Pharmacology Biochemistry & Behavior, Vol. 11, pp. 493–498. Printed in the U.S.A.

Postnatal α-Methylphenylalanine Treatment Effects on Adult Mouse Locomotor Activity and Avoidance Learning

MARVIN W. LUTTGES AND RICHARD A. GERREN

Deparmtnet of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309

Received 26 March 1979

LUTTGES, M. W. AND R. A. GERREN. *Postnatal α-methylphenylalanine treatment effects on adult mouse locomotor activity and avoidance learning*. PHARMAC. BIOCHEM. BEHAV. 11(5) 493–498, 1979.—Neonatal mice were injected for five days with a combination of α-methylphenylalanine and phenylalanine to determine the influences of excess phenylalanine during development upon the behavior of these mice as adults. Spontaneous activity, bolus production, passive avoidance learning, simple active avoidance learning and complex active avoidance learning were tested in mice treated at two different postnatal periods. The results show that the treatments during development produced adult behavioral alterations compared to controls. The effects were most pronounced in mice treated in the postnatal period immediately after birth. The behavioral effects can be summarized as increased emotionality and generalized, stimulus-induced activity as well as decreased passive avoidance performance and complex active avoidance performance. These behavioral deficits are consistent with those usually reported in various models of human phenylketonuria.

α-Methylphenylalanine Phenylketonuria Phenylalanine Development Learning and memory deficits

MANY recent studies have demonstrated p-chlorophenylalanine (PCPA) often administered together with large doses of *l*-phenylalanine (PHE) early in neonatal development produces persistent behavioral deficits in the treated animals when tested as adults [3, 4, 19, 20]. These deficits are reminiscent of the behavioral consequences of phenylketonuria (PKU) in humans and have been taken as evidence that such treatments yield an induced model of PKU. A considerable amount of biochemical evidence seems to support the usefulness of such an animal model of human PKU [1, 5, 6, 8, 10, 11, 21].

Several recent studies have raised questions about the specificity of PCPA treatments in yielding a biochemical model of PKU [12, 13, 14, 15, 21, 22]. When contrasted to the newer analogue of PHE, α-methylphenylalanine (αMPHE), PCPA was observed to produce toxicity unrelated to direct effects upon phenylalanine hydroxylase inhibition. In particular, it was shown that unlike αMPHE, PCPA was capable of direct translational toxicity mediated through the disaggregation of brain polysomes. Unlike PCPA, αMPHE does not decrease serotonin levels. Also, αMPHE had been reported to produce no differences in weight gain of experimental versus control animals.

The biochemical specificity of αMPHE suggested that an animal model of PKU or hyperphenylalaninaemia might best be produced by this new PHE analogue. The following studies were undertaken to determine whether αMPHE and PHE administered together to neonatal mice, as used in biochemical experiments, would lead to behavioral alterations in such mice as adults. Spontaneous locomotor activity, passive avoidance learning and active avoidance learning tasks were used for testing purposes. In addition, several other ancillary measures were used.

METHOD

Ten litters of eight neonatal mice each were selected for use based on matched neonate weights and dates of birth. An outbred strain, HS, (cf., [2,7]) was used. Each litter was housed in a separate cage with the maternity-experienced female and all mice were allowed free access to food and water except during testing. During the course of the experiments some neonates died and in some cases equivalent losses were exacted randomly upon the paired litter to maintain comparable litter sizes between experimental and control groups.

Between 16–19 days of age all mice were sexed and litters were subdivided accordingly into separate male or female cages. At 60 days of age testing was initiated for all groups.

All neonates were weighed and injected (intraperitoneal, 30 ga needle) daily. Control animals received 10 ' cc volume of 0.15M NaCl per gram body weight and experimental animals received the same volume of solvent containing both αMPHE (4.3 mg/cc) and PHE (20 mg/cc). The final dose for the experimental mice was 2 gm/Kg PHE and 430 mg/Kg αMPHE daily. Although care was exercised to prevent the escape of injected solution from the peritoneal cavity of the neonates, small amounts of solution were occasionally lost. During daily injections a whole litter was removed briefly from the home cage and following all injections the whole litter was returned. This procedure minimized maternal disturbances and assured daily injections for each neonate.

