ter the change in head velocity. Figure 2C shows the same three traces, but now with the normal and low-gain traces shifted vertically so that the three averages are superimposed just before the change in head velocity. The initial trajectory of the response is the same in all three gain states, whereas the adaptive changes are evident at later times. Figure 2D shows the early part of the eye velocity responses at high magnification. To estimate the time at which the high- and low-gain averages of eye velocity deviated from the normal eye velocity, we have measured magnified records like those in Fig. 2D. For increases in VOR gain, the averaged eye velocity records deviated from the normal averages at latencies averaging 18.6 msec after the onset of the head velocity stimulus (4.4 msec after the onset of the VOR); for decreases the eye velocity deviated from normal at latencies averaging 19.9 msec [5.7 msec after the onset of the VOR (*10*)]. Similar results were obtained when the VOR was tested with gentler changes in head velocity that had exponential trajectories and required 100 msec to reach a final value. The high- and low-gain responses did not deviate from the normal averages until 6.5 msec (4.25 and 8.75 msec in the two monkeys) after the onset of the VOR.

An intriguing feature of Fig. 2 is the sequence of inflections in the eye velocity records taken when VOR gain was low. Similar but smaller inflections occur at the same times in the normal and high-gain records, but none are apparent in the head velocity record. If the inflections were a response to some undetected component of the stimulus, we would have expected them to be larger when VOR gain was higher (*11*). Therefore, we believe that the inflections are a genuine part of the response and that they reflect the contributions of VOR pathways having different latencies.

Our data imply that the VOR is driven by several parallel pathways having different latencies and that the site of motor learning is in pathways having latencies of at least 19 msec, roughly 5 msec longer than the pathways with the shortest latency. Although it is not yet possible to be specific about the anatomical site of changes, our data may exclude the classical disynaptic reflex arc, which would be expected to drive the earliest part of the VOR (*12*). If we assume that synaptic delays are on the order of 0.5 msec, as is generally found with electrical stimuli, the 5-msec difference between the latency of the VOR and the latency of the modifiable pathways would allow for a rather large number of intervening synapses. However, it may take much longer for presynaptic activity resulting from natural stimuli to affect the firing of a postsynaptic cell. For example, Purkinje cells in the flocculus of the cerebellum receive di- or trisynaptic inputs from the vestibular nerve, but respond to sudden changes in head velocity with latencies 10 msec longer than was seen in vestibular primary afferents (*13*). Thus, the modifiable pathways may contain as few as one or two extra synapses (*14*). Definitive interpretation of our data, however, must await neurophysiological studies using the same rapid changes in head velocity used here.

