adrenergic receptors inhibits the heat production mechanism. On the other hand, activation of central serotoninergic, dopaminergic or cholinergic receptors facilitates the heat loss mechanism, while activation of central adrenergic receptors inhibits the heat loss mechanism.

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Glutamate decarboxylase activity in brain regions of differentially-housed mice; a difference in the olfactory bulb

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Summary. Glutamate decarboxylase (GAD) activities were determined in homogenates of 8 brain regions of mice that had been differentially housed ('isolated' vs 'grouped') for 4–9 weeks. GAD activity was lower in whole forebrains and in olfactory bulbs of isolated mice, changes which might be associated with their increased aggressiveness.

L-Glutamate-α-decarboxylase (L-glutamate-1-carboxylyase; E.C. 4.1.1.15; GAD), the rate-limiting enzyme in the synthesis of γ-aminobutyric acid (GABA), is present mainly in synaptosomes[1,2]. GABA-α-oxoglutarate transaminase (GABA aminotransferase; E.C. 2.6.1. c; GABA-T), the main enzyme involved in GABA catabolism, is associated, in part, with synaptosomal mitochondria[1,3,4]. Thus, changes in the activities of these enzymes could underlie certain behavioral modifications. In this regard, an inverse correlation has been found between cerebral GAD activity and exploratory activity in mice[5]. Changes in the central GABA-ergic system can also be produced by environmental modification; e.g., GABA binding to synaptosome-enriched fractions occurred to a lesser extent in 'isolated' (aggressive) male mice[6] and cerebral GABA content was lower in 'isolated' mice than in their 'grouped' counterparts[7,8]. Furthermore, olfactory deafferentation can induce mouse-killing in previously 'non-killer' rats, but isolation of the rats is necessary after the operation to elicit this behavior[9]. The GABA content of the olfactory bulbs is much lower in spontaneous 'killer' rats than in non-killers[10], and bilateral injections of GABA into the olfactory bulbs inhibits muricidal behavior[11]. Taken together, the above-mentioned results have revealed that a central GABA-ergic system, localized especially in the olfactory bulb, appears to be involved in the control of aggressive behavior, and that this system is subject to environmental regulation. Since this system provides a model for negative (inhibitory) control of behavior, we have compared the GAD activities of several brain regions of isolated vs grouped mice.

Materials and methods. Post-weanling, male mice (28 days of age) of the C3H/He/orl strain were used. 'Isolated' mice were kept singly in small (14 × 20 × 26 cm) cages and were prevented from seeing each other ; 'grouped' mice were kept in groups of 10–12 in large (28 × 31 × 50 cm) cages furnished with various objects which provided environmental enrichment. After 4–9 weeks of differential housing, the mice were decapitated and their whole forebrains (rostral to the inferior colliculi, excluding cerebellum) and regions of their brains were excised rapidly, weighed (regions from 2 brains/sample), and homogenized at 0 °C in 10 vols of K^+-phosphate buffer (5 mM, pH 7) containing 2-aminoethylisothiouronium bromide hydrobromide (1 mM), EDTA (5 mM), and pyridoxal phosphate (10 mg/l).

Aliquots (20 µl) of homogenates (representing about 0.2 mg protein) were incubated together with 100 µl of 0.1 M K^+-phosphate buffer (pH 7.0) which contained 0.5 mM pyridoxal phosphate, 1.0 mM 2-aminoethylisothiouronium bromide hydrobromide, 0.1 mM EDTA, and 15 mM L-glutamate (10.4 mCi/mmole) (final concentrations). Unlabelled L-glutamate (Sigma Chem. Corp.) and [U-^{14}C]L-glutamate (CEA, France; sp. act. 250 mCi/mmole) were used. Solutions were placed into 6-ml conical tubes, and the reaction was initiated by addition of homogenate. Tubes were sealed with rubber stoppers in which were fixed small cups containing 200 µl of hyamine hydroxide. After incubation at 37 °C for 35 min, 0.1 ml of 0.2 M sulfuric acid was injected through each stopper to terminate the reaction and samples were allowed to stand for an additional 60 min at 37 °C. Blanks were run under identical conditions using 20 µl of homogenizing buffer in place of homogenate. GAD activities were determined by 2 methods[12]. CO_2 evolution was measured by a modification of the method of Albers and Brady[13,14]. The contents of cups containing hyamine-plus-trapped $^{14}CO_2$ were washed into scintillation vials with 9 ml Omnifluor scintillation fluid (New England Nuclear Corp.; diluted 4 g/l of toluene). To determine [U-^{14}C]GABA (formed from labelled glutamate), incubated solutions were placed onto columns (0.5 × 4 cm) of Dowex AG-1 × 8 (200–400 mesh) anion exchange resin, previously equilibrated with 2 M Na^+-acetate[15,16]. After applying the samples to the columns, the tubes were washed with 2 ml of water, and the columns were washed with 3 × 1 ml of water; eluates were collected in scintillation vials containing 5 ml of Instagel (Packard Instr. Co.). Quench curves were constructed for both scintillation fluids to convert counts/min to disintegrations/min. An Intertechnique scintillation spectrometer was used to determine radioactivity.