Two separate age groups of neonates were used. One group received either control or experimental treatments beginning during the first neonatal day. The other received appropriate treatments beginning during the sixth postnatal day.

Regardless of treatment group, all subjects received the same comprehensive test schedule: 10 days of spontaneous locomotor activity testing, 3 days of passive avoidance testing using the step-through apparatus, 5 days of simple active avoidance testing using a wheel-turn apparatus and 15 days of discriminated, active avoidance testing using the same wheel-turn apparatus.

Spontaneous locomotor activity was evaluated [7] daily across 10 consecutive, 10 min test sessions in a small Plexiglas cage with nine separate metal plates serving as the floor. As the test animal bridges two plates, a counter circuit is activated such that the total number of plates traversed is counted for each 10 min test. These activity counts also were paralleled to an event recorder so that patterns of locomotor activity could be evaluated. The test cages were washed and dried between tests, thus it was possible to observe and count the bolus production for each animal during each test session. The order of daily testing followed a Latin square design so systematic effects due to test order or associated variables were eliminated.

The simple passive avoidance testing began four days after activity testing was completed (age, 74 days). The testing was conducted as described earlier [17]. A double-blind approach was used so that the experimenter did not know the treatment group assignments of any of the test mice. During training, each mouse was placed carefully on a small, highly illuminated, metal platform extending horizontally from a smooth, vertical wall. The mice could move from the platform into a darkened chamber via a small hole in the vertical wall. The floor of the chamber was one electrical lead and the platform, another of a constant current (5 mA) circuit. Thus, each mouse received a single, large shock when escaping from the platform to the darkened chamber. Initial step-through response latencies were recorded during training and then again during testing conducted first at 24 hours and then repeated seven days later.

Simple active avoidance training began six days after the completion of step-through testing (age, 89 days). The training apparatus and procedures were quite similar to those reported earlier [18]. Animals were placed individually into small Plexiglas test chambers with two frontmounted wheels and a bar grid floor. A sound (≈ 800 Hz, 85 dB (C)) from a top-mounted speaker signalled the beginning of a trial (the interval between trials was variable with a mean of 20 sec). Four seconds later, shock (scramble, 1.0 mA, constant current) was applied through the floor grids and was continued together with the sound until the mice turned either of the two wheels. During training the mice go from poorly directed escape behavior which involves attempts to get off the electrified grid floor by jumping to the top of the wheels to more directed avoidance behavior involving the rapid turning of the wheel prior to shock delivery. In this apparatus, the mice were allowed 50 daily trials. Both successful avoidances and total wheel turns were recorded for each of four test apparatuses which were programmed to run automatically. Testing was continued through five successive days.

Discriminated active avoidance testing started two weeks later (age, 108 days). The apparatus and training parameters were exactly the same as outlined above. The only difference in this task was the requirement that each of two different sounds be associated with the appropriate wheel (left or right). A 400 Hz (85 dB(C)) signal indicated the selection of the right wheel while a 1000 Hz (90 dB(C)) sound indicated the selection of the left wheel. Turning the wrong wheel did not terminate the trial. Using 50 daily trials, these tests were continued for 15 consecutive days.

Test performance evaluation was done a posteriori using analyses of variance. Individual comparisons were done subsequently, where justified, using the student t test. All p values were determined for two-tailed tests.

