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Early postnatal maternal deprivation in rats induces memory deficits in adult life that can be reversed by donepezil and galantamine

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ABSTRACT

Early postnatal maternal deprivation is known to cause long-lasting neurobiological effects. Here, we investigated whether some of the cognitive aspects of these deficits might be related to a disruption of the cholinergic system. Pregnant Wistar rats were individually housed and maintained on a 12:12 h light/dark cycle with food and water freely available. The mothers were separated from their pups for 3 h per day from postnatal day 1 (PND-1) to PND-10. To do that, the dams were moved to a different cage and the pups maintained in the original home cage, which was transferred to a different room kept at 32 °C. After they reached 120–150 days of age, maternal-deprived and non-deprived animals were either sacrificed for brain acetylcholinesterase measurement, or trained and tested in an object recognition task and in a social recognition task as described by Rossato et al. (2007) [Rossato, J.I., Bevilaqua, L. R.M., Myskiw, J.C., Medina, J.H., Izquierdo, I., Cammarota, M. 2007. On the role hippocampal synthesis in the consolidation and reconsolidation of object recognition memory. Learn. Mem. 14, 36–46] and Lévy et al. (2003) [Lévy, F., Melo. A.I., Galef. B.G. Jr., Madden, M., Fleming. A.S. 2003. Complete maternal deprivation affects social, but not spatial, learning in adult rats. Dev. Psychobiol. 43, 177–191], respectively. There was increased acetylcholinesterase activity in hippocampus and perirhinal cortex of the deprived animals. In addition, they showed a clear impairment in memory of the two recognition tasks measured 24 h after training. Oral administration of the acetylcholinesterase inhibitors, donepezil or galantamine (1 mg/kg) 30 min before training reversed the memory impairments caused by maternal deprivation. The findings suggest that maternal deprivation affects memory processing at adulthood through a change in brain cholinergic systems.

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1. Introduction

In rodents and other mammals, alterations of the infant–mother relationship cause long-term changes in the neurobiology and behavior of the offspring (Levine, 2000; Huang et al., 2002; Cirulli et al., 2003; Renard et al., 2005; Benetti et al., 2007; Uriarte et al., 2007). In particular, postnatal maternal deprivation (3–6 h daily deprivation of pups from the dam) is one of the most potent natural stressors during development. Indeed, when the deprived animals become adult they show increased hypothalamic–pituitary–adrenal axis (HPA) responses to stressors (Kuhn and Schanberg, 1998; Wigger and Neumann, 1999; Renard et al., 2005, 2007), high corticosterone levels (Huot et al., 2002, 2004; Plotsky et al., 2005), increased anxiety and behavioral inhibition by fear (Ogawa et al., 1994; Madruga et al., 2006; Aisa et al., 2007), a reduced capacity to copulate (Rhees et al., 2001; Greisen et al., 2005), increased brain apoptosis, decreased neurotrophic factor expression and reduced mossy fibre density (Lee, 2008; Huot et al., 2002; Roceri et al., 2002; Mirescu et al., 2004) and neuronal survival in the hippocampus (Bredy et al., 2003).

In humans, parental maltreatment or neglect or familiar strife can compromise cognitive development (Voorhees and Scarpa, 2004). Likewise, non-human primates (Suomi, 1997) and rodents show profound effects of maternal deprivation on memory parameters and responsiveness to stress (Liu et al., 2000; Mirescu et al., 2004), two functions commonly associated with the hippocampus (Mirescu et al., 2004; Izquierdo et al., 2006).

The hippocampal formation receives abundant regulatory inputs from the basal forebrain cholinergic system, and acetylcholine (ACh) plays a crucial role in a number of physiological processes, including learning and memory (Prado et al., 2006). The cognitive deficits seen in aging and Alzheimer's disease (Winkler

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et al., 1998; Mohapel et al., 2005) have been associated with brain cholinergic deficits (Blokland, 1995; Winkler et al., 1998; Gold, 2003; Mohapel et al., 2005; Prado et al., 2006).

