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Altered Development of Responsiveness to Clonidine in Severely Malnourished Rats[1,2]

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GOODLETT, C. R., M. L. VALENTINO, O. RESNICK AND P. J. MORGANE. *Altered development of responsiveness to clonidine in severely malnourished rats.* PHARMACOL BIOCHEM BEHAV **23**(4) 567–572, 1985.—To examine the effects of malnutrition on the ontogeny of α_2 noradrenergic receptor function, we compared the effects of clonidine during early development in severely malnourished and well-nourished rat pups. Independent groups of pups from dams given either 6% or 25% casein diets received one of five doses of clonidine at 5, 10, 15, 20 or 25 days of age and dose-response relationships for motor activity were determined. In the 25% pups the clonidine-induced locomotor activity was greatest at 5 and 10 days, intermediate at 15 days and not elevated at 20 and 25 days. The malnourished pups exhibited a significant delay in the transition from hyperactivity to hypoactivity, being activated by clonidine until at least 25 days. Wall-climbing measures indicated similar developmental trends as overall activity. These results are discussed in terms of the proposed mechanisms mediating the developmental change in the effects of α_2 receptor stimulation.

Malnutrition Clonidine Developmental psychopharmacology α_2 Agonist Wall-climbing Motor activation

EARLY malnutrition is known to delay many aspects of development of the nervous system. For example, the DNA synthesis phase of the cell cycle of neurogenesis is prolonged [21], the rate of cellular migration in the olfactory bulb is reduced [8] and the onset of sensory and motor functions is delayed [1]. In addition, malnutrition produced by restriction of dietary protein to rat dams has also been reported to produce chronic and irreversible elevations of brain norepinephrine, serotonin and their metabolites [18, 25, 30]. However, little is known about the effects of malnutrition on the ontogeny of monoaminergic receptor function. Given the general delays in development and the permanent alterations in norepinephrine metabolism resulting from early malnutrition, it is possible that malnutrition alters the development of noradrenergic receptor function.

One approach that has proved useful in delineating the ontogeny of neurochemical systems is a developmental psychopharmacological analysis [5,27]. Changes in the effects of specific receptor agonists or antagonists during development may provide ontogenetic markers which indicate functional changes in neuronal systems. For example, one of the most striking demonstrations of age-related differences in behavioral effects elicited by a drug is seen with clonidine [19, 24, 28], an α_2 adrenergic agonist [2, 3, 26]. In the first two weeks of life the predominant behavioral effect of clonidine is an increase in locomotor activity including stereotypic wall-climbing when a vertical edge is encountered. Between 15 and 20 days of age, there is a dramatic change in the effects of clonidine, with cataleptic hypoactivity becoming the predominant behavioral effect. Since both the activational effect of clonidine early in life and the behavioral sedation produced in older animals can be blocked by specific α_2 antagonists, it appears that both effects may involve α_2-adrenoceptors in the central nervous system [9, 10, 11, 19, 20].

The present study compared the effects of clonidine in severely malnourished and well-nourished rat pups during early development, including the period of transition from motor activation to behavioral sedation. The dose-response curves obtained across early development allowed us to address whether malnutrition caused a delay in the loss of the motor activational effects of clonidine.

METHOD

Dietary Treatments

Subjects were 296 pups of the first litters of Sprague-Dawley dams obtained from Charles River Breeding Laboratories. The dams were maintained in our laboratory on diets containing either 6% casein or 25% casein (Teklad Mills, Madison, WI), with the diets instituted five weeks before mating and lasting throughout gestation and lactation. (For a more detailed description of the diets see [18 and 25].) On the day of birth (Day 0), each litter was weighed and the litter weight and the number of live pups were recorded. All pups born on the same day within a particular diet group were placed together and eight pups were randomly assigned to each dam. Those litters tested after

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TABLE 1
NUMBER OF SUBJECTS PER GROUP

Age*	5		10		15		20		25	
Diet+	6%	25%	6%	25%	6%	25%	6%	25%	6%	25%
Saline	5	5	6	5	5	6	4	5	6	5
0.1 mg/kg	4	6	6	7	6	8	7	6	5	8
0.5 mg/kg	5	7	7	7	7	6	7	6	5	5
1.0 mg/kg	5	6	4	7	7	6	6	5	6	7
2.5 mg/kg	7	5	5	6	6	6	8	6	6	$6_{N_1} = 296$

* Age in days.
+ % Casein of the two isocaloric diets provided the dams and offspring.