RESULTS

During daily injection of either αMPHE - PHE or saline solutions, most neonates continued to show body weight gains. However, the αMPHE - PHE mice always exhibited significantly ($p<0.001$) slower weight gains than saline animals. These observations were the same whether injections began at one or six days of age. Typical (one control and one experimental litter) comparisons of daily weight gain are shown in Fig. 1. Where αMPHE - PHE neonates died and comparable numbers of saline neonates were removed randomly from matched litters, the difference between control and experimental neonate weight gains was not as significant ($p<0.01$) as when litter sizes where allowed to vary. Inspection of the data showed that the lightest mice died in αMPHE -PHE litters, whereas the random selection of neonates from control litters resulted in various sizes of individuals, sometimes including the largest of the litters, being discarded. This systematic bias tended to keep the average individual neonate weights of αMPHE -PHE litters closer to the average weights of saline mice than might otherwise have been the case. Overall, the mortality levels for αMPHE -PHE were approximately 11% and for saline 3%. There was no significant difference in the mortality rates of male versus female neonates.

At the time of testing as adult mice there was no significant difference between the weights of the mice in experimental compared to control groups.

Activity Tests and Bolus Counts

Throughout the ten consecutive days of spontaneous locomotor activity testing and the measurement of bolus production, no significant trend developed. Accordingly, the data for each group were analysed without regard to days. A summary of average daily activity and bolus measures for each of the four test groups is provided in Fig. 2.

Activity measures of αMPHE -PHE mice whether injected during neonatal days 1–5 or 6–10 did not differ significantly from those of saline mice. On the average each mouse traversed approximately 200 to 225 floor recording plates during each daily 10 min test. Each mouse exhibited very consistent levels of activity across test days. When all of the mice, regardless of treatment, injected during neonatal days 1–5 are compared to those injected on days 6–10, there is a significant ($p<0.05$) difference in spontaneous locomoter activity. The mice receiving injections on postnatal days 1–5 exhibited more overall activity than those injected on days 6–10.

The bolus counts indicated significant differences between saline and αMPHE -PHE mice for both injection periods. The 1–5 day injection mice showed significantly ($p<0.005$) higher bolus counts than mice injected during the 6–10 day period, regardless of treatment. Within the 1–5 day

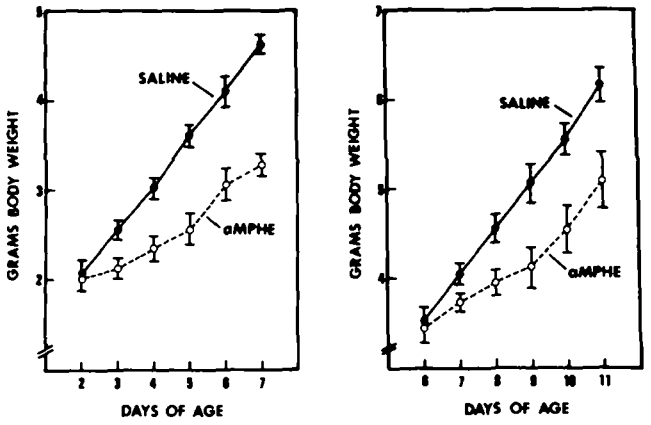

FIG. 1. Daily average body weights for saline and αMPHE-PHE injected mice. In these randomly selected, weight matched litters the injections were started on day 2 and day 6, respectively, and were continued through 6 daily injections. All mice received 10^{-1} cc/gram body weight of solution injected intraperitoneally. Eight neonates per litter. No deaths occurred in either litter throughout treatment. Vertical bars are standard error of mean (SEM).