Here, we study the possible role of cholinergic deficits in the cognitive impairment caused by maternal deprivation in rats. Wistar rats that experienced 3-h daily deprivations from the dam during the first 10 days of life were tested in adulthood for object and social recognition in animals treated or not with the clinically effective acetylcholinesterase (AChE) inhibitors, donepezil and galantamine at doses well-known to have effects on memory in rats. AChE activity was measured in normal and deprived animals in hippocampus and perirhinal cortex. Donepezil and galantamine revert the memory deficits of aged rats (Barnes et al., 2000).

2. Material and methods

2.1. Subjects

Virgin young adult female Wistar rats (age, 60–70 days; weight, 200–220 g) were obtained from the colony of the Federal University of Rio Grande do Sul. They were housed for one week in the presence of sexually experienced male Wistar rats. At the end of this period and 7 days before delivery, pregnant female rats were individually housed with bedding and *ad libitum* access to food and water. The day of birth (day 0) was non-deprived. All dams and pups were maintained in light/dark cycle (lights on at 07:00 a.m., off at 7:00 p.m.) and the temperature (23–24 °C) and humidity (60%) were kept constant.

2.2. Maternal deprivation

On postnatal day 1 (PND 1), litters were culled to eight pups (four males and four females when possible) per dam. One or at the most two animals per litter were used in each experimental group (see below). The rat pups were daily deprived of their mother for 3 h during the first 10 days of life. Deprivation consisted of removing the mother from the home cage. The pups were maintained in the original homecage (grouped in the nest in presence of maternal odor), which was transferred to a different room kept at 32 °C to compensate for the mother's body heat (Renard et al., 2005). Deprivation was carried out between 08:00 a.m. and 13:30 p.m. Non-deprived rats remained undisturbed in the home cage with their mothers. The first bedding was changed only on PND 11 for both the groups (non-deprived × deprived rats) studied. Rats were weaned on PND 21 and only males were chosen for the present work. The females were donated to other research groups. All subsequent experiments were performed when the animals were adult (100–120 days of age).

2.3. Behavioral observations

All behavioral observations were performed in an isolated room at 23–24 °C. Animals were brought in 1 h before training or testing began. All behavioral observations were carried out in the light part the light/dark cycle.

Before the experiments each animal was handled by experimenter. This consisted of gently touching and holding the rat with two hands using gloves during approximately 5 min for 3 consecutive days.

2.3.1. Open field exploratory activity

Rats were placed in a 40 cm wide, 30 cm deep and 50 cm high wooden box pained white, with a frontal glass wall. Black lines were drawn on the floor to divide it into 12 equal rectangles. Crossings and rearings, which measure locomotor and vertical exploration, respectively, were counted (Barros et al., 2000, 2002).

2.3.2. Elevated plus maze and anxiety measurement

The elevated plus maze consisted of a central platform (5 cm × 5 cm), two open arms (50 cm long, 10 cm wide, 0.5 cm high borders) and two enclosed arms (same, with 10 cm-high walls), elevated 50 cm above the ground. Rats were placed in the platform facing the open arm and were watched for 5 min. Total number of entries in open and closed arms, time spent in the open and closed arms and the total number of "lookdowns" were measured (Pellow et al., 1985; Barros et al., 2000).

2.3.3. Object recognition

The object recognition task was adapted from Ennanceur and Delacour (1988) as described by Rossato et al. (2007). The apparatus was similar to the open field described above. The first procedure of the experiment consisted in habituation of the animals. Each subject was placed in the apparatus for 20 min of free exploration per day during 4 consecutive days before the first trial of object recognition. On day 1 of the training period, two different objects (A and B) were placed in the apparatus; animals were allowed to explore them freely for 5 min. The objects were constructions made with Lego toys not more than 10 cm high. In the test phase one of the objects was randomly exchanged for a novel object (C) and rats were reintroduced into the apparatus for an additional 5 min period. The test phase was performed 24 h after the sample phase (to analyze long-term memory, LTM). To avoid confounds by lingering olfactory stimuli and preferences the objects and the arena were cleaned after testing each animal with 70% ethanol.