TABLE 2
BEHAVIORAL CATEGORIES USED FOR THE TIME SAMPLING SCORING PROCEDURE

1. Lying Still - On Ventrum or Side
2. Lying Still - On Back
3. Locomoting or Turning
4. Rolling or Kicking on Back
5. Angled Against Wall - No Treading
6. Wall-Climbing - Front Paws Treading
7. Paddling - Front Paws Treading on Floor
8. Paddling - Back Paws Treading
9. Twitching or Rhythmic Jerks
10. Grooming
11. Rearing
12. Sniffing
13. Yawning
14. Chewing
15. Defecating
16. Crouching

weaning (21 days) were provided the appropriate diet and housed four to a cage with their same-sex littermates until the day of testing. All rats were given food and water ad lib and maintained on a 12 hr light:12 hr dark cycle (lights on between 0700 to 1900 hr).

Design

The study used a completely randomized factorial design having diet, age and dose as the three between-group factors. Thus, independent groups of rat pups from the two nutritional treatments were tested at 5, 10, 15, 20 or 25 days of age and were given one of five drug doses. Within each age group dose-response relationships were determined using the saline vehicle or 0.1, 0.5, 1.0, or 2.5 mg/kg of clonidine (see Table 1 for summary of design and group n's). All pups from a litter were tested on the same day and each pup was given a single injection and tested once. Pups were assigned to the drug dose groups such that no more than two pups within a litter received the same dose, and at least four litters were represented at each dose within each age. The drug solutions were coded so that the observer was not informed of the dose given. Behavioral observations were made for 90 minutes beginning 5 minutes after injection of the drug (see below).

Procedure

All testing was done between 0800 and 1600 hours in a room separate from the vivarium. Due to the length of the testing a maximum of two litters could be tested during this period. When possible one litter of each diet was tested per day.

The pups were observed in one of four individual chambers having wire mesh floors and Plexiglas walls ($18 \times 18 \times 12.5$ cm for 5 and 10 day old pups and $18 \times 18 \times 19$ cm for 15, 20 and 25 day old pups) placed inside a clear plastic incubator (Marsh Manufacturing, Inc.). As in previous experiments (e.g., [24]), the ambient temperature was maintained at 33°C for the 5 and 10 day olds and 31–32°C for the older pups. On the day of testing the first group of four pups from a litter were weighed and placed in the chambers for a five minute adaptation period. Following the adaptation period each pup was injected subcutaneously with the appropriate, coded dose in a volume of 10 μl/g body weight. Five minutes after the last pup was injected a 90 minute time sampling of behavior was begun with observations recorded once every 30 seconds. Each observation was recorded as one of sixteen mutually exclusive behavioral categories (see Table 2), yielding 180 total observations per pup. At the end of the 90 minute test period the pups were returned to the dam and the remaining pups of the litter were tested in the same manner.

Data Analysis

The data were initially examined as a function of 15 minute intervals following injection. In general, the drug effects declined over time for both groups. Since these functions reflected essentially the same results as the total scores summed across the 90 minute interval, for the sake of clarity only the total scores will be reported. The number

TABLE 3
BODY WEIGHT (IN GRAMS)

Age / Diet	5	10	15	20	25
6%	8.1 ± 0.7*	9.2 ± 0.4	11.9 ± 1.2	15.1 ± 0.9	17.5 ± 1.3
25%	13.6 ± 1.3	30.0 ± 1.1	46.6 ± 1.1	61.5 ± 6.1	83.1 ± 6.3

* Mean ± standard error of the mean for pups tested at each age.

of observations were totaled for each behavioral category and an overall activity score was obtained by summing the counts for locomoting, rolling, wall climbing and paddling (categories 3–8 of Table 2). Due to the low incidence of the grooming, rearing, sniffing and mouthing, no further analyses were performed in these measures. A three-way analysis of variance was performed on the scores of overall activity and on the wall-climbing measure, with age, dose and diet as the three factors. Tukey's critical range test was used to compare group means in all *post hoc* multiple comparisons.

