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Lateralization of Zinc in Rat Brain and Its Relationship to a Spatial Behavior

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VALDES, J. J., S. W. HARTWELL, S. M. SATO AND J. M. FRAZIER. *Lateralization of zinc in rat brain and its relationship to a spatial behavior.* PHARMAC. BIOCHEM. BEHAV. 16(6) 915–917, 1982.—Essential metals are differentially accumulated within the brain and have been related to normal neurotransmitter metabolism. Hippocampal glutaminergic pathways have the highest zinc levels in the brain, and lesions to these pathways disrupt behaviors with a spatial component. Zinc distribution may thus reflect glutaminergic activity or innervation and may have functional consequences for spatial behavior. The present data support this hypothesis, indicating that the lateral distribution of zinc between the right and left hippocampus is strongly correlated with the spatial preference of the animal ($r=+0.72$). Other parameters tested but shown not to be significantly correlated with spatial preference were zinc in corpus striatum and cortex, and copper in hippocampus, corpus striatum and cortex.

Zinc Copper Lateralization Hippocampus Cerebral asymmetry

THE essential metals copper and zinc are selectively accumulated by neuronal elements [8] but the functional significance of this phenomenon is presently obscure. The metabolic pathways of several neurotransmitters are sensitive to metals. Choline acetyltransferase is sensitive to inhibition by Cu^{++} [20] and glutamic acid decarboxylase to Zn^{++}. Essential metals are also present in the form of metalloenzymes; copper is required by the norepinephrine synthesizing enzyme dopamine-β-hydroxylase and zinc is required by glutamic acid dehydrogenase. Zinc has been suggested to form complexes with amino acids [3], ATP [10], and neurotransmitters [11], and a functional interaction between the zinc ion and opioid systems has been proposed [18]. Thus, essential metals have been associated with processes which are capable of modulating regional neurotransmitter efficacy.

The hippocampus is an allocortical structure which selectively accumulates copper and zinc [4, 8, 13], concentrating zinc in the mossy fiber boutons of area CA3 as much as ten times over levels in other brain tissue [5]. Hippocampal zinc has been associated with the maturation and function of the mossy fibers [2], a glutamic acid system, and zinc deficiency produces abnormal neurotransmission in this system, possibly as a result of diminished glutamic acid release [9]. The differential accumulation of zinc within this region may reflect the distribution or activity of zinc-dependent neurotransmitter systems and may therefore have functional consequences for behaviors mediated by this system.

The hippocampus is uniquely involved with behaviors having a spatial component [14,16], and lesions to the hippocampus [15,17] and, more selectively, to the glutaminergic pathway within the hippocampus [7], disrupt spatial memory. Lateralization of hippocampal [19] and striatal [6,22], neurotransmitters has been related to spontaneous turn preferences which appear to be unlearned. Since zinc and copper are essential for the normal functioning of several neurotransmitters located in regions implicated in spatial behaviors, the lateral distribution of these metals may reflect their functional relevance to behaviors with spatial components.

The objective of this study was to investigate the relationship between copper and zinc levels in the left and right hippocampus and striatum—two areas implicated in specific aspects of spatial behavior—and the cortex, a control area, and performance on a task thought to have a spatial component. The hypothesis to be tested was whether the distribution of these metals was related to behavioral asymmetries.

METHOD

Animals

Twenty male Long-Evans hooded rats were used in this study (268±11 g). All rats were housed in individual stainless steel cages with ad lib access to food (Charles River RMH-1000 Chow) and water in The Johns Hopkins Medical Institutions animal facilities for 7 days prior to experimentation. Rats were obtained from Blue Spruce Farms, Altamont, NY.

