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Open Field Behavior and Gross Motor Development in Offspring of Nursing Rat Mothers Given Penfluridol[1]

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AHLENIUS, S., J. ENGEL, E. HÅRD, K. LARSSON, P. LUNDBORG AND P. SINNERSTEDT. *Open field behavior and gross motor development in offspring of nursing rat mothers given penfluridol.* PHARMAC. BIOCHEM. BEHAV. 6(3) 343–347, 1977. – Nursing rat mothers were injected with penfluridol, a dopamine receptor blocking agent, at Day 1, 3, 5 and 7 after delivery, and their offspring were investigated at 6–18 days of age for various aspects of motor behavior development, and at 4, 8 and 12 weeks of age for open field behavior. No deficits were found in the development of locomotion, air righting, startle response and eye opening. Open field ambulation decreased from an abnormally high level at 4 weeks of age to an abnormally low level at 8 and 12 weeks of age. The ability to habituate to an open field decreased from a normal level at 4 weeks of age to an abnormally low level after 8 weeks of age. The behavior deficits were related to a decreased functional activity of the mesolimbic dopamine neuron system, and the possible relation to a clinical dysfunction among children – minimal brain dysfunction – was discussed.

Open field behavior Development Penfluridol Catecholamines

PHARMACOLOGICAL evidence indicates that central dopamine (DA) containing neurons in the rat are not mature at birth, but gradually develop their function during the first week postnatally [14,19], suggesting that the first postnatal week may be a vulnerable period for the functional development of central DA neurons. Treatment with the DA-receptor-antagonist penfluridol during this period caused a decreased synthesis and decreased release of DA in the mesolimbic DA neurons when measured at the age of 4 weeks [9,10]. This treatment also markedly impaired the acquisition of a conditioned avoidance response [1]. A similar treatment of rabbits with haloperidol, another catecholamine receptor blocking agent, produced gross behavior abnormalities in the early postnatal period [17].

The present studies were undertaken to further characterize the behavioral changes following neonatal penfluridol treatment. The learning deficits seen in our previous work may reflect an impaired motor development or an altered reactivity to environmental stimuli. Therefore, the gross motor development during the first weeks after birth were observed after treatment with penfluridol. Further, the open field behavior was analyzed following this treatment.

EXPERIMENT 1: GROSS MOTOR DEVELOPMENT

METHOD

Animals

The animals were 64 infant Sprague-Dawley rats of both sexes which were the offspring of 8 females obtained from Anticimex Breeding Co., Stockholm. At birth, infants of all litters born at the same day were mixed and randomly assigned to 8 litters, with each litter reduced to 8 infants. The litters were housed in individual cages with the floor covered with shavings, with access to nesting material, and with continuous access to food and water. They lived in air-conditioned colony rooms at a temperature of 24 ± 1°C and at an air humidity of 50–60%. The dark-light cycle was reversed with light out at 11.00–23.00. The observations were begun approximately 4 hr after onset of darkness.

Drug Treatment

The mothers of the newborn rats were randomly assigned to the two treatment conditions. The nursing mothers in 4 litters were given penfluridol (1 mg/kg) by gastric intubation on Day 1, 3, 5 and 7 after delivery (Day

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1: within 24 hr after birth). The nursing mothers of the other 4 litters received 5.5% glucose with a few drops of glacial acetic acid orally at the same intervals. Penfluridol was dissolved in a few drops of glacial acetic acid and the final volume was made up with 5.5% glucose. The pH and administration volume was kept constant at around 5.0 and 5 ml/kg, respectively.

Behavior Testing Procedure

Locomotion development. Between Days 6 and 13, daily 1-min tests were performed to study the gross motor behavior development. Immediately before the observation, the infant was taken out of the cage and placed on the rough side of a masonite board. No further stimulation was administered to the animal during testing. The experiments were performed under constant room temperature conditions (23°C). Several different response patterns were recorded and then grouped into three classes of response patterns in accordance with a previous study [11]. When showing at least one response pattern belonging to one of three classes, the animal was included in this class. The same animal could thus be included in more than one class. Table 1 lists the classes and response patterns used.