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References and Notes

1. The characteristics of the adaptive mechanism have been studied in a number of species: human [A. Gonshor and G. Melvill Jones, *Proc. Can. Fed. Biol. Soc.* **14**, 11 (1971)]; rhesus monkey (*2*); squirrel monkey [G. D. Paige, *J. Neurophysiol.* **49**, 152 (1983)]; cat [D. A. Robinson, *ibid.* **39**, 954 (1976)]; rabbit [H. Collewijn, *Trends Neurosci.* **1**, 98 (1979); M. Ito, P. J. Jastreboff, Y. Miyashita, *Exp. Brain Res.* **37**, 17 (1979)]; goldfish [J. O. Schairer and M. V. L. Bennett, in *The Vestibular System: Functions and Morphology*, T. Gualtierotti, Ed. (Springer-Verlag, New York, 1981), pp. 463–477]; chicken [J. Wallman, J. Valez, B. Weinstein, A. E. Green, *J. Neurophysiol.* **48**, 952 (1982)].
2. F. A. Miles and B. B. Eighmy, *J. Neurophysiol.* **43**, 1406 (1980).
3. S. M. Highstein, *Exp. Brain Res.* **17**, 301 (1973); M. Ito, N. Nisimaru, M. Yamamoto, *ibid.* **24**, 257 (1976); W. Precht and R. Baker, *ibid.* **14**, 158 (1972).
4. A. A. Skavenski and D. A. Robinson, *J. Neurophysiol.* **36**, 724 (1973).
5. R. Baker, W. Precht, R. Llinas, *Exp. Brain Res.* **15**, 364 (1972); S. M. Highstein, *ibid.* **17**, 301 (1973); M. Ito, N. Nisimaru, M. Yamamoto, *J. Physiol. (London)* **265**, 833 (1977).
6. Details of our methods can be found in Miles and Eighmy (*2*) and in S. J. Judge, B. J. Richmond, F. C. Chu, *Vision Res.* **20**, 535 (1980).
7. E. L. Keller, *Vision Res.* **18**, 311 (1978).
8. To obtain voltages proportional to eye velocity, the eye position signal was processed by an operational amplifier circuit that was an effective differentiator up to 50 Hz. A voltage proportional to head velocity was taken from the tachometer output of a turntable (Contraves Goertz). Both signals were digitized at sampling rates 500 or 1000 Hz; results did not depend on sampling rate.
9. This value agrees with the data of J. Lanman, E. Bizzi, and J. Allum [*Brain Res.* **153**, 39 (1978)].
10. The numbers given here represent average values for the two monkeys, for leftward and rightward head accelerations, and for changes in head velocity of 10 and 30 deg/sec. In one monkey, the values were smaller for decreases in VOR gain than for increases, while in the other monkey the opposite situation obtained. Across both monkeys and all stimuli, the delay between the onset of the VOR and the deviation of eye velocity records from normal ranged from 2 to 9 msec.
11. To obtain an independent monitor of the trajectory of the head velocity stimulus, we analyzed the eye velocity records obtained from a calibration search coil that was held stationary in the world while the chair and field coils underwent rapid changes in velocity. The resulting records mirrored the head velocity monitor from the tachometer on the shaft of the turntable, and showed no evidence of extra inflections during the rapid change in head velocity.
12. The conduction times in disynaptic VOR pathways are very short: the total delay from labyrinth to abducens motoneuron is 0.9 to 1.7 msec in rabbit [S. M. Highstein, *Exp. Brain Res.* **17**, 301 (1973)] and 1.2 to 2.0 msec in cat [R. G. Baker *et al.*, *Brain Res.* **15**, 577 (1969)]. Thus it would be difficult to argue that any other pathway was responsible for the earliest part of the response to rapid changes in head velocity.
13. F. A. Miles, D. J. Braitman, B. M. Dow, *J. Neurophysiol.* **43**, 1477 (1980).
14. Because relatively little is known about the multisynaptic VOR pathways, it would be premature to argue the anatomical site of changes or even whether the site of adaptive modification is in a feedback or a feedforward circuit.
15. Supported by NIH grant EY03878, BRSG grant 05355, the McKnight Foundation, and the Alfred P. Sloan Foundation. I am grateful to L. E. Westbrook for her attention to the monkeys, to M. Stryker and A. Lander for their comments on an earlier version of the manuscript, and to F. Miles for lending me his set of magnifying spectacles.

25 January 1984; accepted 5 April 1984

Phencyclidine-Induced Immunodepression

Abstract. *Phencyclidine ("PCP" or "angel dust") and some of its derivatives are psychotomimetic drugs that have been used in general anesthesia for some time. This drug blocks potassium ion channels in brain tissue, and there is a specific PCP binding to lymphocytes. In a study of the effects of this drug on immunocyte function, it was found that humoral and cellular immune responses in vitro were depressed when immunocytes were treated with PCP before biological assay. This finding has implications for PCP abuse and also for the use of its derivative in general anesthesia, where it may contribute to postoperative infection.*

Phencyclidine ("PCP" or "angel dust") is a psychotomimetic drug that is abused in epidemic proportions in many areas (*1, 2*). The schizophrenia-like syndrome that sometimes occurs with PCP abuse (*3*) or that is seen as a hallucinogenic reaction to general anesthesia (*4*) has stimulated a search for the mechanisms producing these adverse effects (*5–8*). Although the neuropharmacological effects of PCP have received attention, the effects of PCP on the immune system have not been studied. We have found that PCP acts as an immunodepressant, at least in the immunological parameters studied in vitro. This finding is important for restoring the mental health of the PCP abuser as well as in situations in which PCP derivatives like ketamine are used for general anesthesia during surgery (*9*).

