The complete disappearance of the ocular dominance columns after monocular deprivation for the first 3 months of postnatal life is not entirely surprising. Hubel, Wiesel, and LeVay (*3*) have studied long-term monocular occlusion begun at 2 weeks and at 3 weeks of age. In both instances it was observed that, while the combined width of adjacent columns remained the same, there was an enlargement of the width of the column representing the intact eye and a corresponding reduction in the width of the column of the deprived eye. A greater discrepancy in column widths was found when the monocular deprivation was initiated at 2 weeks rather than at 3 weeks of age, and it was suggested that the difference in severity of the effects of monocular deprivation was related more to the age at which the monocular deprivation was initiated rather than to the total period of deprivation. The newborn animals that we studied were monocularly deprived on the first day of life. The complete disappearance of the ocular dominance columns and the functional preemption of the territory normally occupied by the column representing the occluded eye by that of the intact eye may reflect this earlier deprivation.

M. H. DES ROSIERS*
O. SAKURADA
J. JEHLE, M. SHINOHARA
Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, Maryland 20014
C. KENNEDY
Department of Pediatrics, Georgetown University School of Medicine, Washington, D.C. 20007
L. SOKOLOFF†
Laboratory of Cerebral Metabolism, National Institute of Mental Health

References and Notes

1. P. Rakic, *Science* **183**, 425 (1974).
2. T. N. Wiesel and D. H. Hubel, *J. Comp. Neurol.* **158**, 307 (1974).
3. D. H. Hubel, T. N. Wiesel, S. LeVay, *Cold Spring Harb. Symp. Quant. Biol.* **40**, 581 (1976).
4. P. Rakic, *Nature (London)* **261**, 467 (1976).
5. L. Sokoloff, M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, M. Shinohara, *J. Neurochem.* **28**, 897 (1977).
6. L. Sokoloff, *ibid.* **29**, 13 (1977).
7. C. Kennedy, M. H. Des Rosiers, J. W. Jehle, M. Reivich, F. Sharpe, L. Sokoloff, *Science* **187**, 850 (1975).
8. C. Kennedy, M. H. Des Rosiers, O. Sakurada, M. Shinohara, M. Reivich, J. W. Jehle, L. Sokoloff, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4230 (1976).
9. D. H. Hubel and T. N. Wiesel, *J. Comp. Neurol.* **146**, 421 (1972).
10. S. LeVay, D. H. Hubel, T. N. Wiesel, *ibid.* **159**, 559 (1975).
11. M. H. Des Rosiers was supported as a Fellow of Le Conseil des Recherches Mèdicales du Canada.
* Present address: Département de Physiologie, Université de Montréal, Montréal, Canada.
† To whom correspondence should be addressed.

22 August 1977; revised 25 November 1977

Brain Noradrenergic Systems as a Prerequisite for Developing Tolerance to Barbiturates

Abstract. *Mice treated with 6-hydroxydopamine before they were chronically fed phenobarbital did not develop functional barbiturate tolerance, measured by duration of the loss of righting reflex and hypothermia. Injection of 6-hydroxydopamine caused significant depletion of brain norepinephrine, while brain dopamine levels were not significantly depleted. Intact brain noradrenergic systems seem to be necessary for developing tolerance to the hypnotic and hypothermic effects of the barbiturates.*

Continual ingestion of sedative hypnotics such as barbiturates or ethanol results in the development of central nervous system (CNS) tolerance to and physical dependence on these drugs (*1*). The neuronal systems that participate in developing this tolerance and dependence have not been defined. We now report that intact brain noradrenergic systems may be necessary to develop tolerance to the hypnotic and hypothermic effects of barbiturates. Male mice of the C57Bl/6 strain (22 to 24 g) were injected intraventricularly with 10 μl of a solution (*2*) containing 50 μg of 6-hydroxydopamine (6-OHDA, free base), or 10 μl of an artificial cerebrospinal fluid (CSF) (vehicle). Seven days after injection, the mice were divided into four groups. Group 1, the vehicle/control group, consisted of mice pretreated with artificial CSF, housed individually, and offered an unlimited diet of ground Purina mouse chow and water. Group 2, the 6-OHDA/control group, consisted of mice pretreated with 6-OHDA, and housed and fed as group 1. Group 3, the vehicle/barbiturate group, consisted of mice pretreated with artificial CSF, housed individually, and offered a diet of ground Purina mouse chow containing phenobarbital (free acid). The drug was present in the diet at a concentration of 3.5 g per kilogram of food for the first 3 days of continuous feeding, and at 4 g per kilogram of food for the next 3 days. Group 4, the 6-OHDA/barbiturate group, consisted of animals first treated with 6-OHDA and then fed the same barbiturate-containing diet as group 3. After 6 days, all mice were fed the control diet (withdrawal).