Air-righting. Observations of the ability for air-righting were performed between Days 11 and 18 according to a method described by Hård and Larsson [13]. The infant was dropped from 30 cm height upon a landing platform of cotton with a sheet of stretched black plastic. The experimenter kept his left forefinger and thumb grasped around the neck of the animal and the right hand grasped around the pelvis. The animal was not released until the observer felt that the rat was relaxed. The behavior was recorded as a complete air-righting response only when the rat landed on the table with all four feet simultaneously making contact with the floor. Testing was performed by two persons, one releasing the animal, and the other observing the behavior.

Startle response. Observations of the startle response were performed between Days 8 and 13. After the infant had relaxed and did not move, it was exposed to a sharp sound produced by knocking a glass bottle with an iron bar.

Eye-opening. From Day 12 the rats were examined for eye-opening which was defined as opening of both eyes.

RESULTS

Figure 1 shows the main results of this study. No statistically significant differences between the groups were found in the gross motor development with the exception

TABLE 1

VARIOUS RESPONSE PATTERNS OBSERVED IN EACH TEST AND THE CLASSES IN WHICH THEY WERE ORDERED

Class of Response Patterns		Response Pattern	
I.	Movements of head and/or forelegs	1.	Unilateral head movement without immediate return
		2.	Unilateral head movement with immediate return
		3.	Bilateral head movement
		4.	Simultaneous movement of the head and one or both of the forelegs
II.	Movements of head trunk and all legs	5.	Simultaneous movement of the head, one or both of the forelegs and one or both of the hindlegs
		6.	Pivoting <360°
		7.	Creeping. Forward progression with abdomen in contact with the supporting surface
		8.	Pivoting>360°
III.	Walking	9.	Alternating creeping and walking. Forward progression accomplished by a continuous series of alternating creeping and walking responses, where the walking phase does not include more than two steps
		10.	Walking. Forward progression with head and abdomen lifted from the supporting surface.

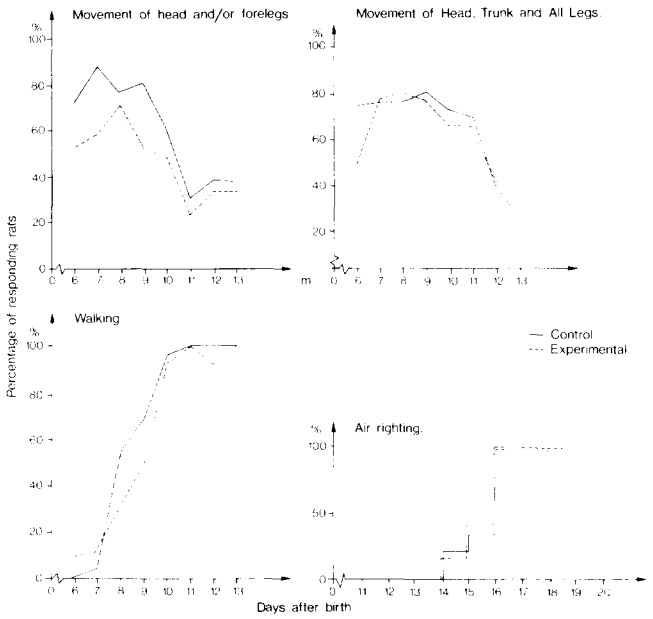

FIG. 1. Main trends in the development of gross motor behavior and air-righting response in offspring of nursing rat mothers treated with penfluridol experimental compared to controls. Group comparisons did not reveal any statistically significant differences except for movements of head and/or forelegs, which showed a difference (χ^2-test, $p<0.05$) at Day 7. The curves represent percentage of animals showing various classes of response patterns.

for movements of head/or forelegs, which was delayed at Day 7 ($p<0.05$, χ^2-test). Neither were any statistically significant differences found in eye-opening and startle response. No statistically significant differences were observed in litter mortality or body weight between offspring of mothers given penfluridol and those of mothers given glucose.