RESULTS

Body Weight

The mean weights for the 25% and 6% pups at each test age are presented in Table 3. Pups from the 6% dams weighed less than the 25% pups from birth and throughout the ages tested, and at 25 days the weights of the 6% pups were only 20% of those reached by the well-nourished pups. These weights agree with data on the development of pups from the 6% paradigm that have been previously reported by our group [25].

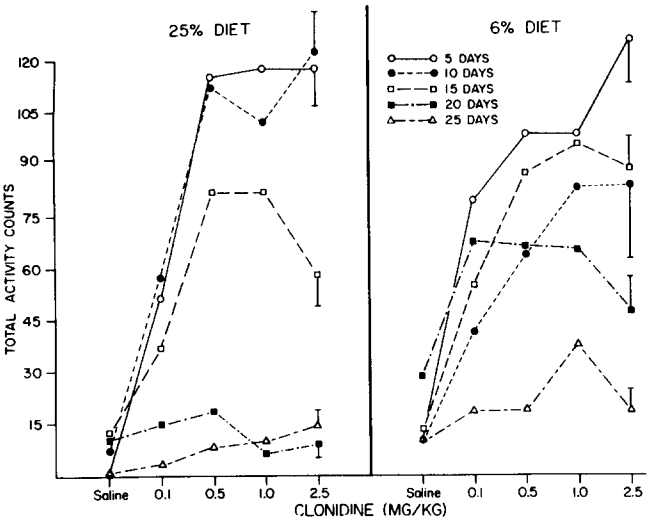

FIG. 1. Mean (and SEM for highest dose) total activity scores as a function of age and dose for pups from dams given the 25% casein diet (left panel) and from dams given the 6% casein diet (right panel).

Effects of Clonidine

As shown in Fig. 1, clonidine induced a dose-dependent motor activation at younger but not older ages, yielding a significant dose × age interaction for the total activity measure, $F(16,246) = 8.3$, $p<0.001$. As seen in the left panel of Fig. 1, the 25% pups' total activity levels induced by clonidine were greatest at 5 and 10 days, and intermediate and significantly lower at 15 days than at 10 days (Tukey test: 0.5 mg/kg, $p<0.05$; 2.5 mg/kg, $p<0.01$). For the 25% pups at 20 and 25 days, clonidine did not induce elevated activity relative to saline controls. Thus, the activity levels at these older ages were significantly lower than at 15 days (Tukey test: $p<0.01$ at the 3 highest doses).

In the 6% group (see right panel, Fig. 1) the loss of clonidine-induced hyperactivity was delayed by about 5 days, producing a significant diet × age interaction, $F(4,246) = 15.79$, $p<0.001$. For the 6% pups the activity levels induced by clonidine at 15 days were not significantly different at any dose level from that induced at 10 days, and a significant reduction in clonidine-induced activity between 15 and 20 days occurred only at the 2 highest doses (Tukey test: 1.0 mg/kg, $p<0.05$; 2.5 mg/kg, $p<0.01$). Furthermore, unlike the 25% pups, a significant reduction in clonidine-induced activity occurred between 20 and 25 days for the 6% pups (Tukey test: 0.1 and 0.5 mg/kg, $p<0.01$, 1.0 and 2.5 mg/kg, $p<0.05$). The observers reported that the older pups (20 and 25 days for the well nourished group, 25 days in the malnourished) had long periods of immobility with short bursts of activity interspersed throughout the test period. Since we did not want to disturb the pups to administer periodic tests for catalepsy, our scoring procedure was insensitive to decreases in activity below saline controls and measures of this apparent cataleptic immobility were not available.

