served in the transplant. However, there was a slight diffusion of the HRP from the host cortex into the transplant.

Functional recovery from brain damage in animals with transplants of neural tissue may be due to factors other than connectivity between the transplant and host brain. It is possible that fetal brain grafts release neuronotrophic substances, such as polyamines and specific nerve growth factors. These may promote functional recovery by altering glial activity or neurotransmitter levels or by changing membrane receptor properties in the tissue surrounding the graft. Although the specific mechanisms remain to be discovered, these findings indicate that transplants of cortical tissue in adult rats are capable of enhancing behavioral recovery after bilateral brain injury.

Dunnett *et al.* (*4*) found that implants of fetal septal tissue promoted recovery of a learned discrimination in rats with damage to the fimbria-fornix system. These animals were able to solve a rewarded, spatial alternation task significantly faster than rats with similar damage but without transplants. However, animals with grafts did not perform as well as intact control animals on the spatial alternation task. These findings are similar to our own, despite the fact that Dunnett *et al.* waited 7 months before beginning behavioral training whereas we began testing just 4 days after transplantation. It is reasonable to conclude that the transplanted tissue begins to mediate behavioral recovery soon after transplantation and remains functional for almost a year, and perhaps for the rest of the animal's life.

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References and Notes

1. S. Finger and D. G. Stein, *Brain Damage and Recovery: Research and Clincal Perspectives* (Academic Press, New York, 1982).
2. A. Bjorklund, S. B. Dunnett, U. Stenevi, M. E. Lewis, S. D. Iversen, *Brain Res.* **199**, 307 (1980).
3. A. Bjorklund and U. Stenevi, *ibid.* **177**, 555 (1979).
4. S. B. Dunnett, W. C. Low, S. D. Iversen, U. Stenevi, A. Bjorklund, *ibid.* **251**, 335 (1982).
5. L. K. McLoon, S. C. McLoon, R. D. Lund, *ibid.* **226**, 15 (1981); M. M. Oblinger and G. D. Das, *ibid.* **249**, 31 (1982).
6. W. C. Low, P. R. Lewis, S. T. Bunch, S. B. Dunnett, S. R. Thomas, S. D. Iversen, A. Bjorklund, U. Stenevi, *Nature (London)* **300**, 260 (1982).
7. C. M. Leonard, *Brain Res.* **12**, 321 (1969); *Brain Behav. Evol.* **6**, 524 (1972).
8. We waited 7 days after inflicting the lesions to implant the fetal tissues because early responses to brain injury may hinder survival of such implants [E. R. Lewis and C. W. Cotman, *J. Neurosci.* **2**, 66 (1982)].
9. General transplant techniques are described in detail by G. D. Das, B. H. Hallas, and K. G. Das [*Experientia* **35**, 143 (1979)] and U. Stenevi, A. Bjorklund, and N. Svengaard [*Brain Res.* **114**, 1 (1976)]. Specific details of our transplant techniques may be obtained on request.
10. G. Patrissi and D. G. Stein, *Exp. Neurol.* **47**, 470 (1975).
11. J. V. Corwin *et al.*, *Neurobiol. Aging* **3**, 69 (1982).
12. Pressure injections of HRP conjugated to wheat germ agglutinin (Sigma) were made into the host cortex or transplant tissue. After 48 hours the rats were perfused transcardially and their brains were prepared by the tetramethylbenzidine procedure [M. M. Mesulam, Ed., *Tracing Neural Connections with Horseradish Peroxidase* (Wiley, Chichester, England, 1982)]. Animals that did not receive HRP injections were perfused with 0.9 percent saline and Formalin and their brains were cut into 40-μm sections and stained with cresyl violet acetate.
13. K. A. Crutcher and F. Collins, *Soc. Neurosci. Abstr.* **53**, 4 (1982).
14. We thank P. Curley, M. L. Valentino, R. Plourde, and D. Gash for their assistance and advice. Supported by United States Army Medical Research and Development Command Contract DAMD-82-C-2205.
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2 May 1983

Effects of Serotonin on Memory Impairments Produced by Ethanol

Abstract. *Subjects treated with low or high doses of ethanol demonstrated impaired memory, particularly in tests involving the recall of poorly learned information. Zimelidine, an inhibitor of serotonin reuptake, reversed this ethanol-induced impairment. The serotonin neurotransmitter system may mediate learning and memory in humans and may determine some of the effects of alcohol on higher mental functions.*