Our investigation of the effects of PCP on the immune system was based on the hypothesis that some forms of schizophrenia could be considered an autoimmune disease of the central nervous sys-

tem and that autoimmune reactions could contribute to the neuropathology of the disease (*10–12*). Thus, PCP can indeed interact with the immune system in that PCP preferentially binds to certain lymphocyte subpopulations—B cells have twice as many PCP receptors as T-helper cells, and T-suppressor cells have one-eighth as many PCP receptors as T-helper cells (*13*). Since PCP blocks K^+ channels in central nervous tissue (*14, 15*), and the functional integrity of these same channels is essential in lymphocyte activation by mitogens (*16*), it is not surprising that PCP inhibited lymphocyte DNA synthesis. However, quite unexpectedly the magnitude of inhibition for all three lymphocyte subpopulations (T-helper, T-suppressor, and B cells) was comparable (Table 1). This suggests that there is no strong correlation between PCP receptor numbers and the DNA synthesis inhibitory activity of this compound, for which the explanation may be the substantial heterogeneity of PCP receptors as reflected in the number of entities that bind PCP. These PCP-binding materials include the well-known opiate sigma membrane receptor (*8*) as well as the alpha and delta subunits of the acetylcholine receptor, the high- and low-affinity binding sites within the synaptosomal complex, a 95-kilodalton synaptosome protein involved in K^+ channeling, and a 43-kilodalton protein in the synaptosome of unknown function (*10–12*). Binding of PCP to one or more of these "receptors" may be instrumental in initiating an antireceptor process.

Table 1. Effects of PCP on lymphocyte DNA synthesis. Peripheral blood lymphocytes were separated by Ficoll-Hypaque centrifugation. Cells forming E rosettes (T cells) were separated as described (*21*). OKT-4– and OKT-8–enriched populations were prepared by lysis with OKT-4 and OKT-8, respectively (*22*). Positive selection was performed by the panning method (*23*), with the use of corresponding monoclonal antibodies. DNA synthesis was evaluated by [^{3}H]thymidine incorporation by 3-day cultures (*24*) in the presence (+) and absence (−) of PCP.

PCP treatment	[^{3}H]Thymidine incorporated (cpm/10^6 cells)
T-8 cell	
+	1246 ± 531
−	4764 ± 176
T-4 cell	
+	676 ± 215
−	2141 ± 837
B cell	
+	507 ± 133
−	1403 ± 451

Table 2. Effects of PCP on lymphocyte 2-deoxyglucose uptake. A cell suspension (100 μl) in complete RPMI [buffered with $NaHCO_3$ and supplemented with L-glutamine (2 m*M*/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and 5 percent fetal calf serum] was placed in each well of a microtiter plate. Phencyclidine was added to each well at the final concentration of $10^{-5}M$ (predetermined). The plate was then incubated in 5 percent CO_2 at 37°C for 18 hours. At the end of the incubation period, cells were washed three times with glucose-free Hanks balanced salt solution (HBSS) and incubated for another 15 minutes in the same buffer. The cells were then centrifuged for 5 minutes at 300*g*. The supernatant was discarded, and 50 μl of glucose-free HBSS containing 5 μCi of [^{3}H]deoxyglucose with a specific activity of 40 Ci/mmol was added to each well. After 90 seconds of incubation, the cells were harvested with a semiautomatic cell harvester, and radioactivity was measured with a liquid scintillation counter.

PCP treatment	[^{3}H]Deoxyglucose uptake (cpm/10^6 cells)
T-8 cell	
+	932 ± 131
−	1684 ± 833
T-4 cell	
+	1416 ± 351
−	3135 ± 920
B cell	
+	1149 ± 651
−	3278 ± 621

Cell glucose metabolism and respiratory burst can be studied via [^{3}H]deoxyglucose uptake (*17*), an event which also correlates directly with immunologic activation (*18*). In this system, a significant reduction of deoxyglucose uptake was observed when lymphocytes were treated with PCP. The degree of reduction for T-suppressor cells was less than that for B cells or T-helper cells (Table 2).