CONCLUSION

Although marked deficits have been produced in the functional development of the dopaminergic system with the drug treatment used in this study (see Introduction), no notable disturbances were found in the gross motor development. The only exception was a delay of the appearance of movements of head and/or forelegs which may have been directly caused by the drug injected on Day 7.

EXPERIMENT 2: OPEN FIELD BEHAVIOR

METHOD

Animals

Seventeen male rats which were offspring of 4 Sprague-Dawley rats were used in this experiment. The nursing mothers of 2 litters were given penfluridol (1 mg/kg) according to the method described in Experiment 1, while the mothers of other litters were given glucose. The animals were weaned at 28 days and were kept under the same conditions as in Experiment 1.

Apparatus and Testing Procedure

The animals were placed in a circular area (0.48 m^2), surrounded by a 75 cm high wall and divided into 13 sub-areas of approximately equal size. A 60 W light was suspended directly over the centre of the field, 90 cm from the floor. The rats were observed at three 3-min sessions, each separated by an interval of approximately 30 min. The locomotor activity was recorded once a minute by counting the number of times an animal moved with all four legs into a new subarea. The number of boluses left in the arena were counted after each test. The offspring were observed at the age of 4, 8 and 12 weeks. All observations were performed in an isolated room approximately 4 hours after onset of darkness.

RESULTS

Figure 2 shows the main results of this study. At 4 weeks of age, the penfluridol treated animals displayed a higher locomotor activity than the controls ($p<0.002$; Mann Whitney U-test). At 8 and 12 weeks of age, when the activity of the control rats had increased markedly in comparison to the activity displayed at 4 weeks of age, the penfluridol treated rats showed a decrease to a level significantly lower than that of the controls ($p<0.002$; Mann-Whitney U-test).

Although the differences failed to reach statistical significance, the trend of the boluses score showed a picture inverse to that of the ambulation score. The penfluridol treated rats deposited fewer boluses at 4 weeks of age than the controls while at 8 and 12 weeks of age, the experimental rats showed more defecation than did the controls.

Marked group differences were found in changes in locomotor activity occurring over the three sessions at the various age levels (Fig. 3). At 4 weeks, both the control and the penfluridol treated rats showed a systematic decrease in ambulation over the three sessions indicating intersession habituation (Page test of trend, $p<0.001$ in both groups, see 20). At 8 and 12 weeks, the control group showed a similar decrease in activity as at 4 weeks ($p<0.05$), whereas the penfluridol treated rats failed to show any sign of habituation, and the activity of the penfluridol treated group was as high in the first session as in the last.

DISCUSSION

Two major findings emerge from the present series of experiments with rats exposed to the DA receptor blocking agent, penfluridol, during early postnatal life: (1) the open field ambulation decreased from an abnormally high level at 4 weeks of age to an abnormally low level after 8 weeks of age whereas, in accordance with earlier reports, normal rats show increased open field ambulation with increasing age [6,17]; (2) the ability to habituate in an open field situation decreased from a normal (compared with the controls) level at 4 weeks of age to an abnormally low level at 8 weeks of age.

Defecation and ambulation in an open field has often been reported to be inversely related [5]. A similar result was obtained in both groups in Experiment 2. Low ambulation in an aversive open field situation combined with high defecation is traditionally thought to reflect increased emotionality or a lowered ability to adapt to novel stimuli [5]. The high activity and low defecation scores shown by the 4-week-old penfluridol treated rats thus suggest a lowered emotional reactivity in these animals. The relatively low reactivity to stimuli displayed by the 4-week old penfluridol treated rats may contribute