While ethanol has been shown to disrupt many higher mental functions, its effects on learning and memory processes have been studied the most extensively. This is due in part to the precision with which these aspects of cognition can be measured. However, we still know little about the behavioral and biological mechanisms underlying ethanol-related changes in learning and memory (*1–3*). At intoxicating doses, ethanol alters many information-processing components, including quantitative and qualitative aspects of how events are encoded, the shift of information from short- to long-term memory, retrieval processes, and memory consolidation (*1–7*). A complete understanding of these complex cognitive changes depends on our knowledge of the psychobiology of memory and learning, which remains incomplete (*8–10*). A better understanding of how ethanol alters information processing would also be useful for elucidating the biology of information processing and might generate new tools for treating ethanol-related cognitive impairments.

This study was designed to ascertain whether zimelidine, a relatively specific blocker of serotonin reuptake (*11*), can attenuate the impairing effects of ethanol on memory and learning. We found that poorly learned but not well-learned information was subject to ethanol-induced memory impairment in a dose-dependent fashion and that treatment with zimelidine prevented this cognitive disruption.

In a preliminary experiment we found that a single dose of zimelidine reversed ethanol-induced impairment of memory, as measured by a battery of standardized procedures that test vigilance, learning, and memory functions (*12*). This prompted us to conduct the present experiment. The subjects were ten male volunteers, all with at least some college education. They were 22 to 27 years old, free of medical or psychiatric illness, within 15 percent of their ideal body weight, and had normal liver enzyme values. Their first-degree relatives were free of alcoholism. The subjects were informed about the nature of the study and gave their signed consent to participate as volunteers.

Study design consisted of a 10-day hospitalization, preceded or (in half the subjects) followed at least 2 weeks later by three successive outpatient days. After receiving an initial 200-mg dose of zimelidine on the morning of admission, the subjects were given 100-mg doses twice a day (morning and evening) throughout their hospital stay. Each subject served as his own control under six different test conditions. On each test day they had a light breakfast between 0700 and 0800. On arrival at the laboratory between 0900 and 1000, the subjects were given breath alcohol analyzer tests to ensure that their systems were ethanol-free. Between 1000 and 1100 each subject ingested two tablets containing a placebo or 100 mg of zimelidine. (Zimelidine would be expected to produce its

peak effects 3 to 5 hours later.) Two hours after drug treatment on inpatient days 8, 9, and 10 and on all outpatient days, each subject received, in random order, a placebo drink or 190-proof ethanol (0.5 or 1.0 g/kg) mixed in 4 ounces of orange juice. The subjects were allowed 30 minutes to consume this cocktail. Two hours later breath alcohol analyzer readings were obtained. The blood ethanol level was 0.014 ± 0.005 percent (mean ± standard deviation) in subjects given the low ethanol dose and 0.057 ± 0.014 in subjects given the high ethanol dose. Zimelidine treatment did not alter blood ethanol levels.

Tests of learning and memory (*12*) were administered 120 to 150 minutes after the subjects had finished drinking and immediately after blood ethanol concentrations were measured. The subjects were asked to listen to highly familiar and categorically related words, such as names of birds, kinds of transportation, or parts of a house, read to them at the rate of one every 3 seconds. Some of the words were repeated while others were presented only once. The first task of each subject was to identify a word that was repeated in a list consisting of six words read once and six words presented twice, a total of 18 words. After this test of vigilance, the subjects were given a distracting task for 10 minutes to prevent rehearsal and then asked to freely recall the words. Finally, the subjects' recognition memory was tested by requiring them to identify the 12 previously presented words from an equal number of semantically equivalent distractors (*13*).

Vigilance performance was not altered by ethanol, zimelidine, or their combination. Furthermore, many other forms of cognitive performance, such as a task requiring continuous execution (*14*), were unaltered by zimelidine and ethanol. No changes in the vestibular, cerebellar, or extrapyramidal motor systems were noted after the zimelidine treatments (*15*). In each test condition the subjects were consistently accurate in identifying repeated words correctly, with an average accuracy rate of 85 ± 6 percent. They remembered more twice-presented than once-presented words [$F(1, 9) = 113.1$, $P < 0.001$] and recognized more words than they could remember freely. They were also very reliable in their free-recall performance, "remembering" few words not presented as stimuli (intrusion rate, less than 5 percent for all conditions).

