behavior of blepharmone on the ion exchange celluloses described above.

From these results it is concluded that blepharmone is a slightly basic glycoprotein and that it has been isolated almost free of other proteins. Although the purity of the sample was not rigorously tested for organic substances other than protein, the purification procedures described above are likely to have removed most of them. Assuming that the 2.0 mg of protein detected in the purified sample is all active blepharmone, we calculate that its specific activity is 1.6×10^7 unit/mg; that is, this glycoprotein can induce cell union at 6×10^{-8} mg/ml in mating type 2 cells (10^3 cell/ml).

The molecular weight of blepharmone was estimated by the pressure filtration method. Blepharmone dissolved in SMB passed freely through the Amicon PM-30 membrane but was retained (about 95 percent) by the PM-10. To avoid absorption of blepharmone to the membrane, a preliminary run was made for 5 minutes with SMB containing 1 percent albumin, which did not appreciably change the flow rate. Since these membranes retain globular molecules with a molecular weight larger than 3×10^4 and 10^4, respectively (*13*), the molecular weight of blepharmone should be 1×10^4 to 3×10^4. This value is consistent with the previous value of 2×10^4 measured by the gel filtration method (*2*).

Both components of a pair of gamones of *B. intermedium* have now been isolated and characterized. Unlike gamone 2 or blepharismone, which is calcium-3-(2′-formylamino-5′-hydroxybenzoyl)lactate, a derivative of formylkynurenine (*4*), gamone 1, blepharmone, is a much larger molecule and is a glycoprotein. This striking chemical difference in a pair of specific substances participating in a cell interaction may be compared to that seen in the feedback interaction between thyroid gland and pituitary gland by thyroxine (amino acid) and thyroid stimulating hormone (glycoprotein).

Gamones are known in other species of protozoa (*14, 15*) but gyno- and androgamones of *Chlamydomonas eugametos* (*16*), a chlorophyll-bearing flagellate, are the only other gamones so far isolated and identified in protozoa. They are both large glycoproteins with a molecular weight of 10^8 and are the material basis of the agglutination between complementary mating types. Blepharmone and blepharismone differ from these gamones not only by their molecular size but also by their function. They are not the material basis of agglutination but are hormone-like substances which transform specific types of cells so that they can form a cell union and also induce them to produce their own gamone. Isolation and characterization of both of these gamones are expected to facilitate the study of cell interaction by means of specific substances as well as the study of the molecular mechanism of conjugation.

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6. The unit activity of the gamone of one mating type was defined as the smallest amount of the gamone that can induce the homotypic cell union in 0.5×10^3 to 1×10^3 cells of the other mating type suspended in 1 ml of SMB (*2*). The gamone activity of a sample was measured by comparing its activity with that of a gamone standard (*2*).
7. The protein content was measured by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)] with bovine serum albumin as standard.
8. When 0.01 percent of albumin was contained in a sample, its gamone 1 activity was four to eight times higher (*2*).
9. Blepharmone is very unstable in a diluted state, and successful purification was achieved only under the continuous presence of albumin. Thus both Bio-Gel and carboxymethyl cellulose columns were eluted with a buffer containing albumin. When albumin was removed in the last step of purification, blepharmone was no longer so unstable probably because its concentration was high enough to protect itself.
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Neurochemical Correlate of a Spatial Preference in Rats

Abstract. *Spatial (left or right) preferences were determined for rats given foot shock in a T-maze. The animals were killed, and left and right striata were assayed separately for dopamine and left and right teldiencephalic regions were assayed for norepinephrine. Dopamine content was significantly higher (by 12 percent) in the striata contralateral to rats' side preferences than in the ipsilateral striata; there was no such difference for teldiencephalic norepinephrine. The small asymmetry in striatal dopamine content is not due to any learning- or stress-related change induced by the testing procedure but is probably inherent in normal rats. Some spatial behavior appears to be the manifestation of a normal and specific difference in the activity of left and right nigrostriatal systems.*