In comparing the effects of clonidine between the two dietary groups at each age, no evidence of differences in general activity were seen at 5 days. The 6% pups were significantly less active than the 25% pups at 10 days (Tukey test: 0.5 mg/kg, $p<0.05$; 2.5 mg/kg, $p<0.01$), and the 10-day old 6% pups were also significantly less active than the 5-day old 6% pups (Tukey test: 0.1, 0.5 mg/kg, $p<0.05$; 2.5 mg/kg, $p<0.01$). This suggests that by 10 days of age the pups from the dams fed the 6% diet had developed a reduced sensitivity to the effects of clonidine in producing hyperactivity in neonates. The most obvious differences between the two dietary groups in response to clonidine occurred at 20 days (Tukey test: $p<0.01$ for all doses of clonidine). Here the 6% animals given clonidine continued to show significantly elevated activity (though less than the

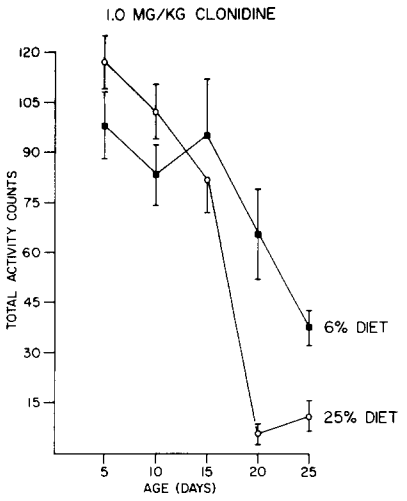

FIG. 2. Mean (and SEM) total activity induced by 1 mg/kg of clonidine plotted as a function of age for the two dietary groups. This illustrates the significant delay in the loss of clonidine-induced hyperactivity that normally occurs in the third week of life.

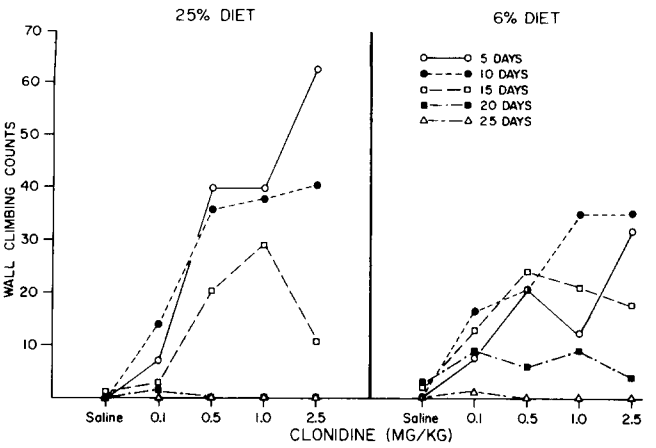

FIG. 3. Mean number of wall-climbing counts (category 6 of Table 2) graphed as a function of age and dose. Note the continued wall-climbing activity induced by clonidine induced in the 6% pups at 20 days of age.

15 day old 6% pups), while the 25% pups did not. Even at 25 days the 1.0 mg/kg dose of clonidine produced a significant increase in activity relative to saline controls in the 6% pups (Tukey test: 1.0 mg/kg, $p<0.05$).

The developmental delay of the 6% pups is clearly illustrated in Fig. 2 showing the mean activity scores for each age at the 1.0 mg/kg dose of clonidine. While there was a sharp decline in activity from 15 to 20 days in the 25% animals, the decline was more gradual for the 6% pups with these animals remaining at elevated activity levels until at least 25 days. This delay in the loss of clonidine-induced hyperactivity in the 6% pups suggests a delay in the functional maturation of the substrate(s) which mediate the reduced locomotor activity in the older pups following clonidine administration.

We analyzed the frequency counts of wall-climbing in addition to the overall activity scores since wall-climbing is one of the characteristic features of clonidine-induced activation (see Fig. 3). In general, the developmental trends were similar to those of overall activity, with more wall-climbing induced at the younger rather than the older ages (dose × age interaction: $F(16,246)=4.2$, $p<0.001$). As with the total activity measure, the loss of clondine-induced wall-climbing was delayed by about five days in the 6% group, contributing to the significant diet × age interaction, $F(4,246)=2.48$, $p<0.05$. Interestingly, the 5-day old 6% pups given clonidine showed significantly less wall-climbing at the three highest doses than their 25% counterparts (Tukey test: 0.5 mg/kg, $p<0.05$; 1.0 mg/kg, $p<0.01$; and 2.5 mg/kg $p<0.01$). It appeared that this resulted from a motoric immaturity of the malnourished pups, since it was observed that the 5 day old malnourished pups were poorly developed, were less coordinated in their movements and less capable of maintaining the balance necessary for long bouts of wall-climbing. This was also apparent as an increase in rolling behavior in the malnourished pups relative to the 5 day old clonidine-treated 25% pups.

