term PCP psychosis develops in immunodepressed and genetically predisposed individuals who encounter a neurotropic virus during the immunodepressed phase.

NEMAT KHANSARI
H. D. WHITTEN
H. HUGH FUDENBERG
Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston 29425

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Intragastric Self-Infusion of Ethanol by Ethanol-Preferring and -Nonpreferring Lines of Rats

Abstract. *An ethanol-preferring line of rats, developed by selective breeding, consumed as much as 9.4 ± 1.7 grams of ethanol per kilogram of body weight per day through intrasgastric self-infusions, yielding blood ethanol concentrations of 92 to 415 milligrams per 100 milliliters. By contrast, the ethanol-nonpreferring line self-administered only 0.7 ± 0.2 gram per kilogram per day. These findings indicate that the reinforcing effect of ethanol is postabsorptive and is not mediated by the drug's smell or taste. Hence the ethanol-preferring line of rats may be a suitable animal model of alcoholism.*

Whereas most rats do not ingest significant quantities of ethanol when ethanol (10 percent by volume in water), water, and food are concurrently available, 1 to 3 percent of the population in some colonies of Wistar rats voluntarily consume 6 to 8 g of ethanol per kilogram of body weight per day or more (*1*). By selectively breeding individuals from such colonies, we developed ethanol-preferring (P) and ethanol-nonpreferring (NP) lines of rats in our laboratory (*2, 3*). Studies of P rats have shown that this line meets almost all the perceived requirements of an animal model of alcoholism (*4*). First, P rats voluntarily drink large quantities of 10 percent ethanol to produce pharmacologically significant blood ethanol concentrations; values as high as 105 mg per 100 ml have been measured (*1, 5*). Second, P rats work through operant responding to obtain ethanol, even when food and water are freely available (*6*). Third, when given the opportunity to drink ethanol over long periods, P rats develop physical dependence (*7*). Studies have also shown that P rats develop tolerance to ethanol's depressant effect more rapidly than do NP rats (*8, 9*), that P but not NP rats exhibit an excitatory response to low doses of ethanol (*10*), and that P rats differ from NP animals in the steady-state concentration of certain monoamines in several brain regions (*11*).

A critical requirement of an animal model of alcoholism (*4*) is that the positive reinforcing feature of ethanol should stem from the postabsorptive, pharmacological actions of ethanol rather than from its taste or smell. We reported earlier (*2*) that some P rats, trained to bar-press for 10 percent ethanol in a dipper, self-administered ethanol intravenously when the ethanol in the dipper was replaced with water. Because the total amount of ethanol self-administered was small and the phenomenon could not be observed in every P rat, involvement of the taste or smell of ethanol as a positive reinforcer could not be ruled out. We now report the results of studies of intragastric self-administration of ethanol by the P and NP lines. It appears that the positive reinforcing effect of ethanol in P rats and its absence in NP rats is mediated principally, if not entirely, by ethanol's postabsorptive effects.

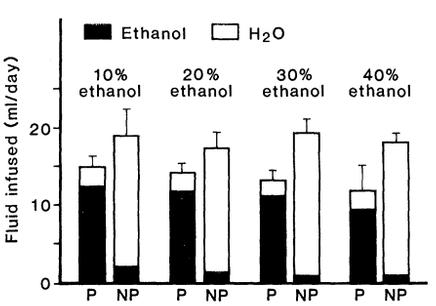

Fig. 1. Volumes of fluids infused by P and NP rats with free-choice drinking of two flavored water solutions paired with intragastric delivery of equal volumes of ethanol or water. Total daily fluid intake is twice that infused.

Male P and NP rats of the S-21 generation, weighing 310 to 475 g, were individually housed in a temperature- and humidity-controlled environment with a 12-hour light-dark cycle. All animals had been tested for ethanol preference at puberty (*12*) and had been ethanol-free for at least 1 month before surgery. The oral intakes of ethanol by the P and NP rats were 6.5 ± 0.4 and 0.4 ± 0.6 g/kg per day, respectively. Standard laboratory feed (Wayne Lab-Blox; Allied Mills) was freely available before and during the experiment. Access to water and ethanol during training and the experiment is described below.

The animals were surgically implanted with a transesophageal catheter (*13*) for the intragastric delivery of fluids. The catheter was held in place with a harness and swivel assembly that allowed the rat to move freely in the cage (*14*). During the 5- to 7-day postoperative period water was freely available. The rats were then trained in an apparatus (*15, 16*) to associate drinking of an aqueous solution of one of two neutral flavors (almond or banana, 0.5 percent by volume; Durkee Foods) from a U-shaped tube with intragastric infusion of an equal volume of water and ethanol (20 percent by vol-

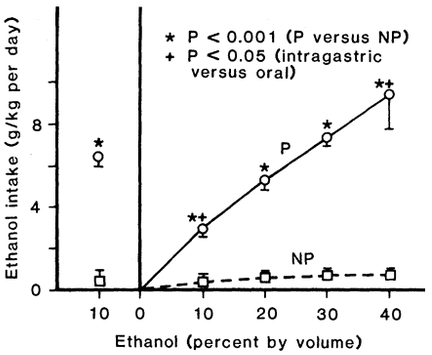

Fig. 2. Amount of ethanol self-infused intragastrically by P and NP rats. Free-choice oral consumption of ethanol by P and NP rats is shown on the left for comparison. Statistical significance was determined with the Newman-Keuls method for multiple comparisons.