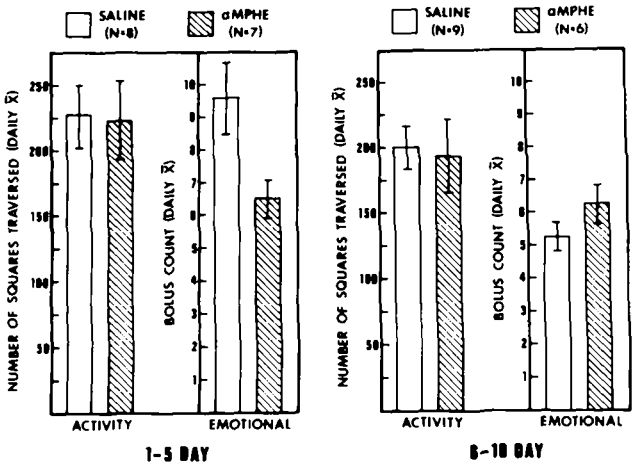

FIG. 2. Daily average amounts of spontaneous locomotor activity and numbers of boluses produced in saline and αMPHE-PHE injected mice across 10 successive test days. Activity counts were determined during 10 min test sessions and bolus counts were obtained from each test chamber immediately following testing and prior to apparatus washes. Vertical bars are SEM.

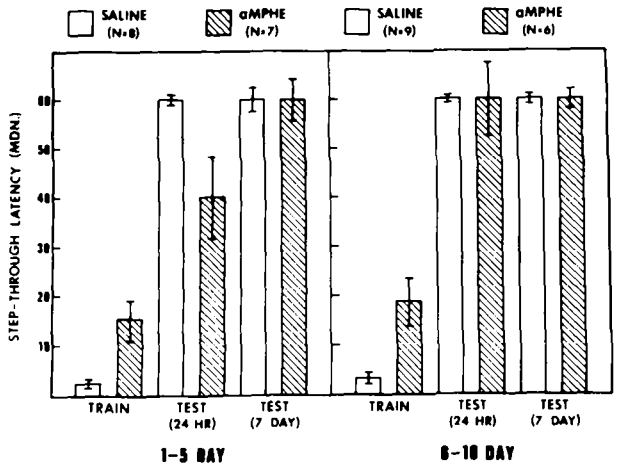

FIG. 3. Performance (median step-through latencies) of saline (clear bars) and αMPHE-PHE (stipled bars) injected mice during single trial passive avoidance training and testing. A 60 sec criterion was used to terminate each trial and to establish complete passive avoidance performance in these tests. Vertical bars are SEM.

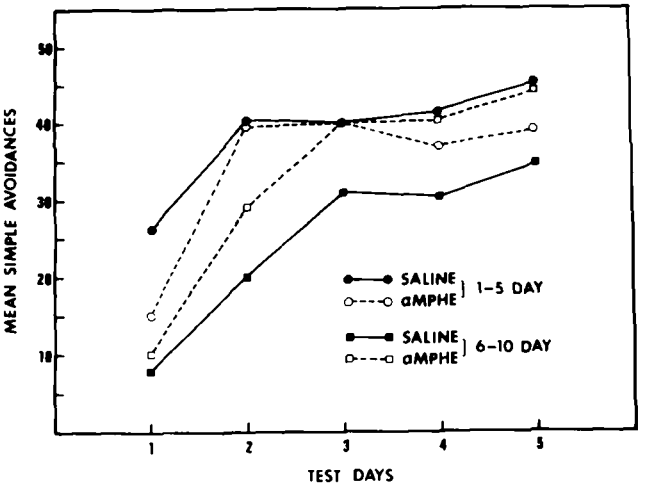

FIG. 4. Average simple avoidance scores for saline and αMPHE-PHE injected mice during simple active avoidance testing in the wheel-turn apparatus. Each mouse was given 50 daily trials and the testing continued for 5 successive days. The performance of mice receiving early postnatal injections (1 day) are plotted separately from those receiving later postnatal injections (6 day).

injection groups, the αMPHE - PHE mice exhibited approximately 40% less bolus production ($p<0.001$) than the saline mice. The treatments have opposite effects in 6–10 day injection animals where the saline mice produce approximately 20% fewer ($p<0.005$) boluses than the αMPHE - PHE mice during the 10 min daily tests.