DISCUSSION

The malnourished rat pups exhibited a developmental delay of the change in the effects of clonidine from hyperactivity to sedation, indicating a delay in the maturation of the substrates mediating this change. While we cannot discount the possibility of pharmacokinetic differences in the distribution or metabolism of clonidine between well-nourished and malnourished rats, it seems unlikely that such potential differences would result in similar, but delayed, effects of clonidine in malnourished rats.

While the mechanisms responsible for the transition in the behavioral effects of clonidine from hyperactivity to sedation during the third week of life are not known, it is clear that both types of effects are related to actions of clonidine at α_2 sites. Both the hyperactivity produced by clonidine in 7-day old rats [19] and the reduced activity seen in 20-day old [20] or adult rats [11] are blocked by a variety of α_2 antagonist drugs.

At least four possible alternatives may account for the developmental change in the effects of clonidine in normal rats which could be delayed in our malnourished rats. First, there may be a developmental onset of pre-synaptic regulation of noradrenergic transmission reflecting the functional maturation of presynaptic receptors on NE terminals [29] or cell bodies [6, 7, 31, 32], or the maturation of epinephrine regulation of noradrenergic neurons via α_2 receptors [22]. However, it seems unlikely that there is a dramatic functional onset of α_2 receptor-mediated inhibition of NE transmission in the third week of life. Kellogg and Wennerstrom [15] showed that clonidine pretreatment prevented the loss of NE from the cortex and brainstem following inhibition of tyrosine hydroxylase as early as 4 days of age, indicating the presence of functional α_2 receptors which can exert presynaptic control over NE release.

It has been suggested that the developmental change in the effects of clonidine may be due to regional differences in the development of α_2 receptors [14,15]. Hartley and Seeman [14] examined α_2 receptor binding in the mesolimbic region, frontal cortex, hypothalamus and hippocampus as function of age and found large increases in α_2 receptor numbers in the mesolimbic area between 7–15 days and later between 21–28 days. It is unlikely that these growth

phases could directly account for the dramatic behavioral change in the effects of clonidine between 15–20, a time when the receptor numbers in the mesolimbic area was at a plateau according to their data. Still, regional differences in the ontogeny of α_2 receptors cannot be excluded as a viable alternative to explain the developmental change in clonidine's effects on activity.

It is also possible that clonidine acts on α_2 receptors located on other neurons (i.e., a post-synaptic effect) and that a developmental maturation of the modulation of these systems occurs. For example, recent studies using tissue from adult rat brain have demonstrated that NE inhibits release of serotonin from cortical slices [13], from hippocampal slices [12] and from cortical synaptosomes [23], and these effects were blocked by phentolamine or yohimbine. Raiteri *et al.* [23] proposed that there are at least two forms of the α_2 receptor, one receptor which inhibits neurotransmission of NE and most likely is located presynaptically to the NE terminal, and one which inhibits release of serotonin and most likely is located on serotonin terminals but post-synaptic to noradrenergic terminals. The suggestion that the clonidine-induced sedation may be related to the development of direct alpha$_2$-adrenergic modulation of serotonin transmission receives support from Zebrowka-Lupina *et al.* [33]. They reported that clonidine produced hyperactivity in adult rats that had been given 6-hydroxydopamine lesions plus reserpine or PCPA but not when either treatment was given alone, indicating that depletion of 5-HT plus the destruction of the noradrenergic cells by 6-OHDA may be necessary for the appearance of clonidine-induced hyperactivity in adult rats.

