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-

solved in 0.9 percent chloride and given in a volume of 50 nl. The behavior of the animals was recorded on videotape. Flank marking was scored by counting the number of times each animal rubbed its flank against the side of the cage during the observation period. Statistical comparisons were made with analyses of variance. Hamsters were exposed to a cycle of 14 hours of light and 10 hours of darkness.

In experiment 1, gonadally intact male ($N = 5$) and female ($N = 5$) hamsters were tested independently with either AVP or 0.9 percent NaCl on different days (Fig. 1). Every animal receiving AVP in the MPOA flank-marked vigorously, whereas only a few of these animals showed any flank marking when tested with saline [$F(1, 16) = 38.56$, $P < 0.001$]. Males and females did not differ significantly in the number of times the animals flank-marked after stimulation. That both male and female hamsters responded to the microinjection of AVP is not surprising since both sexes have flank glands and naturally display flank marking.

In experiment 2, intact male hamsters ($N = 4$) received microinjections into the MPOA of AVP, oxytocin, NT, and AII. Each hamster received each of the four peptides over a course of 4 days. The sequence was counterbalanced to control for order and number of injections. Two-way analysis of variance revealed significant differences in flank marking ($P < 0.01$) (Fig. 2). Four times as many scent marks were observed after the microinjection of AVP as after oxytocin. The AII and NT had little or no effect on flank marking. That these two peptides, like AVP, are vasoactive and yet were unable to stimulate flank marking suggests that the response was not being indirectly mediated by changes in vascular permeability or blood flow in the area of the microinjection.

In experiment 3, intact male hamsters were divided into two groups: group 1 ($N = 4$) received AVP in the lateral cerebroventricle, and group 2 ($N = 7$) received AVP in the area of VMLH. Microinjections of AVP into the lateral ventricle and VMLH had no effect on flank marking (Fig. 2). The inability of AVP to induce flank marking when injected into the lateral ventricle shows that the response is probably controlled from a discrete area of the hypothalamus near the site of microinjection and not some other area of the central nervous system that may have been stimulated as a result of the possible spread of AVP through the ventricular system.

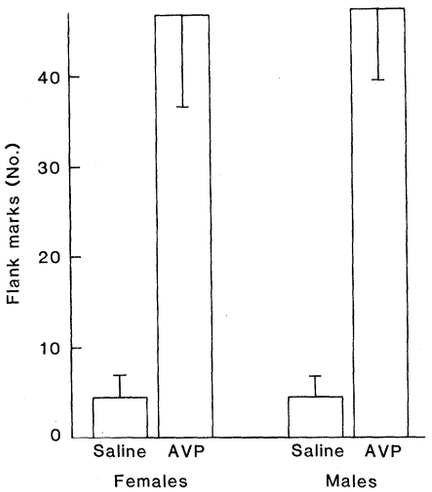

Fig. 1. Effects of microinjections of 0.9 percent NaCl and AVP on the number of flank markers scored over a 10-minute observation period. Vertical lines denote standard errors of the means.

In the original experiments, we studied male hamsters maintained under constant illumination. Each of these animals ($N = 5$) was tested independently with either AVP or NT on different days. More flank marking occurred when AVP was injected into the MPOA (mean ± standard error of the mean, 16.3 ± 5.6) during the 5-minute observation period than when NT was injected into the same site (1.4 ± 0.9) [$F(1, 8) = 6.68$, $P < 0.005$]. Thus animals maintained in constant light can also respond with an increase in flank-marking behavior when injected with AVP.

Three hamsters that had been blinded and castrated 6 weeks before this study for use in circadian studies were also responsive to AVP injected into the MPOA; these animals made 16.0 ± 4.0 flank marks during the 5-minute observation period. This result suggests that the response to AVP is independent of the gonadal status of the animal. Testosterone is necessary for odor-elicited flank marking in golden hamsters (*7*), but is not required for flank marking elicited by aggressive behavior by conspecifics (*8*).

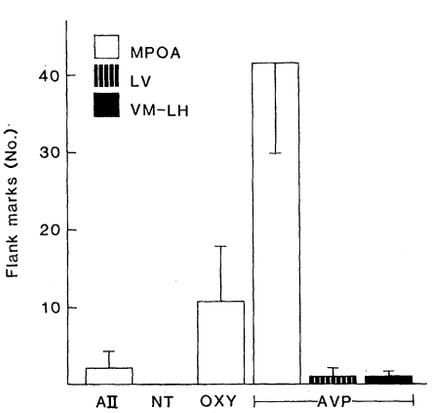

Fig. 2. Effects of microinjections of AII, NT, oxytocin (*Oxy*), and AVP on the number of flank marks scored over a 10-minute observation period. Vertical lines denote standard errors of the means.

