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EFFECT OF "ENRICHED ENVIRONMENT" DURING DEVELOPMENT ON ADULT RAT BEHAVIOR AND RESPONSE TO THE DOPAMINE RECEPTOR AGONIST APOMORPHINE

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Abstract—Enriched housing conditions (enriched environment, EE) during development has been shown to influence adult rat behavior and transmitter systems, especially dopamine function. We were interested in how different degrees of enrichment during development would affect adult rats' behavior and response to dopamine receptor challenge.

Two groups of male Wistar rats (n=11–12) were raised under two different degrees of EE, i.e. "high enriched" and "low enriched" groups. A third group was kept under standard conditions and served as "non-enriched" control. As adults, rats were tested for anxiety (elevated plus-maze), for spatial learning (four-arm-baited eight-arm radial maze), and for motivation (breakpoint of the progressive ratio test). Finally, locomotor activity (activity box) and sensorimotor gating (prepulse inhibition (PPI) of the acoustic startle response (ASR)) were tested with and without challenge with the dopamine receptor agonist apomorphine.

The time spent on the open or enclosed arms of the elevated plus-maze did not differ between groups, but the high enriched group showed higher rearing activity on the open arms. The breakpoint did not differ between groups. Learning and memory in the radial maze task only differed on the first few trials, but high enriched rats run faster compared with the other groups. In contrast, in the activity box enriched groups were less active, but apomorphine had the highest effect. Between groups, no difference in PPI and startle amplitude was found, but in the high and low EE group startle amplitude was enhanced after administration of apomorphine, while the PPI deficit induced by this drug was not different between groups.

Altogether, we found no evidence that different amounts of environmental enrichment without differences in social EE affect rats' cognitive, emotional or motivational behavior. However, motor activity seems to be enhanced when rats are behaviorally or pharmacologically challenged by dopamine receptor agonists.

Key words: learning and memory, motivation, emotion, motor activity.

Enriched environment (EE) has been shown to affect rat behavior, with improved learning and memory being the most consistent finding, together with enhanced markers for synaptic plasticity and long term potentiation (Leggio et al., 2005; Rönnbäck et al., 2005). Additionally, EE has been shown to affect neurotransmitter systems, especially mesocorticolimbic DA function (Leggio et al., 2005; Neugebauer et al., 2004), which is accompanied by altered response to pharmacological manipulation (Brillaud et al., 2005). Environmental manipulation has also been used to study the biological mechanisms underlying behavior, to model symptoms of human neuropsychiatric disorders, or to restore the effect of experimentally induced behavioral deficits.

In general EE describes a combination of social interaction (single or group housing) and equipment of the cage (i.e. space, toys, climbing and nesting material), two features that are often intermingled. Recent work that compared different enrichment conditions has shown both similar and opposite effects of environmental and social enrichment on rat behavior and neurobiological changes, especially with respect to social isolation, suggesting that these conditions influence different neuronal networks. However, a comparison between these studies is difficult, because animals raised under enrichment conditions are usually housed in groups and get toys, whereas the animals of the control group are often single-housed (Elliot and Grunberg, 2005). Moreover, the degree of climbing and nest materials, opportunities to take cover and toys differ among the studies (Gardner et al., 1975; Kempermann et al., 1997; Paban et al., 2005), and the components used for enrichment were rearranged or exchanged in different time intervals or not at all (Gardner et al., 1975; Leggio et al., 2005; Paban et al., 2005).

Additionally, EE has increasingly been used to improve the well-being of laboratory animals. However, since enrichment has been shown to affect rats' behavior and the dopamine transmitter system, there are concerns about using EE for this purpose, especially when using EE for models of neuropsychiatric disorders that are often based on experimentally induced manipulation of the dopamine transmitter system. However, to our knowledge, the behavioral outcome of different amount of physical enrichment on rat behavior and response to pharmacological manipulation of the dopamine system has not been investigated so far.

Our working hypothesis was that different amount of enrichment may differentially affect dopamine-related behavior and response to dopamine treatment. To test this

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Abbreviations: ANOVA, analysis of variance; ASR, acoustic startle response; CRF, continuous schedule of reinforcement; EE, enriched environment; FR, fixed ratio; PND, postnatal day; PPI, prepulse inhibition; PR, progressive ratio; RME, reference memory error; SPL, sound pressure level; WME, working memory error.

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hypothesis rats were raised under different conditions of enrichment. As adults, they were tested in different behavioral paradigms often used for testing of neuropsychiatric animal models, i.e. anxiety (elevated plus-maze), learning and memory (four-arm baited eight-arm radial maze task), and on motivation (progressive ratio (PR) test of operant behavior), which largely depend on dopamine function. To test whether EE sensitizes the dopamine transmitter system, we additionally tested these rats for locomotor activity (activity box) and sensorimotor gating (prepulse inhibition (PPI) of the acoustic startle response (ASR)) after treatment with the dopamine receptor agonist apomorphine.

EXPERIMENTAL PROCEDURES

Subjects

A total of 34 male Wistar rats (offspring of three parents from Harlan-Winkelmann, Borchen, Germany) were used in this study. The day of birth was designated as postnatal day (PND) 0. After weaning on PND 21 the rats were randomly divided into three different groups: *non*-enriched, *low* enriched and *high* enriched groups.