Free recall of poorly learned words (words presented only once during the vigilance procedure) was less complete after ethanol treatment, and this impairment in memory was dose-dependent (Fig. 1). Zimelidine reversed the impairment 80 percent in subjects given the low dose of ethanol and 65 percent in subjects given the high dose [$F(1, 9) = 5.64$, $P < 0.05$]. Ethanol did not disrupt memory of well-learned information (words presented twice), nor did zimelidine alter this aspect of mnemonic performance. Zimelidine did not affect free recall when subjects were administered a placebo drink instead of ethanol. Recognition memory of poorly processed words but not twice-presented words was also disrupted by the high dose of ethanol [$F(2, 20) = 7.4$, $P < 0.01$]. The average number of once-presented words that were correctly recognized after ethanol treatment was 4.0 ± 0.3; 4.6 ± 0.3 such words were recognized after treatment with zimelidine and ethanol [$F(1, 9) = 7.9$, $P < 0.05$]. Correct recognition of distractor words was not altered by ethanol or zimelidine. In no test session were subjects able to tell whether they had received zimelidine.

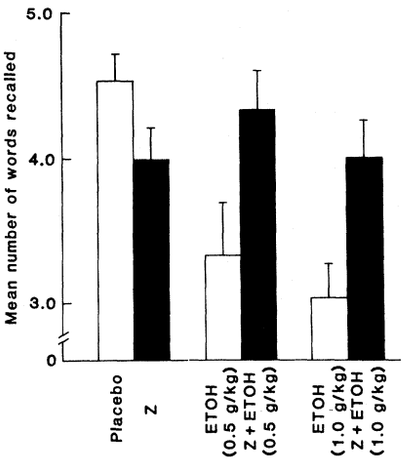

Fig. 1. Memory performance after treatment with ethanol (*ETOH*), zimelidine (*Z*), or both.

Zimelidine may attenuate the disruptive effects of ethanol on memory and learning either directly by stimulating serotonergic activity or indirectly through secondary effects on noradrenergic functions (*16*). However, the latter mechanism of action is unlikely to contribute to the ethanol-antagonizing potency of zimelidine. This is because desipramine, a relatively specific inhibitor of norepinephrine reuptake, does not attenuate ethanol-induced impairment in the same memory paradigm (*17*). Moreover, the zimelidine-ethanol antagonism of memory seems to be a specific phenomenon, because zimelidine did not attenuate deleterious effects of ethanol on other behaviors, such as body balance and visual-motor tracking (*14*).

Antagonism of the effect of ethanol by zimelidine is qualitatively different from other treatments, such as ingestion of food or injection of an imidazoline derivative, which can affect blood ethanol levels by changing the absorption or distribution of ethanol, or administration of large amounts of fructose, which can facilitate the elimination of ethanol (*18–20*). Other types of pharmacodynamic antagonists that have been tested (with generally weak effects in reversing cognitive impairments) include hyperbaric oxygen treatment (*21*); cholinergic drugs such as physostigmine (*22*); sympathomimetic agents such as amphetamine; dopaminergic drugs (*23*); neuropeptides, including thyrotropin-releasing hormone (*24*) and vasopressin (*25*); and opiate antagonists such as naloxone (*26*). These treatments by themselves affect learning and memory, suggesting a role for both the cholinergic and catecholamine systems in mediating these aspects of cognition. Recent evidence has also demonstrated the importance of the serotonin system in memory functions (*27*). While it is known that serotonin reduces ethanol tolerance in the rat (*28*), its role in altering the cognitive impairment produced by ethanol in humans has not been investigated. The zimelidine-ethanol interaction may suggest new strategies for reversing state-dependent effects of ethanol on mental functions. Further research in this area should advance our understanding of ethanol addiction and of the psychobiological bases of learning and memory.

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References and Notes

1. N. Butters and L. S. Cermak, *Alcoholic Korsakoff's Syndrome: An Information Processing Approach to Amnesia* (Academic Press, New York, 1980).
2. H. Weingartner, E. Adefris, J. E. Eich, D. L. Murphy, *J. Exp. Psychol. Hum. Learn. Mem.* **2**, 83 (1976).
3. D. A. Overton, *The Biology of Alcoholism* (Plenum, New York, 1972), vol. 2, p. 193.
4. D. W. Goodwin, E. Othmer, J. A. Halikas, F. Freemon, *Nature (London)* **227**, 201 (1970).
5. R. L. Alkana, E. S. Parker, H. B. Cohen, H. Birch, E. P. Noble, *Psychopharmacology* **55**, 84 (1977).
6. R. S. Ryback, *Alcohol and Memory* (Wiley, New York, 1977).
7. E. S. Parker *et al.*, *Psychopharmacology* **69**, 219 (1980).
8. J. B. Brierley, *Amnesia* (Butterworth, London, 1977).
9. A. J. Dunn, *Annu. Rev. Psychol.* **31**, 343 (1980).