ume), and to associate drinking of the other flavor with intragastric infusion of an equal volume of water alone. Both P and NP rats readily learned this discrimination task. During training and the experiment the banana flavor was paired with intragastric infusion of ethanol and the almond flavor with intragastric infusion of water in half of the animals. Pairing was reversed in the other half. In both P and NP animals the volume of ethanol infused with the ethanol-banana pairing was similar to that infused with the ethanol-almond pairing. Similar results were observed with respect to the intragastric infusion of water. Earlier tests had not shown any preference for either flavor.

After being trained, the P and NP rats were given free access to both flavored solutions in the U-tubes 24 hours per day. Every 5 days the concentration of ethanol in the solution for infusion was increased. Since the infusions were yoked to the drinking of an equal volume of flavored water, the intragastric concentration of ethanol self-administered was half that of the infused solution. P rats consistently self-infused greater volumes of ethanol than did NP rats (Fig. 1). As expected, the volume of ethanol infused decreased in both P (25 percent) and NP (55 percent) rats as its concentration increased from 10 to 40 percent. The volume of water infused did not change significantly in either group.

Figure 2 shows that the amount of ethanol self-administered each day by P rats increased from 3.0 ± 0.3 g/kg with 10 percent ethanol to 9.4 ± 1.7 g/kg with 40 percent. In contrast, NP rats consistently infused less than 1.0 g/kg, the maximum mean value being 0.7 g/kg at 40 percent. The difference between P and NP rats was evident at all concentrations of ethanol. With the 20 and 30 percent solutions, the amount of ethanol self-administered intragastrically by P rats was similar to that consumed orally by the same animals in free-choice drinking of 10 percent ethanol and water (Fig. 2). That with 40 percent ethanol was higher. It took 15 days to advance from 10 to 40 percent ethanol-infused; tolerance may have developed in this period.

To determine what blood ethanol concentrations can be produced in P rats by intragastric self-administration, we made measurements 30 to 40 minutes after observing individual drinking episodes in randomly selected animals. With unlimited access (24 hours per day) to the infusion solution of 20 percent ethanol, blood ethanol ranged from 116 to 303 mg per 100 ml (mean, 199 mg per 100 ml; $n = 4$). Under similar conditions, self-infusion of 40 percent ethanol induced blood ethanol concentrations of 92 to 415 mg per 100 ml (mean, 231 mg per 100 ml; $n = 6$). These blood ethanol levels attained with intragastric self-infusion are considerably higher than those found in P rats with free-choice drinking (*5*). The results suggest that the taste of ethanol may be slightly aversive even in P animals and thereby limit oral consumption.

Behavior supported by a putative positive reinforcer should be extinguished when the reinforcer is discontinued. This phenomenon was demonstrated in P rats when the flavor originally yoked to the intragastric infusion of 20 percent ethanol was paired with the infusion of water (Fig. 3). The volume of ethanol self-administered decreased drastically over a 5-day period. Concurrently, the infusion of water increased. Restoration of the original flavor-ethanol pairing evoked recovery of ethanol self-administration behavior within 4 days, and the amount of water self-infused returned to preextinction levels.

Although a preference for the drinking of alcoholic solutions over water is well documented in some rats and in other animal species, the possibility that the preference might stem from the gustatory or caloric value of ethanol has not been ruled out. Consequently, the validity of animal models of alcoholism involving oral self-administration of ethanol has been questioned (*17*). Our results implicate a postabsorptive, pharmacological mechanism (presumably mediated by the central nervous system) as the major factor underlying ethanol preference in P rats and nonpreference in NP rats. This finding and the other features of these animals indicate that the P and NP lines provide a suitable animal model for alcoholism research. The fact that these lines were developed by selecting

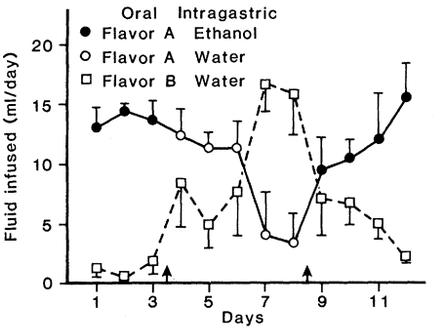

Fig. 3. Extinction and recovery of ethanol self-administration by P rats ($n = 5$) during free-choice drinking of one of two flavored water solutions (flavors A and B) paired with intragastric delivery of 20 percent ethanol or water. The arrows indicate when the pairing of the intragastrically infused fluid to flavor A was switched.

for ethanol preference and nonpreference underscores their usefulness, since there now is evidence that genetic factors contribute to the development of alcoholism in humans (*18*).