Animals were kept under controlled ambient conditions (22 °C, 12-h light/dark cycle, lights on at 7:00 a.m.). They had free access to tap water and were fed *ad libitum* until reaching a body weight of approximately 180 g at the age of PND 50. Thereafter, a restricted diet of 12 g standard laboratory chow (Altromin GmbH & Co. KG, Lage, Germany) per day was given to each rat keeping the animal's body weight on approximately 85% of the free-feeding weight throughout the whole testing period. The amount of food was given in a bulk to the group-housed rats. Notably, in all cages the racks for the food pellets were large enough for all rats to feed at the same time. However, to ascertain that each rat got its allocated share, the rat's body weight was controlled once a week.

In order to keep the experimenter's influence as low as possible, rats were not handled before starting with the experiments except for weighing and gently transferring them once a week to a fresh cage by grabbing them around their chest. Handling of the rats during behavioral testing was similar, i.e. they were merely handled as necessary for the given paradigm. Behavioral studies were conducted during the light period between noon and 6 p.m. During the light cycle a softly playing radio was used to provide a continuous background noise and to minimize the disturbing effects of sudden noise at the animal facilities and experimental rooms.

All experiments were done in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the local ethical committee. All efforts were made to minimize the number of animals used and their suffering.

Housing conditions

Three groups of male Wistar rats ($n=11–12$) were raised and maintained under different EE: (1) The high enriched rats were housed in groups of 11 in a voluminous cage made of a metal frame with wire mesh covers (height: 60 cm, width: 100 cm, depth: 60 cm), which was provided with climbing material (ramps, ropes, a second elevated level), opportunities to take cover (houses, tubes, buckets), different toys and nest materials (cellulose paper). Three different sets of equipment were used with three different materials (wood, plastics and metal). Once a week the current equipment was replaced with elements of another material. Additionally, the elements were rearranged twice a week. (2) The low enriched rats were kept in groups of six in standard cages of Macrolon® type IV (Tecniplast, Hohenpeiβenberg, Germany) that were provided with one shelter, nest material and one or two pieces of the equipment used for the high enriched group. The equipment was replaced once a week, similar to the high enriched group. (3) The non-enriched rats were housed in groups of five to six in standard cages of Macrolon® type IV without any enrichment.

Experimental design

From PND 70 on rats were behaviorally tested for anxiety (elevated plus-maze), for spatial learning and memory (four-arm baited eight-arm radial maze) and for motivation (operant behavior system). Finally, locomotor activity and PPI of the ASR were tested with and without challenge with the dopamine receptor agonist apomorphine.

Elevated plus-maze

Anxiety was tested in the "elevated plus-maze", which was made from black plastic. It consisted of four arms, two open arms (76×12 cm), and two arms (76×12 cm) that were enclosed with walls 26.7 cm high. The four arms were crosswise connected at a central platform with the two open arms opposite to each other. The maze was elevated to a height of 76 cm. For testing, the rat was placed onto the central platform, facing an open arm. During the next 10 min (1) the total number of entries, as well as the number of entries into open and enclosed arms, (2) the time spent in both types of arms, (3) the total number of rearings, i.e. rising on the hind limbs, as well as the number of rearings in both types of arms, and (4) the number of head dippings was recorded. An entry into an arm was counted when both hind paws of the rat were placed into the respective arm. The experimenter monitored the movements of the rat via a video camera mounted above the maze and a TV-screen outside the experimental room. The alleys of the maze were cleaned with a mild disinfectant between the tests.

PR test of operant behavior

For the PR test, rats were trained and tested in standard operant conditioning chambers (29.5×28.5×23.5 cm; Coulburn Instruments; Modular Test Cage System US Pat. 3830201) that were located in sound-attenuated cubicles (62×45×57 cm). The walls and the ceiling were made from metal, the front door from Plexiglas. Each chamber had two levers and a pellet dispenser in between that delivered food pellets to a food cup. First, all animals were accustomed to the test chamber, the taste of the food reward (45 mg casein pellets, Dustless Precision Pellets, BioServ, Frenchtown, NJ, USA), and the noise of the pellet dispenser. After this accommodation, the rats were trained for lever pressing under a continuous schedule of reinforcement (CRF, fixed ratio [FR] 1), where the rat had to press the right lever once to receive one food pellet. The left lever was inactive. The rats were trained until they received 200 pellets within 30 min on two consecutive days.

After completing FR training, rats were placed on a PR-schedule of reinforcement whereby successive reinforcement could be earned according to the following number of lever presses: from 1 to 10, one additional lever press per ratio (i.e. 1, 2, 3, . . . , 10), from 10 to 20, two additional lever presses per ratio (i.e. 10, 12, 14, . . . , 20) and so forth. The "breakpoint" was defined as the first PR ratio where an animal did not respond for 5 min. Additionally, the number of lever presses per 10 min intervals was evaluated.

Four-arm baited eight-arm radial maze task

The radial eight-arm maze was constructed of black plastic with a central platform (33.5 cm diameter) and eight arms (each 76 cm long and 12 cm wide provided with a 1.5 cm rim) projecting at equal angles (45°) from the platform. The maze was elevated to a