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NEONATAL LESION OF FOREBRAIN CHOLINERGIC NEURONS: FURTHER CHARACTERIZATION OF BEHAVIORAL EFFECTS AND PERMANENCY

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Abstract—Intraventricular injections of 192 IgG-saporin in the neonatal rat caused severe loss of basal forebrain cholinergic neurons and ectopic hippocampal ingrowths. These were evident at 24 months of age and thus, were lifelong consequences of the 192 IgG-saporin treatment. When tested as young adults on a novel water-escape radial arm maze, the rats with this lesion were slower to learn the task, committing significantly more working and reference memory errors before they achieved control level of performance. It is unlikely that this was a result of attentional impairment as the lesioned rats performed as vigilantly as controls in a five choice serial reaction time task. When tested in the Morris water maze at 22 months of age, they were slower at learning the hidden platform location. This contrasts with previous studies which have repeatedly shown that they normally acquire this task as young adults. It was concluded that this neonatal cholinergic lesion has modest but discernable effects on problem solving in young adulthood that are consistent with the reported effects of the lesion on cortical pyramidal neurons. The cognitive effects of the lesion may become more severe in aging, perhaps as a result of the added effects of aging on these neurons. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: 192 IgG-saporin, neonatal, radial water maze, problem solving, spatial learning, aging.

Neonatal intraventricular administration of the cholinergic immunotoxin 192 IgG-saporin (192 IgG-S) (Wiley et al., 1991, 1995) causes severe loss of basal forebrain cholinergic neurons and their terminals in the hippocampus and a lesser but still substantial loss in the cortex (Leanza et al., 1996; Pappas et al., 1996, 2000; Robertson et al., 1998; Sherren et al., 1999). This perinatal loss of cholinergic afferents has a long-lasting if not permanent effect on the morphology of cortical pyramidal neurons. Golgi staining indicates dendritic regression in the apical tuft of layer V pyramidal cells from the medial prefrontal cortex and layer III pyramidals from the anterior cingulate cortex (Sherren and Pappas, 2003) in adulthood. This is accompanied by decreased dendritic spine density.

Despite the loss of cholinergic afferents and the subsequent alteration of cortical pyramidal neuron apical dendrites, there are only modest and subtle behavioral sequelae. With one exception (Mattsson et al., 2002), these rats have not been consistently found to be impaired in adulthood on spatial learning and memory tasks such as the Morris water maze task (Leanza et al., 1996; Pappas et al., 1996, 2000; Sherren et al., 1999) and delayed spatial alternation (Pappas et al., 2000). Also with the exception of a reduced exploratory response to spatially re-arranged objects (Ricceri et al., 1997), there has been no effect of this neonatal cholinergic lesion on tasks such as object novelty and open field (Ricceri et al., 1997), locomotor activity in a novel apparatus (Leanza et al., 1996) and elevated plus apparatus (Pappas et al., 1996). However, they are moderately impaired on a passive avoidance task at 15 days of age (Ricceri et al., 1997, 2002) and, as we have previously reported, they commit more baited arm re-entries in a radial arm maze (RAM) (Pappas et al., 2000). While this effect on the RAM is consistent with a working memory impairment, the task was complex: there were 12 arms and the rats were required to revisit the center hub to gain arm entry. Hence, the effect of the neonatal 192 IgG-S lesion could reflect not only a working memory problem but also other difficulties such as an attentional or problem solving impairment.

The current study was designed to provide additional information on the complex maze performance of these rats and as well to assess their attentional function. In the first instance, we employed a novel five arm water escape radial arm maze (WRAM) task which was developed in our laboratory as a variant of previously described tasks (Hyde et al., 1998). Unlike the appetitively motivated land RAM, this task is aversively motivated and stressful, thus providing additional information about the generality of maze solving under drastically different motivating conditions. Secondly, we assessed their performance on a vigilance task (Carli et al., 1983) that required the animals to detect brief light flashes in one of five spatial locations. The rats were progressed through a set of increasingly demanding procedures, finally with a protocol where the light presentations were brief (0.5 s) and at varying interstimulus intervals from 7 to 23 s. This was intended to tax the vigilance of the rats and also to assess their propensity to inhibit

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Abbreviations: Ach, acetylcholine; GP, globus pallidus; nBM, nucleus basalis magnocellularis; $p75^{NTR}$, low affinity neurotrophin receptor; RAM, radial arm maze; VLDB, vertical limb of the diagonal band; WRAM, water escape radial arm maze; 192 IgG-S, 192 IgG-saporin.

