S-100 Protein: Regional CNS Concentrations in Rats Raised in Different Environments

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The S-100 protein was measured in 7 discrete brain areas from rats raised in an enriched, social, or isolated environment for 21, 42, or 84 days after weaning. S-100 was measured at significantly higher concentrations under enriched than under social environmental conditions in cerebellum and brainstem at 21 days but not thereafter. The protein in corresponding areas of brain in isolates was significantly less concentrated than in social controls. The results suggest changes, in turnover of the protein as a result of stimulation and activation of functionally specific brain regions, of the animals' responses to and interaction with environmental stimuli and adaptation to those stimuli.

The S-100 protein (Moore, 1965) is present in central and peripheral nervous tissues in numerous species of vertebrates and invertebrates (Levine & Moore, 1965; Moore, Perez, & Gehring, 1968; Perez & Moore, 1968). The protein is primarily glial in origin (Augusti-Tocco & Sato, 1971; Benda, Lightbody, Sato, Levine, & Sweet, 1968; Cicero, Cowan, Moore, & Suntzeff, 1970; Haglid & Carlsson, 1971; Perez, Olney, Cicero, Moore, & Bahn, 1970), although Hydén and McEwen (1966), Sviridov, Korochkin, Ivanov, Maletskaya, and Bakhtina (1972), and Haglid, Hamberger, Hansson, Hydén, Persson, and Ronnback (1974) have reported the presence of S-100 in neuronal perikarya and nuclei but Haglid *et al.* (1974) have pointed out that no evidence suggests synthesis of the S-100 protein by neurons.

Brain chemistry and morphology are altered by environmental conditions to which rats are subjected during growth and development (Altman & Das, 1964; Bennett, Diamond, Krech, & Rosenzweig, 1964; Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech, & Bennett, 1966; Ferchmin, Bennett, & Rosen, 1975; Greenough & Volkman, 1973; Rosenzweig, Krech, Bennett, & Diamond, 1962). The major morphological changes measured have been increases in glial populations in cerebral cortex, size of neurons and density of dendritic processes, and cortical thickness, all of which are associated with

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environmental enrichment. Considering those data, the fact that S-100 is primarily a glial protein, and that its function(s) remains unknown, we designed the experiments reported here to ascertain if the amounts of the protein measured regionally in rat brain are altered by conditions of environmental enrichment or isolation and, if so, to determine whether the changes are permanent or transient.

Methods

Animals

Young adult female Holtzman rats (*Rattus norvegius*; Holtzman, Madison, Wisconsin) were purchased when 7 to 14 days pregnant. Litters were born in the laboratory which was maintained at 23-25°C and on a schedule with 10 hr of darkness and 14 hr of light (0500 to 1900 hours). Food and water were given ad lib.

Procedure

Within 24 hr after birth litters were culled to 8 pups each. Pups in all litters were weaned at 21 days old, numbered by earpunch, and segregated by gender. Males and females from each litter were randomly assigned by split litter to 1 of 3 environmental conditions: enriched (EC), social (SC), or isolated (IC). The enriched environment was provided in cages measuring 30 x 45 x 120 cm. Each cage housed 12 males or females and contained an activity wheel; aluminum cans of various sizes which were painted with horizontal or vertical black and white stripes and which were either suspended from the roof of the cages or placed on the cage floor; a metal runway, 1.3 cm wide, extending the length of the cage and suspended between 2 small elevated platforms; and 2 small ladders which the animals could use to reach the platforms and elevated runway when not climbing the wire-mesh cage walls to do so. The contents and arrangement of the objects in the complex environment remained unchanged throughout the course of the experiment. In the SC group, 12 males or females were housed in cages identical in dimensions to those of the EC animals except that none of the objects used in the EC situation was present. Animals randomly assigned to the IC group were housed individually in cages measuring 18 x 18 x 25 cm. The rack was covered with a green hospital sheet in such a way as to limit vision to the cage interior and at the same time to allow full circulation of air through the cages. Auditory stimuli for all animals were limited to those of other animals in the room and to that of routine daily laboratory activities of the animal caretakers.