10. H. Weingartner, P. Gold, J. C. Ballenger, S. A. Smallberg, R. Summers, D. R. Rubinow, R. M. Post, F. K. Goodwin, *Science* **211**, 601 (1981).
11. S. O. Ögren, S. B. Ross, H. Hall, A. C. Holm, A. L. Renyi, *Acta Psychiatr. Scand.* **63** (Suppl. 290), 127 (1981).
12. H. Weingartner, D. Langer, J. Grice, J. Rapoport, *J. Psychiatr. Res.* **6**, 21 (1982).
13. W. F. Battig and W. E. Montague, *J. Exp. Psychol. Monogr. 80* (1980), No. 3, part 2.
14. M. Linnoila, C. W. Erwin, W. D. Cleveland, P. E. Logue, W. D. Gentry, *J. Stud. Alcohol* **39**, 745 (1978).
15. M. Linnoila, F. Karoum, H. M. Calil, I. J. Kopin, W. Z. Potter, *Arch. Gen. Psychiatry* **39**, 1025 (1982).
16. M. V. Rudorfer, M. Linnoila, T. Dubyoski, J. Johnson, unpublished observations.
17. M. Linnoila, J. Johnson, R. J. Ross, M. S. Buchsbaum, unpublished observations.
18. P. G. Welling, L. L. Lyons, R. Elliott, G. L. Amidon, *J. Clin. Pharmacol.* **17**, 478 (1966).
19. L. Goldberg, A. W. Jones, A. Beri, *Blutalkohol* **16**, 439 (1979).
20. A. W. Jones, R. D. Jennings, J. Adolfson, C. M. Hesser, *Undersea Biomed. Res.* **6**, 15 (1979).
21. S. Griffin, D. Woodward, R. Chanda, *J. Neurochem.* **28**, 1269 (1977).
22. M. Linnoila and M. J. Mattila, *Pharmacol. Ther.* **15**, 99 (1981).
23. R. L. Alkana, E. S. Parker, H. B. Cohen, H. Birch, E. P. Noble, *Psychopharmacology* **55**, 121 (1977).
24. G. R. Breese, B. R. Cooper, A. J. Prange, J. M. Cott, M. A. Lipton, *Life Sci.* **14**, 1053 (1974).
25. P. L. Hoffman and B. Tabakoff, *Drug Alcohol Depend.* **4**, 249 (1979).
26. A. I. Mackenzie, *Lancet* **1979-I**, 733 (1979).
27. E. R. Kandel and J. H. Schwartz, *Science* **218**, 433 (1982).
28. J. M. Khanna, H. Kalant, A. D. Le, A. E. LeBlanc, *Alcohol. Clin. Exp. Res.* **3**, 353 (1979).

4 April 1983

Light and Propranolol Suppress the Nocturnal Elevation of Serotonin in the Cerebrospinal Fluid of Rhesus Monkeys

Abstract. *Markedly elevated nighttime concentrations of serotonin in rhesus monkey cerebrospinal fluid were reduced to daytime levels by exposing the monkeys to continuous light or to the β-adrenergic antagonist propranolol. Nighttime elevations of melatonin in cerebrospinal fluid were also suppressed by propranolol and light. Serotonin released in large quantities at night appears to be regulated like melatonin, and may act as a cerebroventricular hormone to influence brain and pituitary function at night.*

Concentrations of serotonin in rhesus monkey cerebrospinal fluid (CSF) reach nocturnal peaks that average 20 ± 7 times the mean daytime values and range up to 70 times higher (*1*). This large diurnal rhythm for serotonin went undetected until the development in 1982 of a sensitive gas-chromatographic mass-spectrometric assay that permitted quantitation of the amine in CSF (*1*). Diurnal rhythms in brain serotonin content have been found in many studies; however, these rhythms vary in phase in different brain regions and nuclei and in different species and are of relatively small amplitude (all less than twofold) (*2*). A diurnal rhythm in another neurotransmitter amine, norepinephrine, has been described in rhesus monkey CSF; peak elevations average 1.7 times higher than the nighttime troughs and occur at midday in coincidence with activity and temperature maxima (*3*).