As a fourth alternative, other neurochemical systems which may not be directly influenced by noradrenergic systems but which influence motor activity in parallel with noradrenergic neurons, may develop inhibitory control over motor activity in the third week of life. A recent report [17] indicates that in 17 day old rats treated with cholinergic antagonists, clonidine produced hyperactivity and wall-climbing, typically not seen at this age in normal rats. Development of inhibitory cholinergic influences over activity and arousal by the end of the third week of life had been demonstrated in earlier developmental psychopharmacological studies [4]. If, as these results suggest, clonidine fails to elicit hyperactivity after 20 days because of the development of some tonic cholinergic inhibitory control, then the delay in the transition from activation to sedation seen in the malnourished pups may be due to a delay in the functional development of cholinergic systems.

While the underlying processes mediating the developmental change in clonidine-induced behavior remain to be clarified, it is clear that the severely malnourished pups were delayed in the loss of clonidine-induced hyperactivity. We are presently examining the development of adrenergic receptor binding to determine whether differences exist in the development of these receptors as a consequence of malnutrition. By correlating changes in the ontogeny of α_2 receptors (or changes in the functional development of other neurotransmitter systems as well, from the above discussion) with the observed delay in the reversal of the effects of clonidine, we may be able to better understand the effects of protein malnutrition on the developing nervous system and, eventually, on the development of behavior.

REFERENCES

1. Altman, J., K. Sudarshan, G. D. Das, N. McCormick and D. Barnes. Influence of nutrition on neural behavioral development. III. Development of some motor, particularly locomotor patterns during infancy. *Dev Psychobiol* **4:** 97-114, 1971.
2. Anden, N-E., H. Corrodi, K. Fuxe, B. Hokfelt, T. Hokfelt, C. Rydin and T. Svensson. Evidence for a central noradrenaline receptor stimulation by clonidine. *Life Sci* **9:** 513-523, 1970.
3. Anden, N-E., M. Grabowska and U. Strombom. Different alpha-adrenoreceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. *Naunyn Schmiedebergs Arch Pharmacol* **292:** 43-52, 1976.
4. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 635-636, 1969.
5. Campbell, B. A. and P. D. Mabry. The role of catecholamines in behavioral arousal during ontogenesis. *Psychopharmacologia* **31:** 253-264, 1977.
6. Cedarbaum, J. M. and G. K. Aghajanian. Noradrenergic neurons of the locus coerulus: Inhibition by epinephrine and activation by the alpha-antagonist piperoxane. *Brain Res* **112:** 413, 1976.
7. Cedarbaum, J. M. and G. K. Aghajanian. Catecholamine receptors on locus coerulus neurons: Pharmacological characterization. *Eur J Pharmacol* **44:** 375-385, 1977.
8. Debassio, W. A. and T. L. Kemper. The effects of protein deprivation on neuronal migration in rats. *Dev Brain Res*, in press, 1984.
9. Delbarre, B. and H. Schmitt. Sedative effects of alpha-sympathomimetic drugs and their antagonism by adrenergic and cholinergic blocking drugs. *Eur J Pharmacol* **13:** 356-363, 1971.
10. Delbarre, B. and H. Schmitt. A further attempt to characterize sedative receptors activated by clonidine in chickens and mice. *Eur J Pharmacol* **22:** 355-359, 1973.
11. Drew, G. M., A. J. Gower and A. S. Marriott. Alpha$_2$-adrenoceptors mediate clonidine-induced sedation in the rat. *Br J Pharmacol* **67:** 133-141, 1979.
12. Frankhuyzen, A. L. and A. H. Mulder. Noradrenaline depolarization-induced ^{3}H-serotonin release from slices of rat hippocampus. *Eur J Pharmacol* **63:** 179-182, 1980.
13. Gothert, M. and H. Huth. Alpha-adrenoceptor-mediated modulation of 5-hydroxytryptamine release from rat brain cortex slices. *Naunyn Schmiedebergs Arch Pharmacol* **313:** 21-26, 1980.
14. Hartley, E. J. and P. Seeman. Development of receptors for dopamine and noradrenaline in rat brain. *Eur J Pharmacol* **91:** 391-397, 1983.
15. Kellogg, C. and P. Lundborg. Ontogenic variations in responses to L-DOPA and monoamine receptor-stimulating agents. *Psychopharmacologia* **23:** 187-200, 1972.
16. Kellogg, C. and G. Wennerstrom. An ontogenic study on the effect of catecholamine receptor stimulating agents on the turnover of noradrenaline and dopamine in the brain. *Brain Res* **79:** 451-464, 1974.
17. Linville, D. G., D. F. Emerich and L. P. Spear. Cholinergic inhibition of clonidine-induced wall-climbing in infant and preweanling rat pups. Paper presented at the meeting of the International Society for Developmental Psychobiology, Baltimore, MD, October, 1984.
18. Morgane, P. J., M. Miller, T. Kemper, W. C. Stern, W. Forbes, R. Hall, J. Bronzino, E. Kissane, E. Hawrylewicz and O. Resnick. The effects of protein malnutrition on the developing central nervous system in the rat. *Neurosci Biobehav Rev* **2:** 137-230, 1978.