The results of the step-through task evaluations are shown in Fig. 3. On the initial training trial, the αMPHE - PHE mice showed longer ($p<0.0001$) response latences than saline mice, regardless of when postnatal injections were given. When retested 24 hr later, all mice showed significant ($p<0.001$) increases in response latencies except the αMPHE - PHE mice injected on days 1–5 during postnatal development ($p<0.10$). Since the mice which did not show complete passive avoidance (latencies $\geq$ 60 sec) received a second shock when stepping inside the darkened hole, they effectively received a second trial. The mice attaining the full 60 sec criterion received no further footshock or additional training.

When tested for retention 7 days later, all mice exhibited the 60 sec criterion for passive avoidance performance.

In the simple active avoidance task, mice injected on 1–5 postnatal days showed significantly ($p<0.01$) better avoidance learning than those injected on postnatal days 6–10. Summary learning curves for each of the four test groups are shown in Fig. 4. Whereas there is no demonstra-

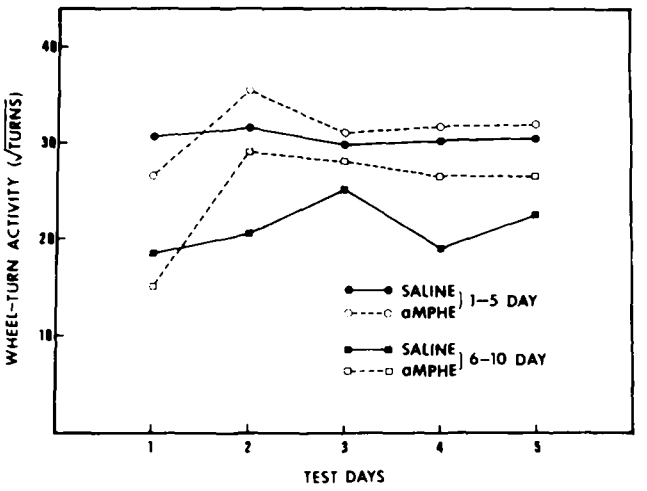

FIG. 5. Average total weel-turn activity (√turns) associated with the avoidance scores of Fig. 4. Total wheel-turn activity was recorded throughout the daily, 50 trial, 20 min test periods for each saline and γMPHE-PHE injected mouse.

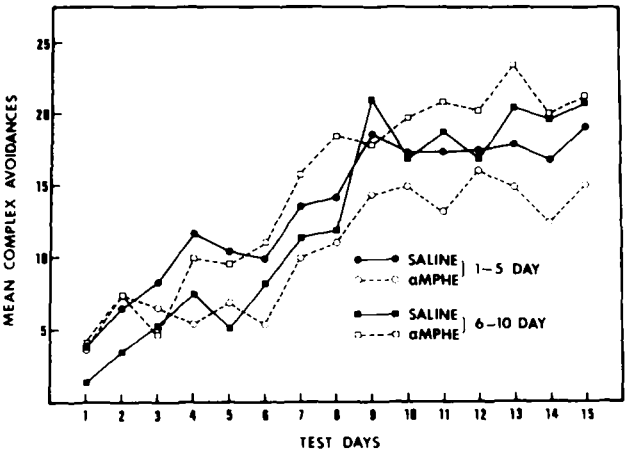

FIG. 6. Average discriminated active avoidance scores for saline and αMPHE-PHE mice for 50 trials of daily testing extended through 15 total test days. These complex avoidance scores are provided for both early and later postnatal injection groups.

ble significant difference ($p>0.05$) between αMPHE - PHE and saline mice injected on postnatal days 1–5, the learning rates are significantly ($p<0.001$) better for αMPHE - PHE mice injected on days 6–10 than for the saline mice injected during the same period. It should be noted that αMPHE-PHE versus saline test groups show widely varying avoidance scores on the first day of testing ($p< 0.05$). An examination of improved performance from the first to the second test day showed the αMPHE - PHE animals increase performance by approximately 20 avoidances compared to a 14 avoidance increment for saline mice during the same period.

