Differential effects of early malnutrition on active and passive avoidance performance by sex of subject

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Female rats were fed a low protein diet (8%) or control diet (19% protein) during pregnancy and for 12 days after birth. Male and female offspring were tested on a go/no-go shuttlebox designed to separate the general learning impairment from a specific one (the inability to inhibit irrelevant responses). The sex, activity levels, and shock thresholds of the subjects were determined. The experimental group was significantly inferior to controls on both the go and no-go components. The malnourished males, in turn, were significantly inferior to the malnourished females on both tasks. Activity differences among the groups were not correlated with learning deficits. The results support a general learning impairment as sequelae of early protein malnutrition. The general inferior performance of the males was considered as support for greater sensitivity of this sex to malnutrition on the behavioral tasks used here.

Does a period of malnourishment produce a general learning impairment or does it lead to specific measurable impairments? Morris (1974) presented data to support the proposition that early malnutrition¹ may lead to an inability to inhibit irrelevant or non-reinforced responses. The theory states that malnourished and supposedly less inhibited animals may perform a wide repertoire of responses which may enable them to score better than controls on certain tasks.

The research paradigm used to confirm the postulate has several methodological flaws. Neither activity levels nor the sex of the subjects were acknowledged or controlled. In addition, the behavioral tasks used could not differentiate a general learning disability from more specific ones.

This study was designed to investigate the effects of an early malnourishing diet on later discriminative avoidance learning tasks. These tasks were chosen to measure specifically a general learning ability against a specific inhibitory one (Rosic & Bignami, 1970). The activity level, sex, and shock threshold levels of malnourished and control subjects were determined.

Activity levels of treatment groups are necessary to distinguish peripheral from central influences on learning. Previous research has shown the differential effects of sex on avoidance behavior (Archer, 1975), and also the differential shock thresholds of malnourished vs. control animals (Smart, Watson, & Dobbing, 1975).

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METHOD

Subjects

Eighty-nine Sprague-Dawley albino rats (*Rattus norvegicus*), 52 females and 37 males, were bred in the laboratory, Animal Section, College of Medicine, Howard University. The control groups, CON, comprised 39 animals (19 males, 20 females) that were nurtured by mothers maintained on a 19% protein diet during gestation and for 12 days following parturition. The experimental group (EXP) comprised 50 animals (18 males, 32 females) that were nurtured by mothers maintained on an 8% protein diet during the same period as Group CON. From the 13th day after birth, both groups were maintained on the control diet for the remainder of the experiment. The animals were weaned at 21 days of age. Testing began when the animals were 150-170 days of age.

Procedure

The apparatus consisted of a 64 × 16.5 × 26.5 cm shuttlebox of black tinted plastic which enclosed a grid floor of stainless steel rods. Each rod was connected to a shock generator/scrambler combination (Tech. Ser. Inc., Model SC-902). The grid floor, without central barrier, was placed on a fulcrum so that as the animal crossed over the midline the floor tilted slightly and thereby closed an electric switch. The apparatus provided several relay switches for activating a light bulb, Sonalert, and each half of the grid floor. The relays were controlled by a FORTRAN program and the use of a Hewlett-Packard 2116B computer with a disk operating system, A-D multiverter, and Teletype.

At age 150-170 days, the animals were tested for acquisition of go/no-go consecutive discrimination avoidance learning. Each subject was given four consecutive daily sessions of avoidance acquisition. Each session consisted of 25 active avoidance trials (go) and 25 passive avoidance trials (no-go) presented in a quasi-random cycle. The conditioned stimulus (CS) for "go" was a tone (90 dB) presented for 5 sec. The unconditioned stimulus (UCS) was .5-.8-mA (see below) constant current delivered through half of the grid floor on which the rat was present. The CS was terminated by an avoidance response (latency < 5 sec) to the other unshocked half of the grid floor. Both CS and UCS were terminated by an escape response (latency > 5 sec) to the other half of the grid floor. Return crossings made during the go signal and for the duration of the go trial were punished, thereby forcing the animal to remain in the safe area for 30 sec.

The no-go parameters included a CS of a 15-W light bulb for 5 sec, followed by the application of .5-.8 mA to the other half of the grid floor from the rat. Crossings made during the no-go signal and for the duration of the no-go trial were punished, again forcing the animal to remain in the safe area for 30 sec. After 30 sec, the computer program initiated a new trial, thereby maintaining a comparable intertrial interval across trials. The location of the animal on the grid floor, the subsequent temporal control of stimulus presentation and shock administration, and the response of the animal were monitored by the computer program. Prior to behavioral testing, detection of shock threshold levels was established for the two groups, CON and EXP. This was done by determining the initial reaction (leg raising, flinching, etc.) to increasing levels of current intensity across the grid floor of the shuttlebox. An average of the meter reading of individual animals in the two groups was used during the actual behavioral tests. The current intensity was established to be .5 mA for the malnourished group, EXP, and .8 mA for the control group, CON. Activity measures (using activity wheels) were taken after the behavioral testing. All testing was conducted at the same time of day.

