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EARLY AMYGDALA DAMAGE DISRUPTS PERFORMANCE ON MEDIAL PREFRONTAL CORTEX-RELATED TASKS BUT SPARES SPATIAL LEARNING AND MEMORY IN THE RAT

L. DIERGAARDE,* M. A. F. M. GERRITS, J. P. W. BROUWERS AND J. M. VAN REE

Rudolf Magnus Institute of Neuroscience, Department of Pharmacology and Anatomy, University Medical Center Utrecht, P.O. Box 80040, 3508 TA Utrecht, The Netherlands

Abstract—Recent studies have demonstrated that the postnatal development of connections between the basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) mature around postnatal days 13–15 (pd13–15), whereas these between the BLA and other structures such as the nucleus accumbens and the mediodorsal thalamus are completed by pd7. Accordingly, it is hypothesized that mPFC cytoarchitecture and hence its function may be specifically affected by neonatal (i.e. on pd7) but not later induced (i.e. on pd21) damage to the BLA. To test this hypothesis, rats received excitotoxic lesions to the BLA on either pd7 or pd21 and were subjected to two tests putatively sensitive to mPFC dysfunction, namely food hoarding and spontaneous alternation. In addition, rats were tested for spatial learning and memory, to determine any possible effects on hippocampal function. Consistent with the documented effects of mPFC lesions, pd7 damage to the BLA impaired spontaneous alternation and food hoarding performance, an effect that was not found in rats with BLA lesions induced on pd21. Spatial learning and memory, however, were not affected by the (neonatal) lesion procedure. Together, these results indicate that neonatal BLA damage affects species-specific sequential behavior and flexibility, which may be attributed to abnormal functioning of the mPFC.

Key words: amygdala, neonatal lesion, rat, neurodevelopmental disorders.

Excitotoxic lesions of the basolateral amygdala (BLA) on postnatal day 7 (pd7) are associated with certain behavioral abnormalities, which are absent in rats lesioned in the amygdala on postnatal day 21 (pd21). For example, alterations in exploratory activity, sensorimotor gating and stress reactivity were demonstrated in rats with neonatal amygdala lesions (Wolterink et al., 2001; Daenen et al., 2001, 2003; Terpstra et al., 2003). Since such effects are absent in animals with later amygdala damage, it was concluded that neonatal amygdala damage interferes with the normal development of connected structures, one of which is the medial prefrontal cortex (rodent homologue of the dorsolateral prefrontal cortex in primates; Uylings and van Eden, 1990). The medial prefrontal cortex (mPFC)—but also the mediodorsal thalamus (MD) and the nucleus accumbens (NAC)—is reciprocally connected with the BLA (Gray, 1999). Shortly after birth, BLA-derived fibers can already be found in these structures (Verwer et al., 1996). Connections between the BLA and the MD and the NAC remain relatively unchanged after pd7, whereas connections between the BLA and the mPFC mature around pd13–15 (Bouwmeester et al., 2002a,b; Verwer et al., 1996). A recent study has shown that BLA damage induced on pd7 results in increased mPFC dopamine metabolism in the adult rat (Bouwmeester et al., 2002a). Furthermore, neonatally BLA-lesioned rats display increased locomotor activity in a novel open field, as was also observed upon pharmacological manipulation of the mPFC (Wolterink et al., 2001; Bubser and Schmidt, 1990; Broersen et al., 1999). Together, these data suggest that lesioning the BLA early in life could interact with the normal maturation of the mPFC, and consequently results in abnormal functioning.

The present study further tested the hypothesis that neonatal amygdala lesions produce mPFC dysfunction. For this purpose, we assessed the effects of neonatal BLA damage on two tasks known to be sensitive to mPFC dysfunction. In the rat, the mPFC has been shown to play a key role in several aspects of executive function, such as planning, behavioral (cognitive) flexibility, response inhibition, and working memory (Kolb, 1984; Tanji and Hoshi, 2001). The tasks employed were food hoarding and spontaneous alternation, tasks that tap behavioral sequencing and behavioral flexibility, respectively. Food hoarding is a species-specific behavior that has a distinct sequence. Normal rats vigorously hoard food. When the mPFC is damaged animals are, however, no longer able to organize the various behavioral elements of hoarding over time (reviews: De Brabander et al., 1991; Nonneman and Corwin, 1981; Lacroix et al., 1998).