Associated numbers of wheel-turns during the simple avoidance testing corresponds reasonably well with avoidance scores (Fig. 5). Mice injected on postnatal days 1–5 do not differ significantly ($p>0.10$) in daily numbers of wheel turns. Those mice injected on days 6–10 do differ significantly ($p<0.05$) with the αMPHE - PHE mice showing greater overall amounts of wheel-turn activity. When all αMPHE - PHE mice are compared to all saline mice regardless of period of injection, the αMPHE mice exhibit significantly ($p<0.001$) more wheel turn activity. The mean acitivity scores ($\sqrt{\text{wheel-turns}}$) show that the difference arises during the last four days of avoidance training (Fig. 5). Within the 6–10 day injection groups, the αMPHE - PHE mice show significantly ($p<0.02$) less first day wheel-turn activity than saline mice. The overall comparison of all 1–5 versus 6–10 day injection mice also shows that a significant ($p<0.01$) difference occurs, with the 1–5 day injection mice exhibiting more wheel-turn activity than 6–10 day injection mice.

Following a two week period without testing, the same groups of mice were returned to the wheel-turn tasks for discriminated active avoidance testing. Day by day plots of successful avoidances are presented in Fig. 6. Among the 1–5 day injection animals, those receiving αMPHE - PHE show significantly ($p<0.0001$) fewer avoidances than the saline mice. The effect is reversed for 6–10 day injection mice where the αMPHE - PHE mice exhibit more ($p<0.001$) avoidances than saline mice. The average performance of all groups over the 750 total trials indicates that task difficulty was pronounced but all groups exhibited significantly ($p<0.002$) improved performance from the beginning to end (1st compared to 15th trials) of training. Throughout these tests the mice which had received injections on days 1–5 exhibited considerably more ($p<0.001$) activity than those injected on days 6–10. In contrast to performance on simple avoidance, the more active mice treated in the early (1–5 day) postnatal period showed fewer ($p<0.001$) successful avoidances than the less active mice treated later (6–10 days).

DISCUSSION

During the injection of neonatal mice with αMPHE - PHE compared with saline, there is substantial evidence of αMPHE - PHE toxicity. All of the drug-treated mice show decreased weight gains during the course of injections. As adults, however, the mice previously treated with αMPHE - PHE do not differ in body weight from those which received saline during the same period of early development. Slight growth decreases are known to be characteristic of human PKU, whereas larger decreases had been associated with dietary restrictions during PKU treatment (cf. [10,11]). Growth deficits have been demonstrated in models of PKU [3, 4, 19, 20] including those induced with PCPA. Unlike reports of body weight decreases which are apparent in adult animals previously treated with both PCPA and PHE [19], the mice treated with αMPHE - PHE show body weights which are comparable to control mouse body weights. In fact, some investigators have not seen any effect of αMPHE upon even neonatal growth rates [9].

The remaining observations were made in adult mice. Spontaneous activity measures only revealed differences between those mice treated during postnatal days 1–5 and 6–10. The neonates treated later in development exhibited less locomotor activity. The treatment age difference persisted in the bolus production analysis where fewer boluses were produced during activity testing by the 6–10 day injection mice

compared to 1–5 day mice. Superimposed upon these effects were bolus decreases in 1–5 and increases in 6–10 day αMPHE - PHE injection animals, respectively, compared to saline controls. Since both activity and bolus measures have been used as indices of emotionality [7], these results suggest that the administration of αMPHE - PHE at various postnatal periods can lead to significantly more or less emotionality depending upon the exact treatment period.

On the passive avoidance task the initial step-through latency of the αMPHE - PHE groups were quite long compared to those of control mice. This initial freezing response occurred in both drug-treatment groups. On the second or test trial conducted 24 hr later, only the αMPHE-PHE group injected on postnatal days 1–5 failed to exhibit a significant improvement in passive avoidance performance. When allowed one additional trial of training (during the 24 hr test), these animals exhibited criterion level avoidance on the test conducted at seven days following the initial test. These results show the αMPHE - PHE treatments produce passive avoidance deficits in adult mice only when the treatments were administered early in neonatal developmemt. Neonates receiving identical treatments between 6–10 days of age did not show passive avoidance deficits as adults.