Electrical stimulation or lesions of the dopaminergic nigrostriatal system disrupt normal motor (*1*) and associative functions (*2*). Unilateral lesions in the nigrostriatal pathway, for example, induce a rotary behavior in rats characterized by circling toward the side of the lesion. Drugs such as amphetamine that release dopamine (*3*) or drugs such as apomorphine that directly activate dopaminergic receptors (*4*) in the striatum potentiate such rotation or elicit rotation when animals have recovered from the initial tendency to rotate spontaneously. The rotation has been attributed to an imbalance of the left and right nigrostriatal systems induced by the unilateral lesion (*3*). Drugs can enhance this imbalance by differentially activating the intact and damaged pathways (*3, 4*). We demonstrated

(5) that systemic administration of *d*-amphetamine also induces rotation in the normal rat, although the rotation is of lesser magnitude than that in the rat with unilateral lesions. The direction (left or right) of this rotation in normal rats was subsequently correlated with the animal's spatial preference for the left or right lever in a two-lever operant task with water as reward (6). These results led to the hypothesis that there is a normal imbalance between the left and right nigrostriatal systems and that this imbalance is related to or is the substrate for certain spatial behaviors. In support of this hypothesis, we report here that there are small differences in left and right striatal dopamine levels which are related to spatial preferences of rats tested in a T-maze.

First, a simple and reliable paradigm for determining spatial preferences was developed. Rats (7) were placed individually in the long arm of a T-maze (8) with scrambled foot shock administered through a grid floor (9). The shock was terminated when a rat entered either the left or right arm of the T-maze; the rat was then removed from the maze. Testing consisted of ten consecutive trials with 5 to 15 seconds between trials. A significant percentage of rats alternated alleys from the first to the second trial; this rate decreased with subsequent trials, and by the tenth trial a significant percentage chose the same alley as in the preceding trial (10). In the ten trials, 92 percent of rats had a preference (that is, an unequal number of left and right choices). To determine the stability of such preferences, three separate groups of eight rats were tested either every day for a week, once a week for a month, or a month apart. Rats exhibited stable side preferences over each of these time periods (11).

The neurochemical experiment involved four groups of rats. The rats in one group ($N = 23$) were tested for T-maze preferences and killed 2 minutes after testing (one rat without a preference was not studied further). The brain of each rat was immediately removed, the striata were dissected, and each left and right striatum was assayed individually for dopamine by fluorometric methods (12); the rest of each left and right teldiencephalon was assayed individually for norepinephrine (12). Rats in the second group ($N = 8$) were treated as those in the first group except that they were killed 10 days after testing. Rats in the third group ($N = 8$) were placed in the long arm of the T-maze and received the same amount of shock as those in the first group but were not allowed to enter the arms or escape the shock. Animals in the fourth group ($N = 8$) were placed and contained in the long arm of the T-maze for the same amount of time as the third group but received no shock.

The results are shown in Table 1. In all four groups, there were small, although nonsignificant, differences in left and right striatal dopamine levels. The importance of these differences was indicated by their relation to spatial preferences: In both groups tested for preferences, dopamine values were significantly higher in the striatum contralateral to the preferred direction than in the ipsilateral striatum. In all groups, dopamine levels were significantly higher in the striatum containing the higher level than in the striatum containing the lower level; that is, side differences in dopamine levels were obtained in the groups that had not been tested as well as in the groups that had. The ratios of the higher to the lower striatal dopamine values were the same in all four groups, and the relation to spatial preferences was the same in rats killed either 2 minutes or 10 days after testing; these results make it appear unlikely that foot shock or learning per se induced the left-right dopamine differences. Rather, it would seem that such neurochemical differences reflect small but intrinsic normal differences in left and right nigrostriatal function which become manifest behaviorally, at least in part, as spatial preferences. Although other regional differences in brain chemistry were not studied, some neurochemical specificity of this relation with behavior is suggested by the entirely negative results with teldiencephalic norepinephrine values.