Three males and 3 females were randomly taken from each of the 3 environmental conditions, each from a different litter. They were weighed and decapitated in groups at 42, 63, or 105 days of age, that is after 21, 42, or 84 days in one of the environmental settings. The brains were removed and at 3°C dissected into frontal cortex (FC), occipital cortex (OC), caudate nucleus-putamen (CPU), hippocampus (HPC), midbrain (MB) including thalamus and hypothalamus, cerebellum (CB), and brainstem (BS) including pons and medulla. Tissues were weighed and homogenized by hand in glass-pestle homogenizers in 15 volumes of isotonic NaCl-veronal buffer containing Ca^{++} and Mg^{++} (Kabat & Mayer, 1967; p. 149) and centrifuged 1 hr at 3°C at 36,900 G. The

tissue pellets were discarded and the supernatant fluids were stored at - 100°C until assay. The S-100 protein was measured in the supernatant fluids by complement fixation (Moore & Perez, 1966). In a single assay, S-100 was measured in samples of 1 brain area in each of the 3 groups and at each of the 3 housing times. Tissue concentrations of the protein were calculated from standard curves of S-100 in each assay.

Results

Amounts of the protein in all brain areas except OC were treated as a repeated measures variable in a 2 (Gender) x 3 (Environments) x 3 (Duration of Environment) x 6 (Brain Areas) split-plot factorial analysis of variance (ANOVA; Kirk, 1968). The data in Table 1 for each Brain Area (BA) and Duration of Environment (DOE) represent the mean concentration of S-100 in 3 males and 3 females. Samples of OC from animals in each environment for 42 days were discarded because of experimental error in preparing the tissues for chemical assay. Therefore, data from OC were analyzed separately by ANOVA.

TABLE 1. S-100 (Mean μg/g Wet Tissue Wt ± SEM) of Rats Raised in Complex, Social or Isolated Environments for 22, 42 or 84 Days.

		Environment			
Tissue	Days Housed	Complex	Social	Isolated	
FC	21	78.9(2.1)	75.4(1.6)	78.5(3.7)	
	42	79.9(3.1)	87.2(3.4)	84.8(4.3)	
	84	88.7(2.9)	89.6(3.7)	92.2(1.8)	
CB	21	150.7(6.1)	122.2(3.3)	109.0(4.4)	$p < .0025$
	42	123.2(3.3)	131.5(3.1)	130.3(2.4)	
	84	141.3(3.1)	153.2(3.5)	151.7(5.0)	$p < .0025$
BS	21	109.2(2.4)	101.5(1.9)	95.6(2.8)	$p < .0025$
	42	114.0(1.9)	125.1(1.8)	120.0(2.9)	
	84	139.4(3.5)	141.5(3.8)	138.4(4.1)	
CPU	21	73.7(.6)	72.0(1.3)	67.0(1.1)	
	42	108.7(2.1)	113.7(2.8)	118.8(3.9)	
	84	84.1(1.1)	81.1(2.8)	80.8(2.4)	
HPC	21	50.0(1.0)	51.1(1.4)	51.2(.6)	
	42	81.2(2.9)	90.4(3.4)	90.7(5.3)	
	84	97.8(3.2)	100.0(1.9)	104.2(2.7)	
MB	21	73.8(2.0)	69.8(2.2)	65.4(1.3)	
	42	95.0(2.7)	97.7(1.3)	97.3(1.8)	
	84	86.3(2.0)	86.1(3.4)	94.3(3.0)	
OC	21	91.5(2.8)	95.0(2.5)	93.4(4.7)	
	42	—	—	—	
	84	97.4(2.7)	99.6(5.2)	98.9(3.0)	

The ANOVA revealed no main effect of gender or any significant interactions with gender ($p > .10$). The factor, DOE, although confounded with age, was significant ($F = 276.7$; $df = 2/36$; $p < .001$), reflecting the overall tendency for concentrations of S-100 to be regionally greater in older than younger animals, with the increase being greater between 21 and 42 than between 42 and 84 days. Mean values of BA at 21, 42, and 84 days were 83.1, 105.0, and 108.4 μg/g, respectively. This increase with age is in agreement with data reported earlier by Moore and Perez (1968) on whole rat brain. The notable exception here is CPU in each of the environmental conditions. We cannot account for the rapid increase in S-100 between 21 and 42 days and the decline thereafter to concentrations at 84 days which are similar to those measured at 21 days in each environment. The amount of protein among brain areas was also highly significant ($F = 483.2$; $df = 5/180$; $p < .001$). Thus, CB (134.8 μg/g) and BS (120.6 μg/g) had appreciably higher concentrations of the protein than any of the other brain areas (FC, 83.9; CPU, 88.9; HPC, 79.6; MB, 85.2 μg/g).