19. Nomura, Y. and T. Segawa. The effects of alpha-adrenoceptor antagonists and metamide on clonidine-induced locomotor stimulation in the infant rat. *Br J Pharmacol* **66:** 531-535, 1979.
20. Nomura, Y., K. Oki and T. Segawa. Pharmacological characterization of central alpha-adrenoceptors which mediate clonidine-induced locomotor hypoactivity in the developing rat. *Naunyn Schmiedebergs Arch Pharmacol* **311:** 41-44, 1980.
21. Patel, A. Undernutrition and brain development. *Trends Neurosci* **6:** 151-154, 1983.
22. Perry, B. D., J. M. Stolk, G. Vantini, R. B. Guchart and D. C. U'Prichard. Strain differences in rat brain epinephrine synthesis: Regulation of alpha-adrenergic receptor number by epinephrine. *Science* **221:** 1297-1299, 1983.
23. Raiteri, M., G. Maura and P. Versace. Functional evidence for two stereochemically different alpha$_2$ adrenoceptors regulating central norepinephrine and serotonin release. *J Pharmacol Exp Ther* **224:** 679-684, 1983.
24. Reinstein, D. K. and R. L. Isaacson. Clonidine sensitivity in the developing rat. *Brain Res* **135:** 378-382, 1977.
25. Resnick, O., P. J. Morgane, R. Hasson and M. Miller. Overt and hidden models of chronic malnutrition in the rat and their relevance to man. *Neurosci Biobehav Rev* **6:** 55-57, 1982.
26. Roach, A. G., J. C. Doxey, D. A. Strachan and I. Cavero. Sleeping times evoked by alpha adrenoceptor agonists in two day-old chicks: An experimental model to evaluate full and partial agonists at central alpha$_2$ adrenoceptors. *J Pharmacol Exp Ther* **227:** 421-428, 1983.
27. Spear, L. P. The use of psychopharmacological procedures to analyze the ontogeny of learning and retention: Issues and concerns. In: *Ontogeny of Learning and Retention*, edited by N. Spear and B. Campbell. Hillsdale, NJ: Lawrence Erlbaum, 1979, pp. 135-156.
28. Spear, L. P. and J. Brick. Cocaine-induced behavior in the developing rat. *Behav Neural Biol* **26:** 401-415, 1979.
29. Starke, K. and H. Montel. Involvement of alpha-receptors in clonidine-induced inhibition of transmitter release from central monoamine neurones. *Neuropharmacology* **12:** 1073-1080, 1973.
30. Stern, W. C., M. Miller, W. B. Forbes, J. P. Leahy, P. J. Morgane and O. Resnick. Ontogeny of the levels of biogenic amines in various parts of the brain and in peripheral tissues in normal and protein malnourished rats. *Exp Neurol* **49:** 314-326, 1975.
31. Strombom, U. and T. H. Svensson. Clonidine: Attenuation of sedative action by facilitated central noradrenergic neurotransmission. *J Neural Trans* **47:** 29-39, 1980.
32. Svensson, T. H., B. S. Bunney and G. K. Aghajanian. Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res* **92:** 291-306, 1975.
33. Zebrowska-Lupina, I., E. Przegalinski, M. Sloniec and Z. Kleinrok. Clonidine-induced locomotor hyperactivity in rats. *Naunyn Schmiedebergs Arch Pharmacol* **297:** 227-231, 1977.