Treatment of T cells, B cells, or monocytes with PCP before culture in vitro for antibody synthesis resulted in a variable reduction of immunoglobulin (Ig) synthesis (Table 3). In these experiments, PCP treatment of monocytes had minimal effects on either IgG or IgM synthesis, whereas treatment of either T cells or B cells resulted in severe inhibition. Whether such inhibition resulted from the ability of PCP to block the membrane sigma receptor K^+ channels (or one of the other PCP-binding entities) or from undefined inhibitory effects on proliferation or maturation of the T or B cells has yet to be determined.

The necessity of interleukin-1 (IL-1) for the culmination of either humoral or cellular immune responses is well established (*19*). Interleukin-1 is produced by cells of the monocyte-macrophage lineage and is reported to act on B cells that are 16 to 20 hours into the G_1 phase of the cell cycle; this interaction promotes the passage of the B cell into the S (DNA synthetic) phase (*20*). We studied effects of PCP on the production of the IL-1 cytokine by monocytes. Our results indicate that PCP treatment reduced monocyte IL-1 production by 50 percent.

Although our observations show that PCP can induce immune depression in vitro, the mechanisms by which these inhibitory effects are exerted and their consequences on PCP abuse in vivo require further experimentation. However, in only a small percentage of PCP users do psychotic episodes progress to chronic schizophrenia. It is possible that long-

Table 3. Effects of PCP on immunoglobulin synthesis in vitro. After one of the lymphocyte subpopulations was treated overnight with PCP (final concentration, $10^{-5}M$), cells were mixed together in a 3:1 ratio of T cells to B cells, plus 5 percent monocytes; the cell mixture (2×10^6 cells) in complete RPMI containing pokeweed mitogen (1:50) was placed in each well of a 24-well microtiter plate and incubated at 37°C in 5 percent CO_2 for 8 days. Cultures were supplemented daily with 80 μl of nutritional cocktail (*25*). The culture supernatants were recovered at the end of the incubation period and assayed for total IgG and IgM by enzyme-linked immunosorbent assay (*26*).

Cells treated with PCP	Immunoglobulin (ng/ml per 10^6 cells)	
	IgM	IgG
T cells	537 ± 187	859 ± 362
B cells	281 ± 105	453 ± 237
Monocytes	989 ± 473	1963 ± 1137
None	1353 ± 615	2043 ± 1011

Table 4. Effects of PCP on monocyte IL-1 production. Three milliliters of a peripheral blood lymphocyte suspension (5×10^6 cells per milliliter) in complete RPMI was placed in each well of a six-well microtiter plate and incubated at 37°C in 5 percent CO_2 for 1 hour. Cells were then washed gently three times with warm medium to remove nonadherent cells. To the wells was added 3 ml of complete RPMI supplemented with 1 percent fetal calf serum containing lipopolysaccharide (25 μg/ml). Phencyclidine was added to the final concentration of $10^{-5}M$ at time zero. The adherent cells were then cultured for an additional 24 hours. The supernatants were then collected, centrifuged to remove any cells, dialyzed against plain RPMI, and filter-sterilized. The IL-1 activity of the supernatants was assayed with augmentation of the thymocyte proliferative response to phytohemagglutinin (*19*); 1a,2p represent 1-acetamide-2-pyrrolidone.

IL-1 (units per milliliter of culture supernatant)		
No treatment	PCP treatment	PCP + 1a,2p treatment
48	21	51
56	15	39
41	13	48
60	25	58
45	12	47

