Rat Brain: Effects of Environmental Enrichment on Wet and Dry Weights

Abstract. *Wet weight of rat cerebral cortex was increased by exposure to an enriched environment, as compared with standard colony or impoverished conditions. Dry weights and wet weights were compared and both yielded identical percentage differences between brains of animals experiencing enrichment and those experiencing impoverishment.*

Exposure to differential environments —enriched, colony, or impoverished— leads to characteristic changes in wet weight of samples of rat brain, in enzymatic activity, and in depth of cerebral cortex (*1–5*). Impoverished animals were caged singly, colony animals two or three per cage, and enriched animals 10 or 12 per larger cage, with toys (*1*). Some of these environmental treatments lead to significant differences in problem-solving ability, although it has not yet been possible to relate behavioral effects strictly to cerebral effects (*4*).

Differences in total protein induced in the cortex by differential experience were as large as those in weight of the cortex (*3*). Furthermore, the differences in activity of acetylcholinesterase and cholinesterase were as large when expressed per unit of protein as when expressed per unit of wet weight of tissue (*1*).

Littermate male rats of the Berkeley S_1 strain were assigned at weaning (about 25 days of age) to either standard enriched or impoverished conditions (*1*). After 30 days each brain was dissected into six standard (*6*) sections: samples of occipital cortex, somesthetic cortex, remaining dorsal cortex, remaining ventral cortex (including hippocampus), cerebellum and medulla, and the rest of the brain. Each sample was weighed to 0.1 mg. Less than 10 minutes elapsed between decapitation and placing the last sample of the brain on dry ice. The analysts did not know the experimental environment of any individual animal.

The brain samples were placed in a cooled desiccator at −30°C, and water was pumped off with an oil vacuum pump and a trap cooled in carbon dioxide and isopropyl alcohol. During the next 6 days the temperature was raised by stages to −10°C, and about 25 ml of water was collected. The refrigeration was then turned off, and pumping was continued until the temperature reached about 0°C. The desiccator was then put on a high-vacuum line, at room temperature, with a trap cooled in liquid nitrogen. Pressure was reduced to an estimated 50×10^{-3} mm-Hg and the samples were dried to constant weight. During the final weighing, only slight gain was observed after the samples were exposed to air, and the weight then stabilized.

In the final weighing, the occipital and somesthetic samples were weighed to 0.005 mg, and the larger samples were weighed to 0.1 mg. The papers alone were then reweighed, and net dry weights of brain were obtained.

Mean wet weights and percentage differences between enriched condition (EC) and impoverished condition (IC) wet weights (Table 1) are similar to those recently reported for 30-day experiments (*3*, *4*). As usual, the largest percentage difference (EC—IC) was found in the weights of the occipital region of the cortex (12.7 percent, $P < .001$), the major differences were found in the weights of cerebral cortex, and the rest of the brain showed little effect.

Drying the tissue reduced the weight of the cortical samples by 78 or 79 percent and that of the rest of the brain by about 77 percent. The slight difference undoubtedly reflects the somewhat lower water content of white matter compared with gray matter of brain. The ratios of dry weight to wet weight are essentially identical for EC and for IC animals (Table 1); thus, the relative water content of EC and IC brains was the same. The percentage differences between dry weights for the EC and IC groups (Table 1) are therefore closely similar to those previously seen for wet weights (*3*, *4*).

Table 1. Wet and dry weights of brain sections of rats from enriched condition (EC) and impoverished condition (IC) (based on 11 littermate pairs of Berkeley S_1 strain rats). For each brain region the table gives the mean wet weight and dry weight for the enriched condition, and the mean wet weight and dry weight for the impoverished condition, as well as the ratios of dry weight to wet weight. Also included are the percentage differences between EC and IC means [100 × (EC − IC)/IC] and the statistical significance of the differences.

Sample source	Mean weights (mg)				Ratio of dry to wet		Percentage differences	
	Wet		Dry				Wet	Dry
	EC	IC	EC	IC	EC	IC		
			Cortex					
Occipital	72.7	64.5	15.7	14.0	0.216	0.217	12.7‡	12.5†
Somesthetic	56.8	54.0	12.6	12.0	.222	.222	5.0*	4.8*
Remaining dorsal	292	270	61.9	57.1	.212	.212	8.4†	8.5‡
Ventral	278	274	59.8	58.9	.215	.215	1.6	1.5
Total	700	662	150.0	142.0	.214	.214	5.7‡	5.7‡
			Rest of brain					
Cerebellum, pons, medulla	384	384	93.0	93.4	.242	.243	−0.1	−0.4
Remainder	490	484	109.2	108.0	.223	.223	1.2	1.0
Total subcortex	874	869	202.0	201.5	.231	.232	0.6	0.3
			Total brain					
	1574	1530	352.2	343.4	.224	.224	2.8*	2.6
			Ratio of cortex to rest of brain					
	0.801	0.763	0.742	0.705			5.0‡	5.3‡

* $P < .05$ † $P < .01$ ‡ $P < .001$

We conclude that our previous reports given in terms of wet weight of tissue would not have changed had dry weights been taken.