In all experiments, the acetylcholinesterase inhibitors (donepezil or galantamine; 1 mg/kg) or saline (controls) were administered PO 30 min before the sample phase (infusion volume, 1 ml/kg).

2.3.4. Social recognition

Animals were housed individually for 2 days before exposition and testing to allow the establishment of the home-cage territory. All animals were transferred to a large cage (45.9 cm × 45.9 cm × 29 cm) made of transparent acrylic to facilitate behavioral observations. The experimental animals remained in this cage during exposure and test. On day 1, a juvenile 20–25-day-old male rat was placed into the cage with the adult male rat for 30 min. The behavioral parameters analyzed were nosing (gently pushing against the nose and face area or flank of another animal with the snout) and sniffing (same as nosing but without directly contact). The behavioral observation by experimenter was performed during the first 5 min of exposure. At the end of the 30 min exposure period, the juvenile rats were removed from the cage and returned to their home cages. On day 2 (test), the animals within each condition were expose to the same juvenile that encountered to day 1 or were exposed to a different juvenile male and observed for the first 5 min of the interaction. The protocol used was adapted from Lévy et al. (2003).

The acetylcholinesterase inhibitors (donepezil or galantamine; 1 mg/kg) or saline were administered PO 30 min before the first exposure to the juvenile rat on day 1.

2.4. Acetylcholinesterase (AChE) assay

Rats were killed at the age of 120 days. The brain was removed and the CA1 region and the perirhinal cortex were dissected out. AChE activity was determined using a standard spectophotometric method according to Ellman et al. (1961) as modified by Lassiter et al. (2003). Hydrolysis rates were measured at an acetylthiocholine concentration of 0.8 mM in 1 ml assay solutions with 30 mM phosphate buffer, pH 7.5, and 1.0 mM DTNB at 25 °C. About 50 μl of rat CA1 or perirhinal cortex supernatant was added to the reaction mixture and preincubated for 3 min. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nm for 3 min. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nM for 3 min with intervals of 30 s with intervals of 30 s. All samples were run in triplicate. Protein determination was by the method of Bradford (1976) using bovine serum albumin as standard.

2.5. Statistical analysis

The results obtained in open field and plus maze are presented as means (±SEM) for each animal analyzed during total time of 5 min. The data obtained in the object recognition task are presented as mean (±SEM) percentage of time exploring a particular object over the total time of object exploration. In the social recognition task, the results are expressed as mean (±SEM) of the percentage of time spent sniffing and nosing the juvenile rats on day 1 or 2, over the total time of investigation. For the sake of comparisons among groups, the only possible parameter to be used in the two tasks is whether there was a difference in the percentage of time spent exploring a particular

Table 1
Maternal deprivation has no effect on locomotor exploratory activity.

	Day 1		Day 2	
	Non-deprived (n = 10)	Deprived (n = 10)	Non-deprived (n = 10)	Deprived (n = 10)
Total locomotion	84.1 ± 12.50	68.2 ± 10.20	45.60 ± 6.89	43.58 ± 4.52
Crossings	57.30 ± 7.76	54.42 ± 5.82	41.50 ± 5.58	39.33 ± 5.86
Rearings	23.80 ± 2.41	20.08 ± 3.14	15.73 ± 2.63	13.25 ± 2.00

Lack of effect of maternal deprivation on the open field test. Deprived rats do not show differences significantly when compared to non-deprived in all behavioral parameters analyzed. Number of animals per group is shown in the table. Results were expressed as mean ± SEM (Student's t-test, $p < 0.05$).

Table 2
Maternal deprivation had no effect on anxiety.