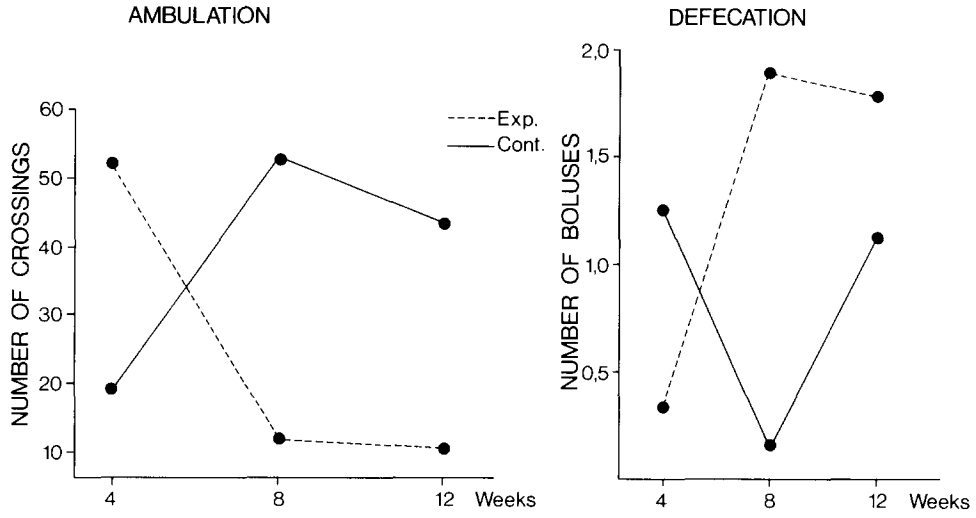

FIG. 2. Mean ambulation score and mean number of boluses in a 3-min open field test shown at 4, 8 and 12 weeks of age by offspring of nursing mothers treated with penfluridol (Exp.) compared to controls (Cont.). Group comparison revealed statistically significant differences in ambulation scores at all 3 age levels (Mann Whitney U-test, $p<0.002$). No statistically significant group differences were observed in defecation scores.

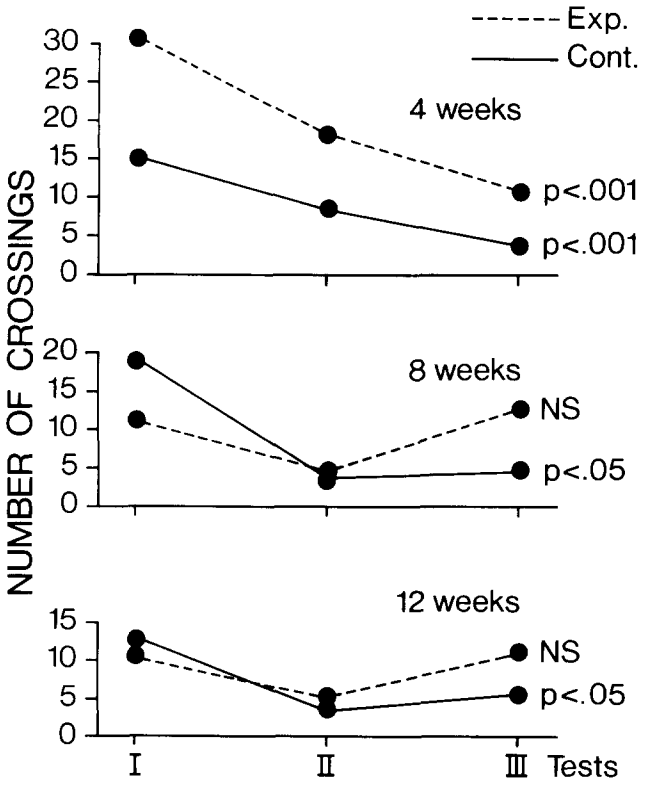

FIG. 3. Ambulation activity during the first minute in each of 3 consecutive 3-min open field tests in tests performed during one day at 4, 8 and 12 weeks of age, displayed by experimental and control rats. Indicated levels of statistical significance refers to results of the Page trend test.

to explain the impaired acquisition of an active avoidance response seen in our previous studies [1] since high reactivity is assumed to facilitate learning in a situation as here used [3]. The lowered ambulation, the increase defecation and the lost ability to habituate at 8 and 12 weeks of age indicate an abnormally heightened emotional reactivity with advancing age as a persistent consequence of the neonatal drug treatment.