Experimental Procedures

Behavioral testing took place in a T-maze 40 cm long, 10 cm wide, and 14 cm high, constructed from clear perspex and painted flat gray. A 25 W Tensor lamp was positioned 5 cm above the start box lid and provided the sole source of

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illumination in the experimental room. Choice performance in the T-maze served to identify the degree of turn preference of the individual rats. The rat was placed in the start arm and a left or right turn choice, judged on the basis of both forelimbs in the cross arm, was scored. The rat was returned to the holding cage for a 30 sec inter-trial interval during which the maze was wiped clean with a wet sponge. A second trial was then initiated and ten trials were administered on each of two consecutive days. The data were expressed as percent right turn preference for twenty trials.

Rats were decapitated 24 hours after behavioral testing and the brains were rapidly removed and placed on an ice cooled dissecting plate. Hippocampus (HIP), corpus striatum (CS) and cortex (COR) were bilaterally dissected from the brain and stored in pre-weighed plastic vials at −20°C until processed for copper and zinc analysis.

Frozen tissues were lyophilized for 16–24 hours. Dried tissue was wet-ashed in 30 ml Kjeldahl flasks with 1.0 ml Ultrex nitric acid (J. T. Baker Chemical Co.) for three hours at a temperature just below the boiling point. After cooling, 0.2 ml of 30% hydrogen peroxide was added and heating again applied for one hour. Sample solutions were cooled and diluted to about 10 ml with deionized distilled water in preweighed plastic vials. The weight of each solution was recorded for calculations of concentrations in tissues.

Zinc analyses were performed by flame atomic absorption spectrophotometry on a Varian AA5 spectrophotometer at the 213.9 nm wavelength. Copper analyses were made by flameless atomic absorption spectrophotometry on a Perkin-Elmer 4000 spectrophotometer equipped with a HGA 500 graphite furnace and AS-40 automatic sampler at the 324.8 nm wavelength. Standards were prepared by dissolving known amounts of copper wire or zinc granules in acid and diluting with distilled deionized water. Stock solutions of standards were stored in plastic bottles and working standards prepared daily. Weighed aliquots of NBS Standard Reference Material 1577—Bovine Liver—were digested and analyzed simultaneously with brain samples for quality control.

All glass- and plastic-ware used during digestion, storage, and dissection was acid-washed and rinsed with distilled deionized water.

Statistical Analysis

All behavioral testing, brain tissue dissections, and trace metal analyses were performed blind by three different experimenters. The behavioral data for each rat were expressed as percent of right turns on the twenty trials. The metal concentration data for the left and right HIP, CS and COR for each rat were expressed as micrograms metal per gram dry tissue weight.

The null hypothesis that turn preference was not related to lateralization of zinc or copper distribution in any of these brain areas was tested in two ways. First, the zinc and copper levels for each rat were expressed as a difference between the right (R) and left (L) side divided by the smaller (S) value for each region: (R−L)/S. A Pearson-product moment correlation was computed for this value and the percent right-turn preference of the rats, for a total of six correlation coefficients. A t statistic was subsequently computed for each correlation coefficient to assess statistical significance of the correlation. Second, where a significant correlation was found, a test for significance of differences between two proportions was computed [1]. This was done by assigning the rats to either a right-turn or left-turn preference group on the basis of their performance on the behavioral test, and then computing the proportion in each group which had higher metal (zinc or copper) levels in the right, relative to the left, area (HIP, CS, or COR).

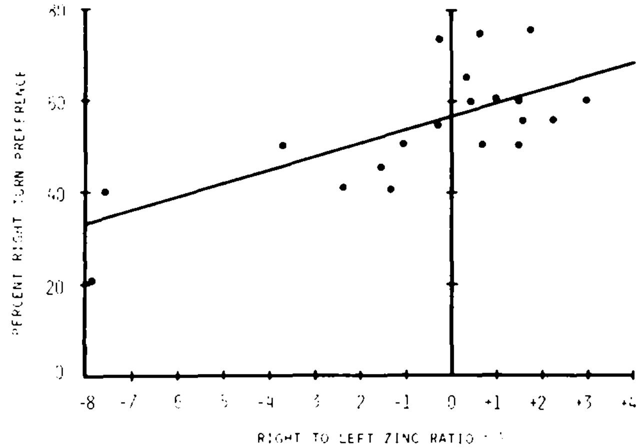

FIG. 1. Scatter plot of hippocampal zinc ratio (Δ=(R − L)/S, where R and L are the zinc concentrations in the right and left hippocampus, respectively, and S is the smaller of the two concentrations) versus turn preference. The regression line is plotted (r=0.72, $p<0.001$).