The origin of CSF serotonin and the basis for its nighttime release are not known. A temporal relation of serotonin to the circadian variation in melatonin concentrations observed in one monkey (*1*) suggested that serotonin, the precursor of melatonin biosynthesis in the pineal gland, is released from the pineal directly into the cerebroventricular system. This seemed possible because the pineal in primates comprises a portion of the roof of the third ventricle and because the pineal contains very high concentrations of serotonin that decrease 80 percent at night in the rhesus monkey (*4, 5*). Other evidence, however, indicates that melatonin from the pineal in sheep, rodents, and man is released into the blood and reaches the CSF compartment secondarily (*6*); such a route would be unlikely for pineal serotonin since serotonin crosses the blood-brain barrier poorly and since there is only slight evidence for diurnal variations in blood serotonin content (*7*).

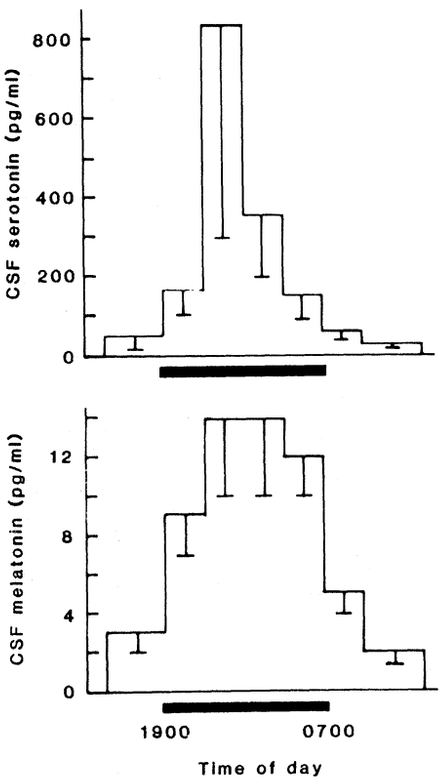

Fig. 1. Diurnal variations in CSF serotonin and melatonin in six monkeys. Lights (approximately 500 lux at eye level) were on from 0700 to 1900. Water was always available and food (Ralston Purina Monkey Chow No. 5038) was provided at 0900 and 1600 and was not withdrawn. CSF samples were collected at 90-minute intervals and pooled for analysis. The following time points are represented, left to right: 1500 to 1930, 1930 to 2230, 2230 to 0130, 0130 to 0430, 0430 to 0730, 0730 to 1030, and 1030 to 1500. Values are means ± standard errors.

To determine whether the diurnal rhythm for CSF serotonin is regulated like the diurnal rhythm for melatonin, we studied rhesus monkeys during normal light-dark cycles and under conditions that alter the release of pineal melatonin. Adult male *Macaca mulatta* were maintained on a 12-hour light-dark cycle and adapted to chair restraint. Under anesthesia a cannula was inserted between the lumbar vertebrae and advanced to the cervical subarachnoid space. After a 48-hour recovery period CSF was collected in 90-minute samples through the cannula, which was encased in a water jacket cooled to 10°C and which led into a fraction collector housed in a freezer at −40°C (*8*). Melatonin was quantified by radioimmunoassay (*9*) and serotonin by capillary gas chomatography and negative chemical ionization mass spectrometry after derivatization with pentafluoropropionic anhydride (*1*).

Serotonin and melatonin rhythms were temporally coincident in all six monkeys studied, with concentrations of both substances remaining low during the day, rising at the beginning of the dark period, and falling to baseline at the onset of light (Fig. 1). The peak concentration of serotonin at nighttime was 841 ± 541 pg/ml (from 2230 to 0130), while the mean concentrations during the entire dark and entire light periods were 378 ± 146 and 46 ± 10 pg/ml, respectively.

Since light suppresses nocturnal secretion of melatonin in rodents and monkeys (*10*), we exposed one monkey to three consecutive 24-hour periods of continuous light, followed by 2 days of 12-hour light-dark cycles. The large nocturnal elevations in CSF serotonin and melatonin were abolished by exposure to constant light (Fig. 2). Returning the animal to a light-dark cycle immediately reestablished the nocturnal rise in serotonin and melatonin. Similar responses to light suppression were seen in other animals (Fig. 3). The marked nocturnal