The spontaneous alternation task measures behavioral flexibility. Behavioral flexibility in rodents and other mammals is defined as the ability to switch strategy under changing environmental conditions. Spontaneous alternation performance is significantly impaired in mPFC-

*Correspondence to and present address: L. Diergaarde, Research Institute Neurosciences Vrije Universiteit, Drug Abuse Program, Center for Neurogenomics and Cognitive Research, Department of Medical Pharmacology, VU Medical Center, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands. Tel: +31-20-444-8101; fax: +31-20-444-8100.
E-mail address: l.diergaarde@vumc.nl (L. Diergaarde).

Abbreviations: AMX, amygdala lesion; ANOVA, analysis of variance; AP, anterior–posterior; BLA, basolateral nucleus of the amygdala; DV, dorsal–ventral; HPC, hippocampus; MD, mediodorsal thalamus; ML, medial–lateral; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; PBS, phosphate-buffered saline; pd, postnatal day; SHAM, sham lesion; vmPFC, ventral medial prefrontal cortex.

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lesioned rats, as they demonstrate an increased number of perseverative errors (Delatour and Gisquet-Verrier, 1996; Divac et al., 1975). Spontaneous alternation in a T-maze is also impaired after hippocampus (HPC) damage; therefore, we also determined the effects of neonatal BLA lesions on spatial learning abilities (review: Lalonde et al., 2002).

Spatial learning and memory capacities were studied using a water maze task, with an invisible platform, including a spatial reversal and a visually cued version of the task. Maze learning by means of spatial cues has been found to be reduced in rats with damage to the HPC, whereas in rats with sustaining mPFC lesions spatial learning is spared (Silva et al., 1998; De Bruin et al., 1994). However, when mPFC-lesioned animals are confronted with a spatial reversal, these animals display certain transient deficits in finding the new platform location (De Bruin et al., 1994; Lacroix et al., 2002). Similar deficits are found when animals have to switch from a spatial strategy to a visually cued task, which may be attributed to a diminished ability to shift to another strategy (De Bruin et al., 1994). The purpose of this task was two-fold: (1) verify that HPC-mediated spatial learning and memory is spared in neonatally BLA-lesioned rats and (2) determine whether lesioned animals can flexibly switch between strategies.

EXPERIMENTAL PROCEDURES

Subjects

Pregnant female Wistar rats (Harlan, CPB, Horst, The Netherlands) arrived at the laboratory at day 16 of gestation and were housed individually in macrolon cages measuring 40×26×20 cm. They had free access to tap water and standard rat chow. The animals were housed in a temperature- and humidity-controlled room under normal light conditions (lights on between 7:00 AM and 7:00 PM). One day after birth, litters were reduced to nine pups. If a litter consisted of less than nine males, the litter was filled up with females. Females were removed after weaning. One group of male pups was operated on pd7, and weaned on pd21. Another group of rats was operated on pd21 and weaned immediately after surgery. Sham-operated and lesioned animals were randomly distributed over the cages. All procedures were in accordance with international guidelines on the ethical use of animals, and were approved by the Ethical Committee on Animal Experiments of the Utrecht University. The number of animals used, as well as their suffering, was kept to the minimum possible needed to accomplish the goals of the study.