Results and discussion. No differences in the protein concentrations of homogenates existed between whole forebrain or brain regions of isolated and grouped mice. GAD

GAD activities in homogenates of brain regions of differentially-housed mice

	GAD activity			
	n-moles $^{14}CO_2$ formed/min/mg protein		n-moles [^{14}C] GABA formed/min/mg protein	
	Grouped mice	Isolated mice	Grouped mice	Isolated mice
Olfactory bulb	5.41±0.08 (21)	4.82±0.05 (21)[a]	4.81±0.08 (21)	4.25±0.08 (21)[a]
Cerebellum	4.03±0.05 (21)	3.63±0.06 (21)[a]	2.59±0.07 (21)	2.63±0.07 (21)
Hypothalamus	9.40±0.12 (6)	9.50±0.34 (6)	8.82±0.14 (6)	8.21±0.32 (6)
Striatum	5.08±0.09 (15)	4.88±0.09 (15)	3.80±0.10 (15)	3.50±0.10 (15)
Pons medulla	3.35±0.12 (6)	3.42±0.13 (6)	2.39±0.11 (6)	2.43±0.07 (6)
Midbrain	5.89±0.18 (6)	5.67±0.16 (6)	4.21±0.20 (6)	4.15±0.14 (6)
Hippocampus	2.91±0.19 (6)	2.89±0.10 (6)	2.10±0.11 (6)	2.07±0.06 (6)
Cerebral cortex	3.68±0.18 (6)	3.47±0.13 (6)	2.59±0.24 (6)	2.62±0.10 (6)

Means±SEM; numbers of determinations in parentheses (e.g., 12 determinations on 6 homogenates, or 6 determinations on 3 homogenates, each homogenate representing regions from 2 brains); [a] indicates $p<0.001$ when these values were compared with corresponding values for grouped mice (Student's t-test; 2-tailed).

activity was significantly lower in the whole forebrains of isolated mice than in those of grouped mice (3.87±0.09 vs 4.18±0.09 nmoles [^{14}C]GABA formed/min/mg protein (means±SEM; 12 determinations in each case; $p<0.02$). GAD activity (as measured by 2 different methods) of the olfactory bulb was also significantly lower in the isolated mice (table). GAD activity of cerebellum was lower for isolated mice, but only when the data were expressed in terms of CO_2 evolution (table).

In accord with previous results on GABA content[7,8] and GABA binding[6], the activities of cerebral GAD of male mice was shown to be sensitive to long-term changes in environment. The localization of these changes in the olfactory bulb further supports the contention that a hypoactive GABA-ergic system (lower GABA levels) might be responsible for muricidal behavior in rats[10,11]. Such a change in the olfactory bulb could also underlie the increased aggressiveness displayed by isolated mice. However, other brain regions not examined in the present study (e.g., thalamus) could also be involved in this behavioral change. In general, the results reported here further support the notion that central GABA-ergic systems are involved in behavioral adaptation and neuronal plasticity.

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Hypothermia induced in mice by enzyme-mediated depletion of serotonin[1]

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Summary. Injection of a purified serotonin (and tryptophan)-degrading enzyme into mice produced a pronounced hypothermia when the mice were maintained at 22 °C, but not at 30 °C. Brain levels of serotonin and tryptophan were strikingly depleted, but concentrations of norepinephrine and dopamine remained unchanged.

The regulation of body temperature in mammals is a complex process which is only beginning to be clarified. Numerous investigators have presented evidence inferring that either dopamine, norepinephrine, acetylcholine or serotonin is the neurotransmitter which is involved in thermoregulation[2]. Also, the sex of the test animal has been claimed to influence the effect of serotonin treatment[3]. We report here the attainment of hypothermia in mice at room temperature by administering a highly purified enzyme which attacks serotonin (and tryptophan), but which is inactive against the catecholamines, dopamine and norepinephrine. These results support the hypothesis that serotonin, or a structurally related indole compound, is involved in thermoregulation.

Female BDF_1 mice, weighing approximately 20 g, were injected i.p. with the indicated dose of highly purified indolyl-3-alkane α-hydroxylase (IAH), a newly-discovered bacterial enzyme which degrades tryptophan, serotonin and related 3-substituted indoles[4]. Rectal temperatures were monitored with a tele-thermometer (Yellow Springs Instruments) while the mice were maintained at room temperature (about 22 °C) or in a 30 °C incubator. At the indicated