	Non-deprived (n = 10)	Deprived (n = 10)
Time in open arms	105.8 ± 21.89	91.25 ± 24.84
Time in closed arms	182.9 ± 24.96	191.0 ± 25.33
Open entries	5.40 ± 1.50	6.08 ± 1.17
Entries in closed arms	4.80 ± 0.72	4.58 ± 0.89
Looksdown	10.30 ± 2.62	11.67 ± 2.50

There were no significant differences in plus maze behavior between non-deprived and maternally deprived animals. Number of animals per group is shown in the table. Results expressed as mean ± SEM (Student's t-test, $p < 0.05$).

object or not between groups; i.e., a qualitative observation (Ennanceur and Delacour, 1988; Lévy et al., 2003; Rossato et al., 2007). Quantitative comparisons between groups would be meaningless. The data obtained in the AChE assay was expressed as mean (±SEM).

3. Results

3.1. Exploratory activity

No significant difference in open field behavior was observed between non-deprived and deprived animals on day 1 and day 2 in crossings or rearings (Table 1).

3.2. Anxiety

No significant difference in plus maze behavior was detected between non-deprived and deprived animals (Table 2).

3.3. Object recognition task

In non-deprived animals, there was a significant reduction of the % of total time spent exploring the familiar object relative to that spent exploring the novel object in the test session. This effect was not seen in control maternally deprived animals, who therefore suffered a reduction of the LTM for object recognition (Fig. 1A). This effect was reversed by donepezil (1 mg/kg PO) or galantamine (1 mg/kg PO) administered 30 min before the sample phase on day 1 (Fig. 1B and C).

3.4. Social recognition task

Non-deprived rats recognized a juvenile (20–25-day old) co-specific they had shared the cage with on day 1, as shown by the reduction of the % of total time spent nosing or sniffing it on day 2 (Fig. 2A1, B1, C1) and by spending more time nosing and sniffing a previously unknown, novel conspecific on day 2 (Fig. 2 A2, B2, C2). These recognition effects were not seen in animals who had been exposed to maternal deprivation early in life (Fig. 2 A1, A2), unless these animals had been treated with donepezil (1 mg/kg PO) or galantamine (1 mg/kg PO) 30 min before training on day 1.

3.5. Effects of maternal deprivation on hippocampal and perirhinal AChE activity

In animals exposed to maternal deprivation during early life, AChE activity was significantly higher in hippocampus and perirhinal cortex of the animals exposed to early postnatal maternal deprivation (Fig. 3).