The BA x DOE interaction was significant ($F = 46.7$; $df = 10/180$; $p < .001$), indicating that the pattern of increase of S-100 with age varied among brain areas. Although the main environmental effect (E) on regional S-100 concentrations was not significant, the E x DOE ($F = 13.1$; $df = 4/36$; $p < .001$), E x BA ($F = 1.93$, $df = 10/180$; $p < .05$) and E x DOE x BA ($F = 3.28$; $df = 20/180$; $p < .001$) interactions were. The 2-way interactions can best be accounted for in terms of further analyses of the 3-way interaction. Because of the importance of ascertaining where in the brain environmental effects altered concentrations of S-100 and of the temporal pattern of change within individual areas, the E x DOE x BA interaction was analyzed for the significance of simple main effects of environment for each BA x DOE combination. This required 18 individual analyses and therefore, alpha (probability of occurrence of Type I error) were set at $p = .0025$ ($\cong .05/18$). Error variance and degrees of freedom were adjusted according to Kirk (1968; p. 291). The results are summarized in Table 1. Environmental conditions had a significant effect on concentrations of S-100 in CB ($F = 88.51$; $df = 2/156$; $p < .0025$) and BS ($F = 9.11$; $df = 2/156$; $p < .0025$) at 21 days. In both of those areas, S-100 was measured in greatest concentrations among EC animals and lowest among isolates. At 42 days, regional differences were not significant in amounts of the protein as related to any of the environments. However, in CB, a significant environmental effect occurred at 84 days ($F = 8.87$; $df = 2/156$; $p < .0025$): SC and IC animals had almost identical concentrations of S-100 which were greater than in the EC animals.

The separate analysis of OC revealed no significant effect of environmental conditions during growth and maturation on concentration of S-100. In the social situation, direct observation of the animals revealed that they typically huddled in corners of the cage and were sleeping during the day but active when the lights were turned out at the start of the 14-hr dark period. This behavior was characteristic of the SC animals throughout the 84 days in that environment. On the other hand, EC animals were active during the day and night, using the activity wheel, running back and forth across the cage on the runway, and climbing to the cage ceiling by means of the suspended objects. This tended to be greater in the dark period than in the light. The EC animals only occasionally huddled and for only relatively brief periods of time. Most noteworthy, however, was the observation that within 2 to 3 weeks after being placed in the enriched environment, EC subjects showed a marked decrease in their use

of the objects in the cage and huddled in the corners as did the SC animals. The behaviors of EC, SC, and IC animals were similar at this time except that the isolates had no other animals with which to huddle. After 21 days in isolation and thereafter, IC animals displayed appreciable aggression during the brief time of handling when they were weighed and killed. Aggressiveness of this sort was not seen in EC or SC animals. Although body weights were not tabulated, isolates weighed more than animals in the other environments at each of the 3 times groups were killed, that is, at 21, 42, or 84 days.

Discussion

The results of this experiment suggest that regional concentrations of S-100 which occur in rat brain with growth and maturation can be affected, if only transiently, by environmental conditions to which the animals are exposed during this time, at least in cerebellum and brainstem. We postulate here that animals respond to environmental stimuli, that responding is representative of activation of functionally specific brain areas, and that this activation regulates the rate of turnover and, therefore, the amounts of S-100 in those areas. The CB is largely involved in the mediation of balance, coordination, and integration of motor activities (Zemen & Innes, 1963), especially those that were clearly part of the behavior patterns of EC animals, evident in the use of the activity wheel, climbing, and traversing the narrow runway. The BS contains the reticular formation which is intimately involved in cerebral arousal and, we assume, arousal of the ascending reticular activating system. The latter was maintained when the animals responded to simuli making up the enriched environment. Therefore, the significant increase in amount of S-100 in CB and BS in EC animals compared with SC rats can at least be tentatively accounted for. Isolates were severely restricted in motor activity and, consequently, CB and BS were only minimally stimulated. This could account for the lower amount of S-100 in those brain areas in IC animals compared with their SC counterparts after 21 days of housing. Indirect evidence supporting the hypothesis relating activation of functionally specific brain areas and amounts of S-100 measured in those areas was obtained in an identical experiment carried out in our laboratory at 16-18°C rather than 23-25°C. Here, EC and SC animals huddled, presumably to keep warm, and EC rats made no use of the stimulus objects, severely reducing any activation of CB or BS. Environmental conditions to which these animals were exposed had no effect on amounts of S-100 measured in any brain area.