In the control animals, ambulation in the open field increased from 4–8 weeks whereas in the penfluridol treated rats an inverse development was observed. This difference in the development of ambulation behavior suggests that the neonatal penfluridol treatment not only interfered with the development in the DA-neurons that functionally mature during the first postnatal week, but also can influence the development of neuronal processes immediately before and after the onset of puberty. In the strain of rats used in these experiments, puberty starts around 60 days of age [15]. The possibility of impaired neuroendocrine relationships warrant further investigation.

In contrast to the observed differences in open field behavior, no abnormalities were observed in the development of the gross motor behavior of the penfluridol treated rats. Thus it seems unlikely that the deficits in the acquisition of an active avoidance response displayed by the penfluridol treated rats [9], were due to impaired motor behavior. The present finding is also in line with our biochemical findings [9,10] indicating that the penfluridol treatment preferentially interferes with development of the mesolimbic DA system, leaving the nigrostriatal system intact. The latter system is generally assumed to be involved in the central control of motor behavior [4, 7, 12]. It should be noted that treatment with the DA-receptor blocking agent haloperidol to neonatal rabbits has been found to produce specific motor distrubances [18]. This discrepancy may be due to species differences in their sensitivity to early treatment with catecholamine-receptor blocking agents and/or that different drug regimens were used.

Since no cross fostering can be performed in the present type of experiments, the possibility cannot be excluded that the behavior deviations observed were due to a change

in the interaction between mother and offspring. However, as assessed by gross observation, penfluridol treated and glucose treated mothers did not differ in their maternal behavior as evidenced by nestbuilding, nursing, and retrieval behavior. Further, no differences were observed in mortality and body weight of the offspring.

The neonatal penfluridol treatment thus produces a dysfunction in mesolimbic DA-neurons and hyperactive behavior at 4 weeks of age in an aversive open field test. Neonatal treatment of rats with 6-OH dopamine (6-OH DA) which caused a persistent damage in central DA-neurons has also recently been shown to produce hyperactivity [21]. Whether the hyperactivity observed is due to supersensitive postsynaptic mechanisms or damage in inhibitory DA-neurons remains to be clarified. With regard to the latter possibility it is interesting to note that in adult rats lesions in the mesencephalo-cortico-limbic dopaminergic A-10 group produced by 6-OH DA has been found to induce hyperactivity [16]. Thus the existence of DA-neurons exerting an inhibitory influence on locomotor activity is suggested.

Summarizing these and other data from this laboratory, we have found that neonatal penfluridol treatment produces: (1) deficits in the acquisition of an active avoidance response; (2) an increased locomotor activity prepuberally followed by an abnormally decreased activity postpuberally, and (3) an impaired ability to habituate to novel stimuli. Associated with these behavior changes we have found a decreased functional activity in the mesolimbic DA system at 4 weeks of age (prepuberal age). In support of the assumption that this behavioral syndrome is related to a dysfunction of the central dopamine system, we have found that the learning deficits could be counteracted by increasing the functional activity of the catecholamine neurons by means of amphetamine [2].

The pattern of behavioral changes produced by the penfluridol treatment resembles a human clinical syndrome, Minimal Brain Dysfunction (MBD). This behavior disorder is characterized by hyperactivity, short attention span and a variety of cognitive and emotional symptoms. The fact that this disorder is successfully treated with amphetamine [22] and the finding of lowered levels of homovanillic acid in cerebrospinal fluid of children with MBD [21] indicate that central catecholamines may be involved in the MBD syndrome.

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