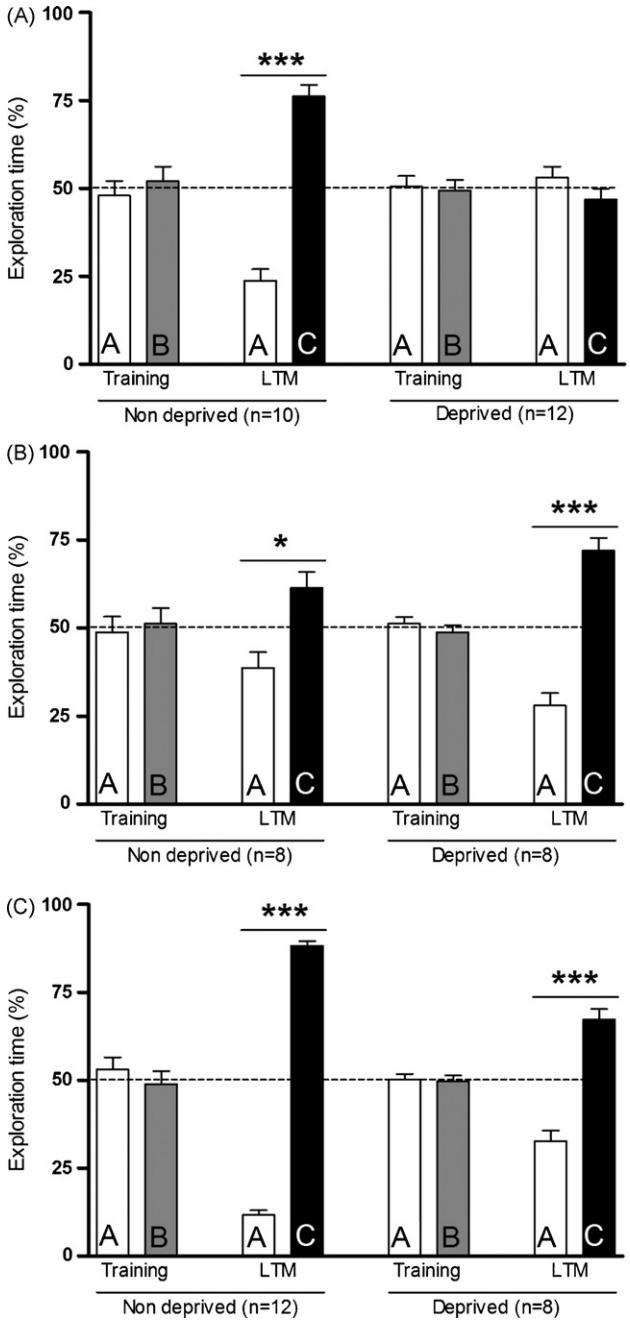

Fig. 1. Effects of neonatal maternal deprivation on the long-term memory of the object recognition task in rats that were given vehicle (panel A), donepezil, 1 mg/kg (panel B) or galantamine, 1 mg/kg (panel C) in 1 ml/kg of saline 30 min prior to training. Rats were placed in the open field containing two different objects (A + B) and left to explore them freely for 5 min. Differences in % of time spent exploring each object is shown. In all cases, there was no difference in the % of time spent exploring objects A and B in the training session. When the latter was replaced by a novel object, C, in the test session, maternally deprived animals did not show a preference for exploring it in control animals (panel A), but did show that preference in the animals treated with donepezil (panel B) and galantamine (panel C). Results were expressed as means (±SEM) of percentage of the total exploration time in each case. Number of animals per group is shown in each panel below each graph; significance levels, $^{*}p < 0.02$, $^{***}p < 0.001$ (Student's t-test).

4. Discussion

Consistent with previous findings on spatial learning (Huang et al., 2002; Lai et al., 2006; Aisa et al., 2007), active avoidance learning, conditioned fear (Lehmann et al., 1999) and social learning (Lévy et al., 2003), we observed that neonatal maternal deprivation leads to long-term memory deficits. This supports the evidence that disruption of mother–infant relationship in the