EDWARD L. BENNETT
Laboratory of Chemical Biodynamics, Lawrence Radiation Laboratory, University of California, Berkeley 94720
MARK R. ROSENZWEIG
Department of Psychology
MARIAN C. DIAMOND
Department of Physiology–Anatomy

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Monosodium L-Glutamate: Its Pharmacology and Role in the Chinese Restaurant Syndrome

Abstract. *Monosodium L-glutamate is the cause of the Chinese restaurant syndrome and can precipitate headaches. In appropriate doses it causes burning sensations, facial pressure, and chest pain. These are pharmacological effects obeying a dose-effect relationship. There is considerable variation in oral threshold doses among individuals.*

Monosodium L-glutamate (MSG) is a widely used food additive. Twenty thousand tons of MSG are manufactured and used in the United States each year (*1*). The labeling of a widely used brand states, "To wake up all the flavor nature put in your food, be sure to use at least the amounts . . . suggested below, adding more as desired." Amounts approximating 1 g per serving are the minimum amounts suggested.

Monosodium L-glutamate is not a wholly innocuous substance. It was proposed as the cause of the Chinese restaurant syndrome in July 1968 (*2*). We report here some aspects of the acute human pharmacology of MSG and, in addition, present evidence that it causes headache.

Many symptoms have been suggested as components of the syndrome (*3*). On repeated observations, we find that three categories of symptoms can be elicited by MSG—burning, facial pressure, and chest pain. Headache is a consistent complaint in a minority of individuals. The MSG response and the syndrome are identical. The symptoms appear only if the meal is taken on an empty stomach by a susceptible individual (*4*).

The proof that MSG is the cause of the syndrome was arrived at with the cooperation of two subjects, both of whom had symptoms in the same restaurant. We found that 200 ml of wonton soup alone was sufficient to provoke an attack (*5*, *6*). Although other foods caused the response, wonton soup was the simplest in composition.

The restaurant prepared soup without MSG and it failed to provoke an attack. The subjects then ingested each of the seven components of the wonton soup separately. Only MSG caused the symptoms. In a blind procedure, MSG was then given to four additional individuals who had symptoms in the same restaurant. It provoked an attack in all four in amounts of 3 g or less. Therefore, we concluded that the Chinese restaurant syndrome was caused by MSG.

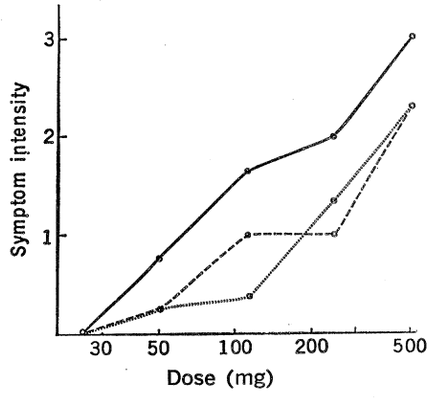

Fig. 1. Relation between intensity of burning (solid line), facial pressure (dashes), and chest pressure (dotted line) and intravenous dose of MSG. Each point represents a mean intensity from three or more responses. The data were obtained from four subjects.

We then determined that L-glutamic acid in several forms could provoke an attack. Symptoms were provoked in our two original subjects by Accent (3 g), MSG, chemically pure grade (C.P.) (3 g), monopotassium L-glutamate C.P. (4 g), DL-glutamic acid (5 g), and L-glutamic acid (5 g). A previous report of failure of L-glutamic acid to provoke an attack (*2*) was due to our using an insufficient volume of water as a vehicle for this poorly soluble substance.

A repeat trial with 5 g of L-glutamic acid, fully dissolved in 500 ml of water at 30°C, provoked an attack. In addition, to eliminate the possibility of an impurity in the commercially available L-glutamate, we synthesized monosodium DL-glutamate (*7*). The resultant product was identified from infrared spectra and by thin-layer chromatography. Five grams of this product were sufficient to provoke an attack.

The following substances did not provoke symptoms: monosodium D-glutamate (7 g), monosodium L-aspartate (5 g), NaCl (10 g), and glycine (5 g).

We next determined that the intensity and duration of the symptoms were related to the dosage of MSG. To define the temporal sequence and nature of the symptoms, we gave MSG, as Glutavene, intravenously to 13 subjects. After oral administration of this substance, the symptoms were perceived by our subjects in a less well-defined order because the onset was less abrupt and the increase in intensity more subtle. After oral administration, many subjects experienced only one or two components of the syndrome. Fifty-six normal subjects (30 male and 26 female) were given oral MSG. The age range was from 21 to 67 years. Symptoms of the syndrome occurred in all but one subject. We gave MSG to 36 subjects at different doses to determine the distribution of thresholds. In the one individual in whom no symptoms could be produced despite large doses of oral MSG (21 g), symptoms were produced with an intravenous dose of 50 mg.

We previously reported that one of the subjects had ingested 25 g of MSG without symptoms (*2*). However, on repeated testing this individual showed a threshold of 5 g. The symptoms had been obscured, in the previous test, by prostration and gastric distress