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

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References and Notes

1. R. S. Burns and S. E. Lerner, *Clin. Toxicol.* **12**, 463 (1978).
2. M. A. Schucket and E. R. Morrissey, *J. Clin. Psychiat.* **39**, 7 (1978).
3. E. D. Luby, B. D. Cohen, G. Rosenbaum, J. S. Gottleib, R. Kelley, *Neurol. Psychiat.* **81**, 363 (1959).
4. E. D. Luby, J. S. Gottleib, B. D. Cohen, G. Rosenbaum, E. F. Domino, *Am. J. Psychiat.* **119**, 611 (1962).
5. R. C. Smith, H. Y. Meltzer, R. C. Aurora, J. M. Davis, *Biochem. Pharmacol.* **26**, 1435 (1977).
6. S. Maayani and H. Weinstein, in *Membrane Mechanism of Drugs of Abuse*, C. Sharp and L. G. Abood, Eds. (Liss, New York, 1979), pp. 91–106.
7. S. Maayani, H. Weinstein, N. Ben-Zui, S. Cohen, M. Sokolovsky, *Biochem. Pharmacol.* **23**, 1263 (1974).
8. J. P. Vincent, D. Cavey, J. M. Kamenka, P. Geneste, M. Lazdumski, *Brain Res.* **152**, 176 (1978).
9. J. Fine and S. C. Finestone, *Anesth. Analg. Curr. Res.* **52**, 428 (1977).
10. A. L. Goldstein, B. Haber, G. H. Cohen, in *Neurochemical and Immunologic Components in Schizophrenia*, D. Bergsma and A. L. Goldstein, Eds. (Liss, New York, 1978), pp. 1–20.
11. H. H. Fudenberg, N. Khansari, P. Arnaud, H. D. Whitten, in *Enkephalins-Endorphins: Stress and Immune System*, N. P. Plotnikoff, A. Murgo, R. E. Faith, Eds. in press.
12. H. H. Fudenberg, H. D. Whitten, E. Merler, D. Farmati, *Med. Hypoth.* **12**, 85 (1983).
13. For PCP receptor enumeration of immunocyte subpopulations, we used a modification of the method of J. Möller Rasmussen *et al.*, [*Scand. J. Immunol.* **16**, 279 (1982)] with increasing concentrations of radiolabeled PCP. The binding data were analyzed by Scatchard plots (assuming one PCP molecule bound per receptor) to determine the amount of labeled PCP needed to saturate the PCP receptors. The number of receptor sites per cell was calculated as $R_0 \times (6.022 \times 10^{23})$ divided by the number of cells per liter, where R_0 is the molar concentration of the PCP receptors in the incubation tubes.
14. E. X. Albuquerque *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1224 (1980).
15. M. P. Blaustein and R. K. Ickowicz, *ibid.* **80**, 3835 (1983).
16. T. E. DeCowsey, K. G. Chandy, S. Gupta, M. D. Cahalan, *Nature (London)* **307**, 465 (1984).
17. D. E. Smith and J. Gorski, *J. Biol. Chem.* **243**, 4169 (1968).
18. J. R. Philp, J. G. McCormack, A. L. Moore, J. L. Johnson III, *J. Immunol.* **126**, 1469 (1981).
19. S. B. Mizel, D. E. Rosenthreich, J. J. Oppenheim, *Cell. Immunol.* **40**, 230 (1978).
20. M. Howard and W. E. Paul, *Annu. Rev. Immunol.* **1**, 307 (1983).
21. J. Grayson, N. J. Dooley, I. R. Koski, R. M. Blaese, *J. Clin. Invest.* **68**, 1539 (1981).
22. N. Khansari and H. H. Fudenberg, *Scand. J. Immunol.*, in press.
23. M. G. Mage, L. L. McHugh, T. L. Rothstein, *J. Immunol. Meth.* **15**, 47 (1979).
24. N. Khansari, M. Petrini, F. Ambrogi, P. Goldschmidt-Clermont, H. H. Fudenberg, *Immunobiology* **166**, 1 (1984).
25. R. I. Mishell and R. W. Dutton, *J. Exp. Med.* **126**, 423 (1967).
26. N. Khansari, H. H. Fudenberg, E. Merler, *Immunobiology* **164**, 42 (1983).
27. Publication 675 from the Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina. Research supported in part by grant J-451 from the Harry Frank Guggenheim Foundation.

12 March 1984; accepted 26 April 1984

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.

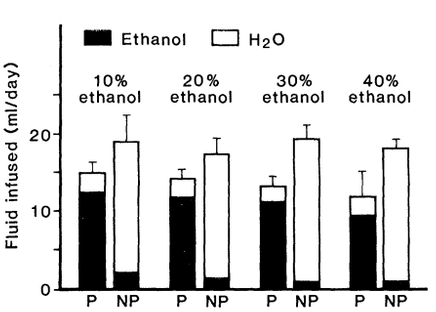

Fig. 1. Volumes of fluids infused by P and NP rats with free-choice drinking of two flavored water solutions paired with intragastric delivery of equal volumes of ethanol or water. Total daily fluid intake is twice that infused.

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-