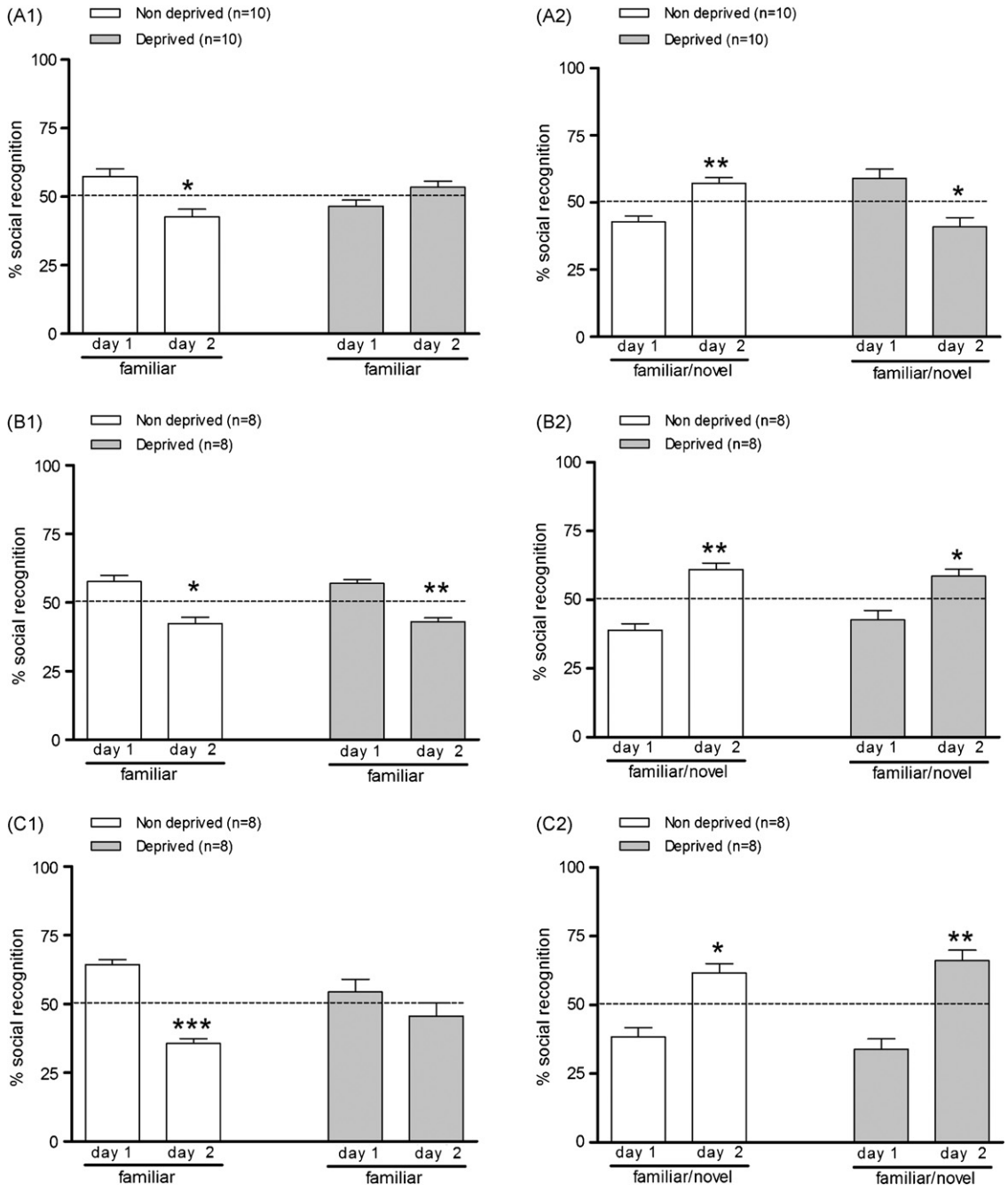

Fig. 2. Effects of neonatal maternal deprivation and of the treatment with donepezil and galantamine on social recognition task. On day 1 (training) a juvenile 20–25-day-old male rat was placed into a cage with the adult male rat for 30 min. On day 2, either the same juvenile (panels A1, B1 and C1) or another, novel juvenile (A2, B2, C2) was placed in the cage with the adult animal. In all cases, adult rats that had not been submitted to early maternal deprivation (non-deprived, white columns) reduced the % of time spent nosing and sniffing the familiar animal on day 2 (A1,B1,C1), and increased the % of time spent nosing and sniffing the novel young animal on day 2 (A2,B2,C2). These effects were not seen in saline-treated control rats that had been exposed to early maternal deprivation (grey columns) (A1,A2), but were seen in animals treated with donepezil (B2) or galantamine (C2). Doses and treatments as in Fig. 1. Data expressed as means (±SEM) % of total time. Significance levels relative to day 1 scores, $^{*}p < 0.02$, $^{**}p < 0.01$, $^{***}p < 0.001$ in Student's *t*-tests.

developing brain period is detrimental to memory processes. The results thus agree with those of Aisa et al. (2007) and Lévy et al. (2003). The results also agree with those of Kosten et al. (2007) who showed cognitive deficits in pups that were isolated from both its conspecifics and their mother at an early postnatal age; i.e., the pups and not the mother was removed and isolated in another room. This raises the possibility that isolation stress, or stress in general at an early age may have unspecific deleterious effects in adulthood; a point that deserves to be further studied (Sarter and Bruno, 2004).