To substantiate the relation between the striatum and spatial behavior, small electrolytic lesions (13) were made in the striata of another group ($N = 15$) of rats. Rats were tested in the T-maze and underwent surgery 4 days afterward. Half of the rats received a lesion in the striatum on the same side as the behavioral preference and half in the

Table 1. Unilateral dopamine and norepinephrine values. Rats in the first two groups were given the preference test and killed 2 minutes or 10 days afterward. Other animals received foot shock without testing for preferences or were placed in the apparatus without receiving shock or being tested for preferences (control). Mean dopamine and norepinephrine levels were computed in three ways: left versus right side, side containing higher level versus side containing lower level, and side ipsilateral to spatial preference versus side contralateral. In addition, the mean ratios of higher to lower values were computed; S.D., standard deviation; Pref., preference test.

Group	Left	Right	High	Low	Preferred	Non-preferred	Ratio (high/low)
			*Striatal dopamine (micrograms per gram) (mean ± S.D.)**				
Pref.-2 min.	7.26 ± 1.11	7.56 ± 1.16	7.87 ± 1.07	6.95 ± 1.06	7.00 ± 1.08	7.82 ± 1.07	1.13 ± 0.09
Pref.-10 day	7.51 ± 1.20	7.13 ± 1.18	7.74 ± 1.10	6.90 ± 1.05	6.94 ± 1.06	7.70 ± 1.11	1.12 ± .06
Shock	7.29 ± 1.19	7.33 ± 1.24	7.82 ± 1.11	6.80 ± 1.09			1.15 ± .08
Control	7.97 ± 1.28	7.58 ± 1.21	8.25 ± 1.15	7.30 ± 1.07			1.13 ± .09
			Teldiencephalic norepinephrine (micrograms per gram) (mean ± S.D.)†				
Pref.-2 min.	0.411 ± 0.051	0.421 ± 0.056	0.428 ± 0.064	0.404 ± 0.058	0.420 ± 0.050	0.412 ± 0.059	1.06 ± 0.05
Pref.-10 day	.417 ± .061	.428 ± .058	.433 ± .055	.412 ± .059	.414 ± .060	.431 ± .054	1.05 ± .05
Shock	.404 ± .048	.416 ± .057	.420 ± .072	.400 ± .051			1.05 ± .04
Control	.405 ± .052	.423 ± .061	.426 ± .062	.401 ± .058			1.06 ± .06

* There were no significant differences ($P > .1$, analysis of variance and *t*-tests) among the four groups in left, right, high, or low dopamine levels or in high/low ratios. Within each group, there was a significant difference (paired *t*-tests) between high and low sides ($P < .01$) but not between left and right sides ($P > .05$). In both groups tested for preferences, the difference between the preferred and nonpreferred sides was significant ($P < .02$ for 2-minute group, $P < .05$ for 10-day group; paired *t*-tests). For each group, high/low ratios were significantly greater for dopamine than for norepinephrine ($P < .05$ in each case, paired *t*-tests). † There were no significant preferences ($P > .1$, analysis of variance and *t*-tests) among the four groups in left, right, high, or low norepinephrine levels or in high/low ratios. Within each group, there was no significant difference ($P > .1$, paired *t*-tests) between left and right sides, high and low sides, or between preferred and nonpreferred sides in the groups tested for preferences.

opposite striatum. Another group ($N = 6$) of rats received sham surgery (*14*) in the striatum opposite to the side of the behavioral preference. After recovery from anesthesia, all rats with lesions rotated in the direction ipsilateral to the lesion for several hours after surgery; spontaneous rotation was entirely absent one or more days after surgery. Three days after surgery, rats were tested in the T-maze again. All rats with lesions had side preferences ipsilateral to the lesion, that is, in the same direction as the initial postoperative rotation; all rats with sham surgery had side preferences in the same direction as they had a week earlier. The relation of spatial preferences to rotation was also confirmed in normal rats. Another group ($N = 18$) of naive rats were tested first in the T-maze, then injected with *d*-amphetamine sulfate (1.0 mg/kg), and placed in an apparatus (*15*) designed to measure rotation automatically. When placed in such a spherical rotometer, rats rotate consistently (that is, to the same direction when tested at different times) after this dose of *d*-amphetamine (*5*). In the present case, rats preferentially rotated in the direction of the spatial preferences determined in the T-maze (*16*). These results, as well as others showing that *d*-amphetamine enhances spatial preferences (*17*), suggest that rotation is a stereotyped form of spatial behavior.