RESULTS

Go Responses

An analysis of variance on the correct responses revealed that both diet and sex affected performance on go responses ($F = 79.2$, $df = 1/85$, $p < .001$, and $F = 15.3$, $df = 1/85$, $p < .001$, respectively). The performance of both male and female EXP groups was significantly inferior to that of their respective CON groups with males demonstrating a greater performance deficit than their female counterparts (Figure 1). Males reared on the low-protein diet differed significantly from those reared on the control diet ($t = 7.7$, $df = 35$, $p < .001$), and performance of the malnourished subjects was also significantly different from that of their controls ($t = 5.9$, $df = 50$, $p < .001$). The scores of the male EXP animals were significantly lower than the scores

of their female EXP counterparts ($t = 3.3$, $df = 48$, $p < .001$).

No-Go Responses

Analysis of variance on correct responses also revealed the effects of diet ($F = 11.7$, $df = 1/85$, $p < .001$) and sex of subject ($F = 7.7$, $df = 1/85$, $p < .001$) on performance of the no-go component. In addition, a significant Diet by Sex interaction revealed the much greater deficit on the male malnourished animals compared with their female counterparts ($F = 18.3$, $df = 1/85$, $p < .001$). The performance of male EXPs was significantly inferior to that of their control counterparts (Figure 1) ($t = 6.41$, $df = 35$, $p < .001$). The female EXP group, however, did not significantly differ from its control group.

Activity Levels

Analysis of variance showed the effects of both diet and sex on activity wheel measures ($F = 6.8$, $df = 1/20$, $p < .001$, and $F = 18.0$, $df = 1/20$, $p < .001$, respectively). The female control group displayed higher activity levels than did the male, and this relationship was further heightened in the malnourished groups.

Body Weights

The effect of the 8% protein diet on both sexes approximated 10%-12% loss of body weight at time of testing.

DISCUSSION

A general performance deficit was exhibited by the protein malnourished animals on both go and no-go components of the avoidance task. This situation was designed to measure opposing tendencies, active vs. passive avoidance performance in the same shuttlebox. Inferior performance by the malnourished group on both components constitutes strong evidence in favor of a general learning deficit in these animals and against the notion of a specific learning disability. The finding that the performance of the malnourished animals was inferior to that of the controls on both tasks also rules out the effects of activity levels as contributing to their performance. The general performance of the malnourished animals was sufficiently poor to negate any advantages that differences in activity levels might have bestowed upon them. In this laboratory, and elsewhere, mere higher activity levels favor performance on the go component and impair performance on the no-go component (Kriekhaus, Miller, & Zimmerman, 1965). The reverse holds true for animals displaying lower activity levels.

The deficits exhibited by the males in the malnourished group compared to females on both com-

Figure 1. Mean total correct responses on go (left) and no-go (right) components of the discriminative avoidance shuttlebox task. The inferior performance by the male EXP (8% protein diet) group compared to the female EXP group and CON (19% protein control diet) is depicted.

ponents of the behavioral task in the presence of their higher activity levels further support the greater effect of malnourishment on males. A relatively large series of findings support the greater sensitivity of the male to malnutrition (Barnes, Neely, Kwong, Labadan, & Frankova, 1968; Williams, Tanner, & Hughes, 1974). There are surprisingly little data on the male/female differences on behavioral parameters after an early malnutrition regimen.

The behavioral performance of the control animals adds to the validity of the behavioral tasks used. In the go component, the female controls were superior to the males, while the male controls were superior in the no-go component. These findings support those of Archer (1975), who used discrete active and passive avoidance measures on both sexes.

In light of the growing evidence in animal and human populations that the male is more vulnerable to early central nervous system trauma (Hutt, 1972; Sikov & Noonan, 1964; Widdowson & McCance, Note 1), the malnutrition model can be considered suitable for further investigations of the mechanisms involved.

REFERENCE NOTE

1. Widdowson, E. M., & McCance, R. A. *Studies on the nutritive value of bread and on the effects of variations in extractive rate of flour on the growth of undernourished children*. Medical Research Council Special Report, Serial No. 287, 1954.

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NOTE

1. Dams were malnourished during gestation and early lactation periods. The offspring of these dams are the subjects of the investigation. The term "low-protein undernutrition" may be more appropriate, but henceforth we align ourselves with the literature definition and use the general term "malnutrition."

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