Table 1. Effect of pentylenetetrazol injection in barbiturate-withdrawn and control animals.

Group	No convulsions	Convulsions
Vehicle/control	11*	1
6-OHDA/control	10	2
Vehicle/barbiturate	1	6
6-OHDA/barbiturate	1	9

*Number of animals exhibiting particular symptoms. Animals having no symptoms or only tremors were placed in the no convulsions category, while the convulsions category included animals showing clonic and/or tonic convulsions, or dying after convulsions. Comparisons of vehicle/control and 6-OHDA/control, $P = .39$ [Fisher exact probability test (*9*)]; vehicle/barbiturate and 6-OHDA/barbiturate, $P = .51$; vehicle/control and vehicle/barbiturate, $P = .002$; 6-OHDA/control and 6-OHDA/barbiturate, $P = .001$; vehicle/barbiturate and 6-OHDA/barbiturate, $P = .51$.

Blood levels of phenobarbital were determined daily while the mice were consuming the phenobarbital-containing diet and every 4 hours after withdrawal began (*3*). Mice were also weighed each morning, and assessed for intoxication by monitoring locomotor behavior and coordination (*4*). Phenobarbital was removed from the diet on the morning of the seventh experimental day (*5*). The mice were kept at 22° ± 1°C and observed at 4-hour intervals for overt signs of withdrawal hyperexcitability; rectal temperature was monitored during each observation period (*4*). After 24 hours of withdrawal, several animals (Table 1) from each group were injected with pentylenetetrazol (50 mg per kilogram of body weight, intraperitoneally), and their behavior (*6*) was continuously monitored for the next 30 minutes. Animals that had been injected with pentylenetetrazol were not used in any subsequent studies.

Some of the remaining mice were injected intraventricularly (*7*) with 200 μg of sodium phenobarbital 44 hours after withdrawal, and the duration of loss of righting reflex (sleep time) and barbiturate-induced hypothermia were monitored (*8*) (Table 2). Upon regaining the righting reflex, these mice were immediately decapitated, and their brains were removed and analyzed for phenobarbital (*3*). Other animals were injected intraperitoneally with sodium barbital (300 mg/kg), and the duration of the loss of righting reflex was monitored. Brain levels of norepinephrine (NE), dopamine (DA), and serotonin in mice of all four experimental groups were determined 44 hours after withdrawal (*8*, *9*).

Mice in the vehicle/barbiturate and 6-OHDA/barbiturate groups had similar

0036-8075/78/0428-0449$00.50/0

Table 2. Effect of 6-OHDA on functional tolerance to barbiturates in mice. Values are means ± standard deviation. Sleep time was defined as the interval (minutes) from the time of injection until the animal could right itself three times in a 30-second period. Change in body temperature (°C) is the difference between the pretreatment temperature and the temperature 5 minutes after injection of phenobarbital. Pretreatment temperatures were vehicle/control group, 36.6 ± 0.4; 6-OHDA/control group, 36.4 ± 0.2; vehicle/barbiturate group, 36.6 ± 0.2; 6-OHDA/barbiturate group, 36.4 ± 0.3. The maximum change in temperature was reached between 5 and 7.5 minutes after injection of phenobarbital.

Group	Mice tested (No.)	Response to phenobarbital injection		Brain level of phenobarbital at waking (μg/g)
		Sleep time	Change in body temperature	
Vehicle/control	13	11.9 ± 3.2	−2.6 ± 0.5	99.1 ± 32.7
6-OHDA/control	9	13.4 ± 4.1	−2.6 ± 0.4	102.9 ± 19.1
Vehicle/barbiturate	16	5.2 ± 2.0*	−1.1 ± 0.3*	228.8 ± 73.9*
6-OHDA/barbiturate	10	13.7 ± 5.0	−2.6 ± 0.4	95.0 ± 32.9

*$P < .001$ compared to the other three groups.