Effects of environmental isolation on concentrations of S-100 during development were transient rather than permanent. The protein was measured in CB and BS in isolates at levels significantly lower than in corresponding areas in SC animals during the 1st 21 days of housing. However, between 21 and 42 days and thereafter, S-100 in those areas in IC animals reached concentrations corresponding to those measured at the same times in SC and EC animals. We should recall that for the EC rats, the environment was left unchanged throughout the experiment. Furthermore, after 2 to 3 weeks, the EC rats made little or no use of stimulus objects in their cages. We suggest, therefore, that the EC animals adapted to the environmental stimuli, being offered no stimulus change to which to respond. Therefore, with adaptation the CB and BS

were only minimally stimulated and no further increase in S-100 occurred in those brain areas in the EC animals. In general, the behavior among EC animals was the same as that among social controls after adaptation. However, at 84 days, S-100 in CB in isolates and SC animals was greater than among EC subjects, an unexpected and inexplicable pattern of change in the amount of the protein.

Altman & Das (1964) described increased numbers of glial cells in cerebral cortex in EC rats. Similarly, Diamond *et al.* (1966) reported an increased glia/neuron ratio in cerebral cortex in EC animals. As previously noted, S-100 is primarily a glial protein. Therefore, a 2nd interpretation of our data is that environmental enrichment increased the glia/neuron ratio in CB and BS during the 1st 21 days of housing and, therefore, the amounts of S-100 in these areas. We have no histologies with which to support such an interpretation nor are we aware of data suggesting that this ratio increases specifically in these areas with environmental enrichment. Altman & Das (1964) measured increased glial numbers in neocortex but not subcortically. The increased glia/neuron ratio reported by Diamond *et al.* (1966) was measured in OC in EC animals. However, no significant differences were found in amounts of S-100 in OC in any of the animals at any time during our experiment. If changes in amounts of the protein were due to a change in glia/neuron ratio, S-100 in CB and BS among isolates should have remained at concentrations lower than in SC and EC animals. This was not the case. The S-100 in brains of isolates reached concentrations equal to those measured in SC and EC animals between 21 and 42 days; it merely took a longer time to do so. Also, after 84 days, S-100 in CB of isolates was greater than EC animals. Under these circumstances, a change in glia/neuron ratio alone would not account for our results: an increase in S-100 may not have been measurable in OC if the increase in cortical thickness and weight (Diamond *et al.*, 1966; Diamond, 1967) were relatively greater than the increased glia/neuron ratio and, presumably, glia S-100 because the protein concentration is expressed in terms of tissue weight.

Hydén & Lange (1970a, b) have suggested that the S-100 protein is involved in learning, reporting an increase in amount of the protein in hippocampus of rats during learning of transfer of handedness. We measured no changes in amounts of S-100 in HPC of any animal. However, Hydén and Lange measured the protein in very specific cells and in histologically well-defined areas of HPC whereas we used most if not all of this tissue site. Therefore, any subtle change in S-100 which may have been due to cellular stimulation in HPC may well have been masked in our experiments. Nevertheless, we are not compelled to interpret our data specifically in terms of learning, although some learning undoubtedly occurred among EC animals when they used the activity wheel, runway, and so on. Our results suggest at this time only that stimulation and activation of cellular populations in functionally specific brain regions are responsible for changes in amounts of S-100. This interpretation is a tentative one. The precise nature of the mechanism(s) by which environmental conditions and ontogenic development of the S-100 protein interact and the transient characteristic of the change remain unclear at this time.

Notes

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