Past studies (*19*) have shown that rats can be induced to self-administer ethanol intravenously or intragastrically; however, not all animals self-administered ethanol by a nonoral route even after extensive training, and the amounts administered were small. The present study indicates that choice of experimental subjects is an important if not crucial variable. The design used here is similar to that reported by Deutsch and coworkers (*15, 20*). Using unselected rats (with respect to ethanol preference), they demonstrated self-administration of ethanol in large amounts only if the rats were first made physically dependent on ethanol by being forcibly administered the drug in large quantities. Ethanol self-administration behavior was quickly extinguished in unselected animals not made ethanol-dependent (*21*). In contrast, P animals do not require a period of forced ethanol administration and, indeed, had been alcohol-free for at least a month before these experiments.

MARSHALL B. WALLER
WILLIAM J. MCBRIDE
Departments of Psychiatry and
Biochemistry, Indiana University School of Medicine, and
Institute of Psychiatric Research, Indianapolis 46223

GREGORY J. GATTO
LAWRENCE LUMENG
TING-KAI LI*
Departments of Medicine and
Biochemistry, Indiana University School of Medicine,
Regenstrief Institute, and
Richard L. Roudebush VA Medical Center, Indianapolis 46223

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* To whom requests for reprints should be sent.

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Postnatal Modification of Hippocampal Circuitry Alters Avoidance Learning in Adult Rats

Abstract. *In rats and mice, the genetically mediated extent of the mossy fiber projection that synapses on the basal dendrites of hippocampal pyramidal cells is inversely correlated with rate of two-way avoidance (shuttle-box) learning. Postnatal hyperthyroidism, induced in 51 rat pups, resulted in marked variations of this infrapyramidal mossy fiber projection. The number of trials required for criterion performance of these rats in adulthood remained correlated with the neuroanatomical trait (r = 0.74, $P < 0.0001$).*

The ability of rats and mice to learn a two-way avoidance task (shuttle-box) is inversely correlated with a discrete and nonpathological variation in the intrinsic circuitry of the hippocampal formation: the more mossy fibers (efferents of the granule cells of the fascia dentata) terminating on the basal dendrites of hippocampal pyramidal cells, the poorer the animal's capacity for shuttle-box learning (*1*). The extent of this intra- and infrapyramidal subdivision of the mossy fiber projection (IIP-MF) is genetically mediated (*2*). To test the prediction that shuttle box learning would remain correlated with an experimentally altered mossy fiber distribution, we injected rat pups with thyroxine—a treatment that produces a variable hyperplasia of the IIP-MF projection that persists into adulthood (3). We now report that such developmental manipulation of neuronal circuitry can change the expression of an inherited talent for avoidance learning. This phenomenon is unlikely to reflect a pathological process but appears to manifest the action of a simple developmental factor that controls the graded and correlated expression of both a neuroanatomical trait and performance in an avoidance learning situation.

We studied rats of a strain that has been selectively bred for more than 40 generations for superior two-way avoidance (Roman High Avoidance, RHA/Verh) (*4*), the behavioral trait being correlated with a modest IIP-MF projection (*1*). One might expect that in these animals a thyroxine-induced hyperplasia of the IIP-MF projection fibers would impair the acquisition of a two-way avoidance response. Although attempting to impair performance is often a questionable strategy, it seemed appropriate for this experiment. A possible dynamic relation between the extent of the IIP-MF projection and two-way avoidance cannot be verified by reducing the IIP-MF in a poorly performing strain. Even with correlated improvement of shuttle-box learning, it does not seem possible to dissociate such an effect from the consequences of possible nonspecific damage inflicted on hippocampal circuitry, for almost any damage of the hippocampus results in an improvement in two-way avoidance. This paradoxical effect reflects a lesion-induced hyperreactivity that is beneficial in a test situation characterized by conflicting cues. The underlying mechanisms, however, are unknown, although several explanatory hypotheses have been offered (*5*). We thus preferred an experimental approach that predicts behavioral effects opposite to those seen after hippocampal lesions. This does not imply an attempt to "correct" a hippocampal malfunction (for

Table 1. Mean (and standard deviation) morphological and behavioral variables in adult RHA rats postnatally treated with saline or thyroxine. Thyroxine values are the pooled means of all treatment groups. Brain weight refers to fixed and trimmed brains before cutting. Data were analyzed with one-tailed t-tests; N.S., not significant.

Variable	Saline controls (n = 24)	Thyroxine (n = 51)	P
Body weight (g)	261 ± 52	238 ± 47	*
Brain weight (mg)	1438 ± 97	1458 ± 111	N.S.
Midseptotemporal volume of CA3-CA4 (mm^3)	0.93 ± 0.08	0.95 ± 0.08	N.S.
Volume ratio (%) of IIP-MF in CA3-CA4	2.9 ± 0.7	4.8 ± 1.3	**
Volume ratio (%) of remaining MF fields and stratum lacunosum–moleculare	32.3 ± 1.3	33.7 ± 1.8	**
Avoidance score (trials to criteron)	16.8 ± 10.2	21.2 ± 9.3	*
Latency to change compartment (shock delivered after 5 seconds)	5.7 ± 1.3	6.0 ± 2.4	N.S.

*$P < 0.05$. **$P < 0.001$.