On the simple active avoidance task all groups, except those receiving saline on postnatal days 6–10, rapidly achieved simple avoidance performance while generating a considerable number of total wheel turns to do so. The 6–10 day, saline control group, however, showed the greatest response effectiveness with a relatively small number of wheel-turns used per successful avoidance. These results we interpret as consistent with αMPHE-PHE treatments and/or early injection experiences leading to stimulus-induced hyperactivity and emotionality. The same kind of performance in this simple task is induced in mice exposed to somewhat higher levels of footshock [18].

The discriminated active avoidance showed that αMPHE-PHE treatments delivered early in neonatal development produce learning deficits in adults. The same treatments given later in development have little effect other than to produce slightly increased amounts of stimulus-induced, wheel-turn activity.

All of the tests reveal that αMPHE-PHE treatments given to neonates can lead to changes in the behavior of these animals when tested as adults. The earlier the treatments are administered, the more pronounced the effects in adults. These observations are quite consistent with those reported earlier [19] using PCPA-PHE treatments administered for a slightly longer period during the postnatal development of rats.

Only one major exception to the existing reports was noted in the present studies, that is, the αMPHE-PHE treatments do lead to considerable developmental toxicity as revealed by lessened neonatal body weights. Greengard and her colleagues [9] showed that similar treatments did not result in changes in mouse body weight. This discrepancy is crucial to resolving the exact nature of the αMPHE - PHE effects. Because of the surprising differences in weight gain, future studies must include nutritionally-matched controls.

In the present studies it is important to recall that αMPHE does not produce marked changes in tryptophan hydroxylase activity and therefore does not yield large changes in serotonergic metabolites [6,16]. Also, the treatments with αMPHE were large enough to inhibit PHE-hydroxylase [9, 12, 21] but presumably do not result in a significant amount of polyribosome disaggregation [15,21]. The question, then of αMPHE -PHE treatments is whether this is a model of PKU and if so, are the residual effects upon mice when tested as adults a consequence of protein synthesis inhibition or perhaps some more subtle developmental consequence such as nutritional deficits.

The behavioral changes in adults produced by neonatal αMPHE-PHE treatments remain to be traced to specific causal factors. Both αMPHE and PHE treatments alone must be evaluated in a study using nutritional controls. Also, the αMPHE-PHE effects discerned in this study should be compared directly to effects produced by PCPA - PHE treatments during the same stages of development.

ACKNOWLEDGMENTS

The authors thank W. Bank and J. Button for their technical assistance. This work was supported, in part, by Public Health Service grant NS-12226 and NSF grant BNS 76-04507, and by the International Chiropractor's Association.