(*10*) morning blood levels of phenobarbital throughout the 6 days of consuming the drug-containing diet. These levels ranged from 50 to 120 μg per milliliter of blood for the 6-OHDA/barbiturate group and 60 to 112 μg/ml for the vehicle/barbiturate group. Barbiturate in the blood was not detectable (< 10 μg/ml) by 16 to 18 hours after withdrawal. Mice removed from the barbiturate-containing diet exhibited withdrawal signs characterized by convulsive episodes and hypothermia; these symptoms became evident 12 to 16 hours after withdrawal and peaked approximately 20 hours after withdrawal. The time course for appearance and termination of overt behavioral signs of barbiturate withdrawal were similar in animals from the vehicle/barbiturate and the 6-OHDA/barbiturate groups. Although animals in the 6-OHDA/barbiturate group had more convulsions during withdrawal than animals in the vehicle/barbiturate group, the difference was not statistically significant [$P > .1$, Fisher exact probability test (*10*)]. Withdrawal-induced hypothermia was also similar in these two groups (*11*).

Mice in the 6-OHDA/barbiturate and vehicle/barbiturate groups exhibited significantly more episodes of clonic and tonic convulsions after injection of pentylenetetrazol (Table 1) than animals in the vehicle/control and 6-OHDA/control groups. Animals in the vehicle/barbiturate group were not significantly different in response to pentylenetetrazol than animals of the 6-OHDA/barbiturate group ($P = .5$, Fisher exact probability test).

Functional tolerance to barbiturates was determined 44 hours after withdrawal, when no overt signs of withdrawal hyperexcitability were evident. Animals of the vehicle/barbiturate group displayed significant tolerance to both the hypnotic and hypothermic effects of intraventricularly injected sodium phenobarbital (Table 2). This tolerance could be ascribed to changes in CNS sensitivity to barbiturate rather than to a change in its metabolism, since brain levels of phenobarbital in the vehicle/barbiturate group, when they regained the righting reflex, were more than twice those in the vehicle/control or the 6-OHDA/control groups. At the time of waking, however, animals in the 6-OHDA/barbiturate group had brain phenobarbital levels comparable to those of animals which had not received barbiturate in their diets (vehicle/control and 6-OHDA/control groups). In addition, when mice of the vehicle/barbiturate group were injected intraperitoneally with (300 mg/kg) sodium barbital, a barbiturate not metabolized by the liver microsomal drug oxidase system (*12*), they slept 173 ± 32 minutes; the sleep time of animals in the 6-OHDA/barbiturate group (226 ± 70 minutes) did not differ significantly from those of the vehicle/control (250 ± 84 minutes) or the 6-OHDA/control (223 ± 72 minutes) groups. The development of metabolic tolerance to barbiturate, however, was not influenced by prior treatment of animals with 6-OHDA. The rate of barbiturate degradation in animals of the two drug-fed groups was significantly increased over rates of barbiturate metabolism in the control groups (*11*).

Although brain DA levels were reduced by administration of 6-OHDA, they were not significantly different between the 6-OHDA and vehicle-treated animals ($P > .1$, t-test). Norepinephrine levels were reduced more than 50 percent in the 6-OHDA–treated animals, and these differences were statistically significant ($P < .05$, t-test) compared with vehicle-treated controls. The brain amine levels (nanomoles per gram of tissue ± standard deviation) of eight mice in each of the groups were: vehicle/control group, NE = 2.4 ± 0.7, DA = 6.6 ± 1.1, 5-hydroxytryptamine (5-HT) = 4.2 ± 0.3; 6-OHDA/control group, NE = 1.1 ± 0.5, DA = 5.2 ± 0.6, 5-HT = 4.3 ± 0.8; vehicle/barbiturate group, NE = 2.9 ± 0.3, DA = 7.0 ± 1.5, 5-HT = 4.3 ± 0.7; 6-OHDA/barbiturate group, NE = 1.2 ± 0.3, DA = 5.6 + 1.4, 5-HT = 4.0 ± 0.5.