In conclusion, the present study provides evidence that rats have a small hemispheric asymmetry in nigrostriatal chemistry which may be related to a behavioral bias of possibly broad significance. This conclusion argues against the generally accepted notion that only man has hemispheric asymmetries (that is, cerebral dominance). The results reported here may eventually be relevant to an understanding of neurological phenomena and disorders in man characterized by unilateral or asymmetric disturbances of function. Regardless of such possibilities, it is important that a relation between a neurochemical index of brain function and behavior can be demonstrated.

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8. The T-maze was constructed of black Plexiglas. The stem was 30 cm long, the arms were each 15 cm long, and the walls were 30 cm high.
9. The floor of the T-maze was a grid of 0.4-cm stainless steel rods, spaced 1.3 cm apart. Scrambled 0.6-ma a-c current was applied to the grid by using a Lafayette A615C shocker.
10. In an initial pilot group of 32 rats, the alternation rate was 72 percent from the first to the second trial and 26 percent from the ninth to the tenth trials, both significantly different from chance ($P < .05$, chi-square tests).
11. Only rats with preferences on the first test were used. Over each time interval, 83 to 85 percent of all rats preferred the same side on subsequent tests as they had on the first test ($P < .005$ in each case).
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14. Sham-operated animals had the electrode lowered but did not receive lesions.
15. The apparatus consisted of a white opaque Plexiglas sphere, 30 cm in diameter, within which the rat rotated. A flexible stainless steel wire, which was connected to a cam positioned on the vertical axis on the outer surface of the sphere, was wrapped around the thorax of the rat. As the rat rotated, the cam closed one of the two microswitches that were positioned so as to indicate left or right turns (*5*).
16. The side preference in the T-maze and the direction of amphetamine-induced rotation was the same in 78 percent of rats ($P < .05$, chi-square test).
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Enduring Learning Deficits and Cerebral Synaptic Malformation from Exposure to 10 Parts of Halothane per Million

Abstract. *Chronic exposure of rats to 10 parts of halothane per million during early life produced later deficits in learning a shock-motivated light-dark discrimination and a food-motivated maze pattern, correlated with enduring synaptic membrane malformation in cerebral cortex. Adult exposure had no effect. Halothane may provide a useful analytical tool for study of brain. The behavioral-ultrastructural techniques also suggest a standard for assessing the safety of trace toxicants with central nervous system effects.*

The possible toxic effects of anesthetic gases on chronically exposed surgical personnel have been of increasing concern in recent years (*1*). We have chosen to study halothane, which, used in conjunction with nitrous oxide, is the most widely employed inhalation anesthetic in the United States. Ambient concentrations of halothane are found to average 10 parts per million (ppm) in the operating theater. The anesthesiologist, stationed near the exhaust of the patient's breathing circuit, may be exposed to a much higher concentration (*2*).

Two different toxicology questions exist for this situation. First, behavioral deficits might occur during the acute exposure of the operating team to halothane. Bruce *et al.* (*3*) found that 4 hours of exposure to 15 ppm halothane (with 500 ppm nitrous oxide) produces significant deficits in cognition, perception, and motor reaction. Such decreases in capacity during surgery are of obvious concern. The second question, and the one we addressed, was whether chronic exposure to 10 ppm halothane produces lasting behavioral deficits and damage to the central nervous system. Such deficits could place an undue burden upon the lives of the surgical team members. Various studies have suggested that anesthesiologists and operating room nurses may suffer long-term, physiological effects (*4*). However, no data exist on possible behavioral deficits from chronic halothane exposure.

We examined the effects of halothane on rats exposed chronically, either during early development, adult life, or both. To obtain sensitive behavioral measures, we utilized two relatively dif-