The AVP-stimulated flank marking was always preceded by grooming behavior. Within a minute of the microinjection the hamster would vigorously rub its eyes and nose with its forepaws and without hesitation start to groom the flank glands. This grooming consisted of tossing and combing the hair with the forepaws while licking and chewing the area. The grooming behavior was observed after the microinjection of all of the peptides tested, but animals tested with oxytocin and AVP spent more time grooming than those tested with either AII or NT (*9*). The flank gland areas became matted and soaked with saliva in the hamsters tested with AVP and oxytocin but not with AII or NT. Whether the wetting of the flank glands is a result of the longer grooming time or whether there is an actual increase in salivary secretion due to the microinjection of AVP or oxytocin is uncertain.

We have demonstrated that discrete microinjections of AVP into the MPOA of all the animals tested ($N = 22$) triggered flank marking behavior. The MPOA has been implicated in the control of scent marking in several species. Lesions in the medial preoptic–anterior hypothalamic region of male cats (*10*) and dogs (*11*) eliminates urine scent marking, whereas extensive lesions of the rostral MPOA near the diagonal band of Broca significantly diminish scent marking in intact gerbils (*12*). Recently, Card and Moore (*13*) observed fibers and perikarya containing immunoreactive vasopressin in the MPOA of the golden hamster, the region that is responsive to the microinjection of the peptide.

Many behavioral responses have been observed after the intracerebroventricular administration of vasopressin in several species (*14*). In mice, vasopressin stimulates hyperactivity, increased grooming, foraging, and squeaking (*15*). In a pilot study using golden hamsters the intraventricular injection of 2 μg of lysine vasopressin "induced a dramatic bout of flank-marking" (*15*, p. 359). These injections may have stimulated the site we have found in the MPOA.

The electrical stimulation of discrete areas of the optic tectum in a freely moving toad initiates a complete se-

quence of prey-catching behavior (*16*). Similarly, stimulation of discrete brain sites in the freely moving cricket elicits different songs (*17*), and activation of certain neurons in the marine gastropod, *Tritonia diomedia*, elicits a complex escape pattern (*18*). These electrical stimulation experiments in lower vertebrates and invertebrates demonstrate that in the central nervous system there are motor programs with fixed neuronal circuits which, when stimulated, result in complex, well-coordinated motor patterns. It is probable that such "hard wired" behaviors exist in mammals. Our studies indicate that the microinjection of AVP into a discrete area of the hypothalamus of the hamster is able to trigger a complex, stereotypic motor pattern that exists normally in the animal's behavioral repertoire, and they suggest that AVP might function as a chemical messenger in the initiation of flank marking in the golden hamster.

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Factors in Ethanol Tolerance

The report by Wenger *et al.* (*1*) reiterates these investigators' important addition to the literature on alcohol tolerance (*2*), but the conclusions of the authors would lead one to believe that learning is the most important, if not the only, factor important for the development of ethanol tolerance. These conclusions ignore a large number of experiments with other techniques, such as liquid diets (*3*), in which ethanol was administered to animals in a manner such that learning could play but a minimal role in the development of tolerance. Yet, functional tolerance to ethanol's physiologic and behavioral effects was clearly demonstrated after such a method of ethanol administration.

Recent work in our laboratories (*4*) has, however, also demonstrated that the use of ethanol in paradigms which constitute conditioning can produce tolerance not only to the hypothermic, but also to the hypnotic effects of ethanol. The demonstration of this "conditioned" tolerance depends on testing the animals in the environment within which they are accustomed to receiving ethanol, and no tolerance can be demonstrated when animals are given ethanol in a novel environment. On the other hand, tolerance produced by feeding animals a liquid diet containing ethanol can be demonstrated within a wide variety of experimental environments. We have, therefore, used the terms "environment-dependent" and "environment-independent" tolerance to refer to forms of tolerance in which learning plays a major and a minor role, respectively (*4*), and we have presented evidence that development of the chronic, environment-independent form of alcohol tolerance requires the presence in the animal of higher levels of ethanol for continuous and extended periods of time compared to the levels of ethanol required for development of environment-dependent alcohol tolerance.

Whether environment-dependent and environment-independent forms of ethanol tolerance are simply dose-related, additive, manifestations of a singular physiological process is not, at present, clearly understood, but more is known in this area than is presented by Wenger *et al.* The pharmacologic manipulations which have been used to investigate learning and memory have been applied to studies of ethanol tolerance. Our studies, with neurotoxins and neurohypophyseal peptides (*5*) have shown certain similarities and some important differences in the way these agents affect the environment-independent form of alcohol tolerance and their effect on learning and memory. Our recent data on the effects of neurohypophyseal peptides or their analogs on development of environment-dependent and environment-independent alcohol tolerance further demonstrate differences in the effect of the peptides on these two forms of ethanol tolerance (*6*).

We would caution against an oversimplification of the alcohol tolerance phenomenon. Learning may be important in the development of some aspects of ethanol tolerance, but may not be important in all forms of ethanol tolerance. One should not ignore the fact that even within the categories of environment-independent and environment-dependent forms of tolerance, a further subdivision into dispositional (metabolic) and functional forms of ethanol tolerance is necessary (*7*).

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