Morgan (*13*) found that the treatment of rats with multiple injections of 6-OHDA, causing a more than 90 percent reduction in NE and a 72 percent depletion of DA, produced an earlier onset of barbiturate withdrawal seizures, and increased the number of convulsions occurring after barbiturate withdrawal. The less extensive destruction of catecholaminergic neurons under our experimental conditions was not found to significantly alter the monitored signs of barbiturate withdrawal. Our results indicate the partial destruction of catecholaminergic systems in the brain prevents or postpones the development of functional CNS tolerance to the sedative and hypothermic effects of barbiturates. We had previously demonstrated (*8, 14*) that destruction of noradrenergic rather than dopaminergic neurons is of primary importance in preventing the development of tolerance to sedation and hypothermia produced by ethanol. Since we did not produce significant destruction of DA neurons in the present studies, our current results support the necessity of intact noradrenergic neurons for development of tolerance to barbiturate as well. Further support for this comes from recent studies (*15*), in which we demonstrated that selective lesions of the NE neurons within the dorsal and ventral bundles could prevent the development of tolerance to barbiturates. Thus, the noradrenergic systems of the brain may play a pivotal role in initiating those responses that result in developing tolerance to sedative hypnotics. In our studies the NE neurons destroyed do not seem to be responsible for determining sensitivity to the sedative and hypothermic effects of an acute dose of barbiturates, since we found no significant differences in either sleep time or hypothermia between mice of the vehicle/control and 6-OHDA/control groups in response to the challenge dose of sodium phenobarbital (Table 2).

Our results also emphasize the need for caution when studying the neural mechanisms that determine the development of tolerance to and dependence on sedative hypnotics. It has been suggested by several investigators (*16*) that the development of tolerance and of physical dependence are equivalent. However, our studies demonstrate that the development of tolerance to certain

effects of ethanol and barbiturates can be prevented, without halting the development of physical dependence, as exemplified by appearance of a withdrawal syndrome upon discontinuation of chronic drug ingestion.

BORIS TABAKOFF, JOSEPH YANAI
RONALD F. RITZMANN

Department of Physiology and Biophysics, University of Illinois Medical Center, Chicago 60612

References and Notes

1. H. Kalant, A. E. LeBlanc, R. J. Gibbins, *Pharmacol. Rev.* **23**, 135 (1971).
2. J. K. Merlis, *Am. J. Physiol.* **131**, 67 (1940).
3. Blood obtained from the tail (50 μl) was added to 5 ml toluene containing 0.1 ml 1*M* phosphoric acid and 50 μl pentobarbital in chloroform (0.25 μg/μl, internal standard). For analysis of phenobarbital in brain, brain tissue was homogenized in 1 ml 1*M* phosphoric acid, and internal standard and toluene were added. After extraction, the toluene layer was carefully removed into Concentra Tubes (Laboratory Research Company). Prior to injection of sample into the gas chromatograph, 20 μl of 25 percent tetramethylammonium hydroxide (TMAH) in methanol (Southwestern Analytical Chemical Company) was added to the toluene extract, mixed thoroughly, and centrifuged. The TMAH solution collected in the reservoir of the Concentra Tubes; 1 μl of this solution was injected slowly (3 to 5 seconds) onto a 6-foot glass column containing 2 percent OV-17 on Gas Chrom Q (Beckman). Helium was used as the carrier gas, and the conditions for chromatography with a Packard Model 421 Gas Chromatograph with a flame ionization detector were injection port temperature, 350°C; oven, 140°C; detector, 240°C. Retention time for phenobarbital under gas flow conditions was 11 minutes. Blood obtained from mice consuming the control diet established blank values.
4. R. F. Ritzmann and B. Tabakoff, *J. Pharmacol. Exp. Ther.* **199**, 158 (1976).
5. Functional CNS tolerance, defined by loss of righting reflex and hypothermia, reached a plateau level after 6 days of phenobarbital feeding.
6. B. Tabakoff and W. O. Boggan, *J. Neurochem.* **22**, 759 (1974).
7. B. Tabakoff, R. F. Ritzmann, W. O. Boggan, *ibid.* **24**, 1043 (1975).
8. B. Tabakoff and R. F. Ritzmann, *J. Pharmacol. Exp. Ther.* **203**, 319 (1977).
9. R. Laverty and K. M. Taylor, *Anal. Biochem.* **22**, 269 (1968); R. P. Maickel and F. P. Miller, *Anal. Chem.* **38**, 1937 (1966).
10. J. L. Bruning and B. L. Kintz, *Computational Handbook of Statistics* (Scott, Foresman, Glenview, Ill., 1968), pp. 43–54; S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956), pp. 96–104.
11. B. Tabakoff, J. Yanai, R. F. Ritzmann, *Abstracts of the Seventh Annual Behavioral Genetics Meeting* (1977) p. 32.
12. A. G. Ebert, G. K. W. Yim, T. S. Miya, *Biochem. Pharmacol.* **13**, 1267 (1964).
13. W. W. Morgan, *Experientia* **32**, 489 (1976).
14. R. F. Ritzmann and B. Tabakoff, *Nature (London)* **263**, 418 (1976).
15. B. Tabakoff, R. F. Ritzmann, G. A. Oltmans, J. E. Comaty, R. Olsauskas, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, in press.
16. M. J. Turnbull and J. W. Watkins, *Eur. J. Pharmacol.* **36**, 15 (1976); J. K. Belknap, G. Ondrusek, J. Berg, S. Waddingham, *Psychopharmacologia* **51**, 195 (1977).
17. Supported in part by NIAAA grant 2696 and the State of Illinois Department of Mental Health grant 720. R.F.R. is an NIAAA postdoctoral fellow.