Surgery

On the day of operation, rats were randomly assigned to lesion (AMX) or sham-operated groups (SHAM) and subsequently received injections of either neurotoxin or vehicle in the amygdala. Lesions were aimed at the basolateral nucleus of the amygdala, and the lesion procedure was performed according to the technique described by Wolterink et al. (2001). Animals operated on pd7 were anesthetized with 0.3 mg/kg sc Fentanyl (Janssen Pharmaceutica, Tilburg, The Netherlands) and immobilized in a David Kopf stereotaxic apparatus. A specially constructed silicone head mold provided stable fixation of the head. A midline skin incision was made and the skull was perforated with the aid of a 1.0-mm dental drill. Excitotoxic lesions were induced by 2-min bilateral infusion of ibotenic acid (3 μg/0.3 μl in 0.1 M pH 7.4 phosphate-buffered saline, PBS, ICN Biomedicals Inc., Aurora, OH, USA) at a rate of 0.15 μl/min by microinfusion pump. Ibotenic acid was obtained from Tocris Cookson (Bristol, UK). Sham operated animals received bilateral vehicle (PBS) injections. The coordinates for the positioning of the needles were: anterior–posterior (AP) −1.0 mm, medial–lateral (ML) +3.8 mm, dorsal–ventral (DV) −6.0 mm, with cannulae at a 4° angle. Four minutes after completion of the infusion, the cannulae were withdrawn and the skin was sutured. Finally, the animals were given 0.3 mg/kg s.c. Naloxone (Du Pont Pharmaceuticals, Wilmington, DE, USA) to antagonize the effects of fentanyl. The animals were returned to their mothers within 30 min after the operation started.

Animals operated on pd21 were anesthetized with 0.3 mg/kg sc fentanyl and immobilized in a David Kopf stereotaxic frame. An operation identical to that described above was performed at the coordinates: AP −2.0 mm, ML +4.0 mm, DV −6.8 mm, with cannulae at a 0° angle. To produce a similarly sized lesion as in animals lesioned on pd7, animals operated on pd21 received a higher concentration of ibotenic acid, namely 4 μg/0.3 μl over 2 min. Postoperatively, this group of animals received 0.3 mg/kg s.c. diazepam (Roche Nederland BV, Mijdrecht, The Netherlans).

For each experiment a separate group of animals (including pd7 and pd21 rats, either sham-operated or lesioned) was used.

Experiments

Two weeks prior to experimentation, animals subjected to the spontaneous alternation and food hoarding task were housed under reversed light conditions, with lights on between 7:00 PM and 7:00 AM. Animals that were tested in the Morris maze were housed under normal light conditions and tested under white light conditions. The animals were handled during 1 week before testing started. Testing took place between 9:00 AM and 3:00 PM and without knowledge of lesion status.

Food hoarding

Rats were subjected to the food hoarding task between the ages of 10 and 12 weeks of life. The test arena consisted of a black acrylic plastic cage measuring 25×25×30 cm, which was used as home cage. An 80 cm long alleyway connected the home cage to the cage serving as the storage room (25×25×30 cm) from which the food could be hoarded. The apparatus was covered with transparent Plexiglas. Twenty-four hours prior to the actual test animals were weighed and then singly housed in their new home cage, allowing them to adapt to the experimental cage. The entrance to the alleyway was closed by a plastic lid. The rats were housed on wood chip, without food, but with water continuously available. Upon testing 100 g of standard rodent pellets was distributed at the far end of the storage room and the plastic lid was removed, thereby giving the animals free access to the storage room. After 1 h, the rats were brought back to their home cages. Cage mates were tested on the same day, to avoid effects of social isolation. After the animals were returned to their home cage the amount of food hoarded (g. food in home cage) and food eaten (100 g−(food hoarded+food left in the storage room and alleyway)) were determined. During the actual hoarding test the apparatus was dimly lit by red light (<10 lux). A radio provided background noise.

Spontaneous alternation

Spontaneous alternation behavior was determined in a plus-maze shaped apparatus, when the rats were 9 weeks of age. The apparatus consisted of four black acrylic plastic arms measuring 40×15×15 cm, with transparent Plexiglas cover. Spontaneous alternation was assessed on 2 consecutive days. The test consisted of placing an animal in the center of the test cage (25×25×30 cm), which it could freely explore for 10 min. After this period the rat was returned to its home cage. For each