Recognition memory confers the ability to discriminate between novel and familiar entities (Rossato et al., 2007). Analysis of amnesic patients as well as lesion studies in non-human primates and rodents indicate that the functional integrity of the temporal lobe is essential for encoding, storage, and expression of this type of memory (Logothetis and Sheinberg, 1996; Riesenhuber

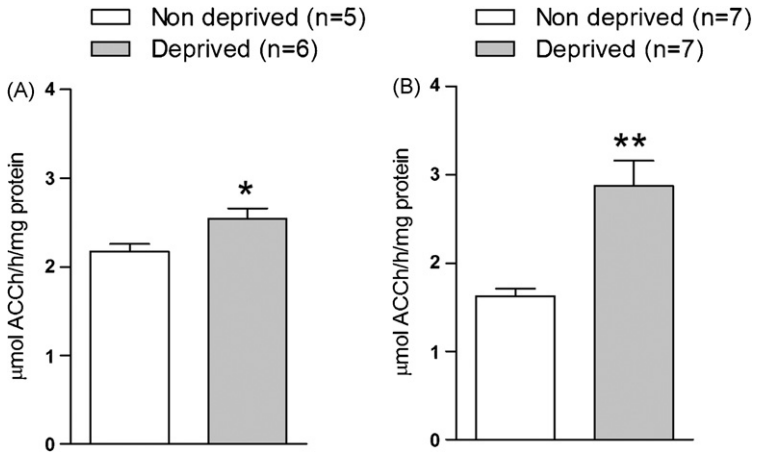

Fig. 3. Effects of neonatal maternal deprivation on acetylcholinesterase activity in adult male rats in hippocampus (A) and perirhinal cortex (B). The results were expressed by mean (±SEM) µmol of acetylcholine (ACh)/h/mg protein in non-deprived rats (white) and in rats submitted to early maternal deprivation (grey columns). Number of animals per group is shown in the graph. Significant levels, $^{*}p < 0.05$, $^{**}p < 0.001$ (Student's *t*-test).

and Poggio, 2002). The hippocampal formation is known to undergo considerable structural changes in response to experience though life (Mirescu et al., 2004).

The present results show that the deleterious effect of maternal deprivation on object and social recognition was accompanied by an increase of hippocampal and perirhinal AChE activity, and that it can be reversed by the cholinesterase inhibitors, donepezil and galantamine. The results suggest that maternal deprivation may induce a disruption of endogenous cholinergic mechanisms. The role of cholinergic mechanisms in cognitive processes memory has been amply ascertained over the years by many authors (Bartus et al., 1982, 1985; Price et al., 1985; Barros et al., 2002; Gold, 2003; Sarter et al., 2003; Sarter and Bruno, 2004; Prado et al., 2006; Kozak et al., 2007). Probably many forms of early postnatal stress also affect adult behavior because of cholinergic deficits in adulthood (Sarter and Bruno, 2004).

The decline of memory that may follow aging or is seen in Alzheimer's disease is very often attributed to a cholinergic malfunction (Bartus et al., 1982, 1985; Price et al., 1985; Mohapel et al., 2005), and it has been suggested to be related to developmental failures in early life (Sarter and Bruno, 2004). Actually, the clinical use of donepezil and galantamine is precisely on the memory failures that accompany aging and on those of Alzheimer's disease, and derives from the fact that both drugs have been repeatedly shown to enhance memory of a variety of animal tasks; including some similar or related to the ones studied here (Lamirault et al., 2003; Prickaerts et al., 2005). In particular, donepezil and galantamine have been shown to improve memory performance in old rats (Barnes et al., 2000).

Here we suggest that the adverse experience of repeated maternal deprivation early in life can induce a dysfunction of brain cholinergic systems, reversible by donepezil and galantamine. The present findings may not be taken to suggest that the complex and manifold cognitive damage brought about by early maternal deprivation or by postnatal stress can be treated solely with memory-enhancing anticholinesterase drugs; these may be viewed, at the most, as possible adjuncts to other appropriate psychotherapeutical and medical treatments.

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