12 September 1977; revised 15 February 1978

Potency in Male Rhesus Monkeys: Effects of Continuously Receptive Females

Abstract. *Ejaculations decreased and mount latencies increased when intact males were paired regularly over a 3.5-year period (3180 tests) with ovariectomized females made constantly receptive by daily injections of estradiol. The deterioration in potency was abruptly and completely reversed by substituting a group of new but similarly treated females for the original ones.*

Although data are not complete and exceptions occur, the majority of primate genera that have been studied show breeding seasonality (*1*). The virtual lack of a sexual season in the human appears to place us in a somewhat special category. Man also differs from other higher primates because the influence of the female's menstrual cycle on the sexual activity of the pair appears to be reduced. The clear-cut sexual rhythms shown by certain Old World monkeys and apes (*2*) are more difficult to detect and quantify in the human (*3*). With the added psychosocial implications of the use of oral contraceptives, which tend to dampen the hormonal fluctuations natural to the woman's cycle, we have a situation in which the human is emancipated from most of the physiological and environmental factors that are responsible for sexual periodicity in lower mammals. In a species such as the rhesus monkey, the males show greater potency than men and are regularly able to ejaculate from one to four times in daily 1-hour tests, but in the wild, they are maximally active for only about 8 to 12 weeks each winter during the height of the relatively brief mating season. Sexual activity is also restricted in the female because a 165-day pregnancy normally supervenes, and sexual activity is virtually abolished during lactation. If pregnancy should fail to occur, there is usually a 3- to 4-month period of summer amenorrhea (*4*). Thus, both the male and female are seasonal; this is emphasized here to draw attention to a major difference between the sexual activity of our own species, in which sexual life is almost uninterrupted, and that of most other primates, for which rhythmicity and periodicity are the rule.

We report here on the decline in the potency of male rhesus monkeys when paired with female partners maintained in a continuously receptive state (to better resemble the human condition) over a 4-year period. We also report on the dramatic restoration of potency brought about by changing the female partners.

Four adult male (weighing 9.8 to 11.3 kg) and eight adult female (weighing 4.6 to 7.2 kg) rhesus monkeys were obtained through dealers directly from India and, after 4 to 10 months of quarantine, housed in individual cages in an animal room where the temperature was maintained throughout between 20.0° and 23.3°C. Artificial lighting was rigorously controlled to give a 14-hour day between 0615 and 2015 hours. Females were ovariectomized and throughout the experiment received daily 10 μg of estradiol benzoate in 0.2 ml of sesame oil injected subcutaneously (*5*, *6*). Once a day, 5 days a week, from December 1972 to March 1975, each male was given 1-hour sexual behavior tests with one of four females. Females were used in rotation on consecutive days so that each female's test with the same male recurred every fifth test day. In this way, each male was paired with four different female partners (16 pairs) to provide variety and to control for the effects of individual partner preferences (*7*). Behavior testing was carried out in large observation cages with one-way mirrors as previously described (*6*, *8*). At all other times each animal was housed in a single cage. In March 1975, the four original female partners ("old" females) were replaced by four similarly treated ones ("new" females) that had not previously been tested with these males. After 4 weeks of testing, the "new" females were replaced by the original "old" females, and testing continued with this latter group without interruption until March 1976. The behavioral indices given quantitative treatment here were (i) mean number of ejaculations per test and (ii) mean latent period (in minutes) to the first male mounting attempt per test. Blood samples (3 ml) were collected weekly at 0800 hours from the saphenous veins of untranquilized males previously adapted to the venipuncture procedure (*9*), and plasma testosterone was estimated by radioimmunoassay without chromatography (*10*).

Figure 1 shows changes in the mean number of ejaculations per test (top) and in the mean latency to the first male mounting attempt (bottom) in four successive years (1973, 1974, 1975A, 1976) during the months of January and February (to control for the effects of season and other long-term variables). By the fall of the second year of regular testing

0036-8075/78/0428-0451$00.50/0