REFERENCES

1. Adesnik, M., M. Lande, T. Martin and D. D. Sabatini. Retention of mRNA on the endoplasmic reticulum membranes after *in vivo* disassembly of polysomes by an inhibitor of initiation. *J. Cell Biol.* **71**: 307–313, 1976
2. Andry, D. K. and M. W. Luttges. Drug-induced dissociation of evoked responses and performance in mice. *Behav. Biol.* **17**: 17–29, 1976
3. Butcher, R. E., C. Vorhees and H. Berry. A learning impairment associated with induced phenylketonuria. *Life Sci.* **9**: 1261–1268, 1970
4. Butcher, R. E., C. V. Vorhees, C. W. Kindt, L. J. Kazmaier-Novak and H. K. Berry. Induced PKU in rats: Effects of age and melatonin treatment. *Pharmac. Biochem. Behav.* **7(2)**: 129–133, 1977.
5. DelValle, J. A. and O. Greengard. The regulation of phenylalanine hydroxylase in rat tissues *in vivo*. The maintenance of high plasma phenylalanine concentrations in suckling rats: A model for phenylketonuria. *Biochem. J.* **154**: 613–618, 1976.
6. DelValle, J. A., G. Dienel and O. Greengard. Comparison of α-methylphenylalanine and p-chlorophenylalanine as inducers of chronic hyperphenylalaninaemia in devoloping rats. *Biochem. J.* **170**: 449–459, 1978.
7. Gerren, R. A., D. E. Groswald and M. W. Luttges. Triethyltin toxicity as a model for degenerative disorders. *Pharmac. Biochem. Behav.* **5**: 299–307, 1976.
8. Greengard, O. and J. A. DelValle. The regulation of phenylalanine hydroxylase in rat tissues *in vivo*.Substrate and cortisol-induced elevations in phenylalanine hydroxylase activity. *Biochem. J.* **154**: 619–624, 1976.
9. Greengard, O., M. S. Yoss and J. A. DelValle. α-Methylphenylalanine, a new inducer of chronic hyperphenylalaninemia in suckling rats. *Science* **192**: 1007–1008, 1976.
10. Holm, V. A. and W. E. Knox. Physical growth in phenylketonuria: 1. A retrospective study. *Pediatrics*, **63**: 694–699, 1979.

11. Holm, V. A., R. A. Kronmal, M. Williamson and A. F. Roche. Physical growth in phenylketonuria: 11. Growth of treated children in the PKU collaborative study from birth to 4 years of age. *Pediatrics* **63:** 700–707, 1979.
12. Hughes, J. V. and T. C. Johnson. The effects of hyperphenylalaninaemia on the concentrations of aminoacyl-transfer ribonucleic acid *in vivo*. A mechanism for the inhibition of neural protein synthesis by phenylalanine. *Biochem. J.* **162:** 527–537, 1977.
13. Hughes, J. V. and T. C. Johnson. Experimentally induced and natural recovery from the effects of phenylalanine on brain protein synthesis. *Biochem. Biophys. Acta* **517:** 473–485, 1978.
14. Hughes, J. V. and T. C. Johnson. Abnormal amino acid metabolism and brain protein synthesis during neural development. *Neurochem. Res.* **3:** 381–399, 1978.
15. Kelly, C.J. and T.C. Johnson. Effects of p-chlorophenylalanine and γ-methylphenylalanine on amino acid uptake and protein synthesis in mouse neuroblastoma cells. *Biochem. J.* **174:** 836–844, 1978.
16. Lasala, J. M. and C. J. Coscia. Accumulation of a tetrahydroisoquinoline in phenylketonuria. *Science* **203:** 283–284, 1979.
17. Luttges, M. W. and J. L. McGaugh. Permanence of retrograde amnesia produced by electroconvulsive shock. *Science* **156:** 408–410, 1967.
18. Luttges, M. W. and J. L. McGaugh. Facilitation of avoidance conditioning in mice by posttraining administration of bemegride. *Agents Actions* **2:** 118–121, 1971.
19. Schalock, R. L., W. J. Brown, J. H. Copenhauer and R. Gunter. Model phenylketonuria (PKU) in the albino rat: Behavioral, biochemical and neuroanatomical effects. *J. comp. physiol. Psychol.* **89:** 655–666, 1975.
20. Schalock, R. L. and F. D. Klopfer. Phenylketonuria: Enduring behavioral deficits in phenylketonuric rats. *Science* **155:** 1033–1035, 1967.
21. Taub, F. and T. C. Johnson. The mechanism of polyribosome disaggregation in brain tissue by phenylalanine. *Biochem. J.* **151:** 173–180, 1975.
22. Weck, P. K. and T. C. Johnson. The influence of brain cytosol on RNA synthesis and RNA products of isolated mouse brain nuclei. *Neurochem. Res.* **3:** 325–343, 1978.