RESULTS AND DISCUSSION

Bilateral zinc and copper concentrations in the three brain regions are shown in Table 1. Copper was relatively evenly distributed across all the regions assessed. Zinc, however, was concentrated within the HIP and, to a lesser extent, in the CS. The variability in zinc concentrations within a given brain region was most prominent in the HIP; however, the range of concentrations observed here was similar to that previously reported [5]. Although there was a statistical difference in mean zinc concentrations between left and right HIP, the lateral effect was much greater than indicated by mean values. This is due to the fact that in most cases studied, the zinc concentration of one side of the HIP was much greater than the other side. The fractional deviations, Δ=(R−L)/S, where S is the smaller of the two values, ranged from −7.9 to +2.9, indicating significant asymmetries in zinc concentrations. Since, some rats had higher zinc concentration on the left side, $\Delta<0$, while the reverse condition held true for other rats, $\Delta>0$, the lateral differences in the means tends to cancel out.

The correlation coefficients computed for the turn preferences and metal differences revealed a lateralization of zinc in the HIP (r=+0.72) but not in the CS (r=+0.08) or the COR (r=+0.01), which was highly correlated with the rats' turn preferences. Specifically, higher zinc levels in the right or left hippocampus were correlated with right- or left-turn preferences, respectively. Subsequent analysis showed this to be statistically significant, $t(18)=4.4$, $p<0.001$. A test for significance of differences between two proportions confirmed these results. The proportion of right-turn preferring rats with greater zinc levels in the right, relative to the left, HIP was significantly greater than the proportion of left-turn preferring rats with this zinc distribution pattern ($p<0.01$). Copper level asymmetries were not significantly correlated

TABLE 1

CONCENTRATION OF ZINC AND COPPER IN LEFT AND RIGHT REGIONS OF RAT BRAIN

Region		Zinc	Copper
Hippocampus	Left	303 ± 68[1]	10.6 ± 2.5
	Right	194 ± 24[1]	9.9 ± 1.5
Corpus Striatum	Left	142 ± 26	8.4 ± 0.8
	Right	184 ± 32	8.5 ± 1.0
Cortex	Left	81.7 ± 3.6	6.2 ± 0.4
	Right	83.3 ± 2.9	6.5 ± 0.5

Data are expressed as Mean ± SE (n=20) μg/g dry weight.

Any two data values with the same numerical superscript are statistically different at the $p<0.05$ level.

with turn preferences in any area studied: HIP (r=+0.32), CS (r=+0.10), and COR (r=−0.10).

Previous investigations [7, 14, 15, 17] have suggested that hippocampal pathways are implicated in behaviors having spatial and cognitive components. Insofar as zinc could influence the hippocampal glutaminergic system via its incorporation into the metalloenzyme glutamic acid dehydrogenase or by inhibition of glutamic acid decarboxylase, the present data are consistent with this hypothesis. It appears that, in the normal brain, hippocampal zinc distribution asymmetries in the lateral dimension reflect an underlying neurobiological substrate for cerebral lateralization which is behaviorally manifest as spontaneous spatial preferences. The fact that both the HIP and the CS are involved with spatial behaviors, but only hippocampal zinc lateralization is strongly correlated with the behavioral task, suggests that these two brain structures may mediate different aspects of spatial behavior which will require more sensitive behavioral tests to define.

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