This difference in sensitivity is comparable to results obtained with other behavioral tests (*18*).

The hypothermic effect of L-PIA reported earlier (*4, 6, 18*) was significantly greater in LS than in SS mice (Fig. 1). Because a reduction in motor activity might be an indirect consequence of hypothermia, we examined behavioral responses to L-PIA under conditions that minimized the decrease in body temperature. Although the behavioral effects of L-PIA were slightly reduced in both lines of mice by warming, the differential sensitivity of the two lines was preserved (*19*).

Our experiments demonstrate that

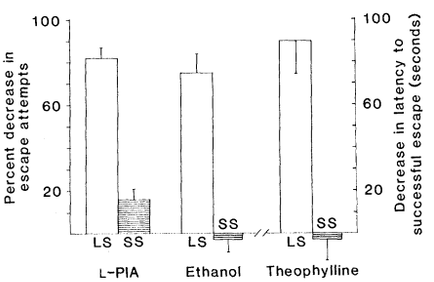

Fig. 3. Behavioral effects of L-PIA, ethanol, and theophylline in LS and SS mice. LS mice showed markedly greater sensitivity to both L-PIA (0.1 mg/kg) and ethanol (1.75 g/kg) as judged by escape attempts. Higher doses produced similar responses in SS mice as well (see text). The theophylline-induced decrease in latency to successful escape was observed only in the LS line at this dose (25 mg/kg). Each bar represents the mean ± S.E.M. for six to nine animals. Control values for SS and LS mice were not significantly different for any of these measures ($P > 0.10$, t-test).

mice selectively bred for ethanol sensitivity also show differential responses to an adenosine receptor agonist, L-PIA. To further characterize differences between these lines of mice, we examined the behavioral effects of theophylline, which antagonizes the effects of endogenous adenosine in the brain (*8, 10, 11*). The behavioral parameter most affected by theophylline in these studies was the latency to successful escape from the elevated platform; escape attempts could not be measured because theophylline-injected mice did not remain on the platform for the duration of the 2.5-minute test. Theophylline (25 mg/kg) caused a 61 ± 13 percent reduction in escape latency in LS mice, but had no significant effect on SS mice (2 ± 7 percent increase; SS versus LS, $P < 0.005$, t-test) (Fig. 3).

Our results show that mice selectively bred for differential sensitivity to the soporific effects of ethanol differ markedly in their behavioral and physiological responses to drugs that exert their effects via adenosine receptors. These data suggest that there may be important differences in purinergic systems in SS and LS mice; for example, in endogenous levels of brain adenosine or in adenosine receptors.

In terms of mechanisms of action, ethanol is probably not a simple purinergic agonist, since adenosine antagonists such as theophylline and caffeine can reduce but cannot block the effects of ethanol (*10, 20*). It is more likely that ethanol interacts in a more complex fashion with purinergic systems to affect behavior. Thus, ethanol and caffeine, two of the most widely used drugs in the United States (*21*), may both act at least in part via purinergic systems in brain.

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Vasopressin Injected into the Hypothalamus Triggers a Stereotypic Behavior in Golden Hamsters

Abstract. *Microinjection of arginine vasopressin into the medial preoptic area of the hypothalamus of male and female golden hamsters triggered a complex, stereotypic behavior—flank marking—a type of scent marking used in olfactory communication. The flank marking was not elicited by saline, oxytocin, neurotensin, or angiotensin II. Vasopressin was ineffective when injected into other areas of the hypothalamus or into the lateral cerebroventricle.*

Golden hamsters have large sebaceous glands on their dorsolateral flanks that are in an area encompassed by dark pigmented hair (*1*). The rubbing of these flank glands against objects is called flank marking (*2*), a type of scent marking that aids olfactory communication (*3*) and that can normally be elicited in intact hamsters by odors or aggressive behavior from other hamsters (*2, 4*). In the course of studies designed to evaluate the effects of peptides on circadian rhythms (*5*), we observed that after microinjection of arginine vasopressin (AVP) into the vicinity of the suprachiasmatic nucleus, the animal rubbed its eyes and nose with its forepaws and then started to lick and chew its flanks, creating large areas on both sides that were matted and soaked with saliva. Immediately afterward the hamster flank-marked by running forward with its back arched, vigorously rubbing its flanks against the sides of the cage. We now report that microinjection of AVP into a discrete area of the hypothalamus of the golden hamster can trigger a complex and well-organized behavior.

Adult male and female golden hamsters were anesthesized with pentobarbital, and a 26-gauge stainless steel guide cannula was implanted stereotaxically into the medial preoptic area (MPOA) above the suprachiasmatic nucleus, the area of the ventromedial and lateral hypothalami (VMLH), or the lateral ventricle (*6*). Animals received microinjections within 3 days after the guide cannulas were implanted.

For each experiment every animal was placed individually in a clean 24 by 32 by 20 cm plexiglass cage and allowed to adapt for 2 minutes. Test solutions were then microinjected, and the animals were observed for either 5 or 10 minutes. All microinjections were done with 33-gauge needles connected to a 1-μl Hamilton syringe through PE 20 tubing. These needles were easily inserted into the guide cannulas while the animals were restrained without the use of anesthesia. Five test solutions were used: control vehicle of 0.9 percent sodium chloride, neurotensin (NT) (50 ng), angiotensin II (AII) (50 ng), AVP (50 ng), and oxytocin (50 ng) (Sigma). All peptides were dis-