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premature responding at the longer inter-stimulus intervals.

In addition to assessing their cognitive function in young adulthood, we also allowed a cohort of neonatal 192 IgG-S rats to age. No information exists concerning either the persistence of this neonatal lesion or its behavioral effects as the brain ages. We tested these rats in the Morris water maze (reference memory version) (Morris et al., 1982) at 22 months of age. Then at 24 months, their brains were examined using an antibody to the low affinity neurotrophin receptor ($p75^{NTR}$), a receptor that is selective for basal forebrain acetylcholine (Ach) neurons (Wiley et al., 1991). In addition, we used stereology and the optical disector technique (West et al., 1991; West, 1993) to count CA1 pyramidal cells. These cells are reduced in Alzheimer's disease (West et al., 2000) and since there is degeneration of forebrain Ach neurons in Alzheimer's (Beach et al., 1997, 2000; Mesulam et al., 1987) we sought to determine if the neonatal lesioning of their cholinergic input could contribute to their loss.

EXPERIMENTAL PROCEDURES

Animals

The animals were male offspring of Sprague–Dawley dams obtained 1 week pregnant from Charles River Laboratories (Montreal, PQ, Canada). Only male rats were used since previous studies have not indicated sexually dimorphic effects of the treatments used here (Sherren et al., 1999). The rats were single housed in polycarbonate cages (26.6×48.3×20.3 cm) containing modest enrichment (e.g. PVC tunnel).

The care and handling of the animals followed the guidelines for humane treatment as established by the Canadian Council of Animal Care. The project was approved by the Carleton University Animal Care Committee. All experiments conformed to Canadian and international guidelines on the ethical use of animals, including the minimizing of animal numbers and their suffering.

Lesions

192 IgG-S (Advanced Targeting Systems, San Diego, CA, USA) was prepared and administered bilaterally into the lateral ventricles on PND 7 as described elsewhere (Pappas et al., 1996, 2000; Sherren et al., 1999). Briefly, the pups were anesthetized with halothane. Using a plaster body mold to hold the pups immobile in a stereotaxic frame, a midline skin incision was made on the skull. Bilateral holes were manually drilled lateral 1.8 mm from bregma. Using a 30 gauge needle connected to a microsyringe, either the vehicle (phosphate-buffered saline, 1.5 μl/site) or 192 IgG-S (300 ng/site in 1.5 μl vehicle) was infused over 3 min at a depth of 3.5 mm from the dura.

WRAM

The WRAM (see Fig. 1) was a black plastic insert that was placed a white plastic pool, 162 cm in diameter and 61 cm deep containing room temperature (22 °C) water. The pool had a false bottom that was elevated 4 cm to accommodate tubing that delivered air to the pistons which controlled the platforms. The RAM insert consisted of eight arms radiating from a 90 cm diameter central hub. Each arm was 28 cm long and 18 cm wide and the arms were spaced 21 cm apart. The space between the ends of the arms and the wall of the pool provided entry for the tubing that controlled the platforms. The maze could be configured into any combination of arms by inserting guillotine doors at the point where each arm radiated from the center hub. For this experiment, the WRAM was configured with four arms for shaping and five arms for the final test. A platform was situated at the end of each arm and there was also a platform in the center of the central hub. The platforms were 11 cm diameter, perforated clear plastic, except for the center platform which was black. The platforms were mounted on submerged pistons. The pistons were activated by compressed air that was directed to them through a system of solenoids that could be individually controlled by the experimenter through a console. The platforms were normally 1.5 cm below the water level. They could be individually lowered to 16.5 cm below the water surface within 3 s by releasing the air pressure within the pistons.

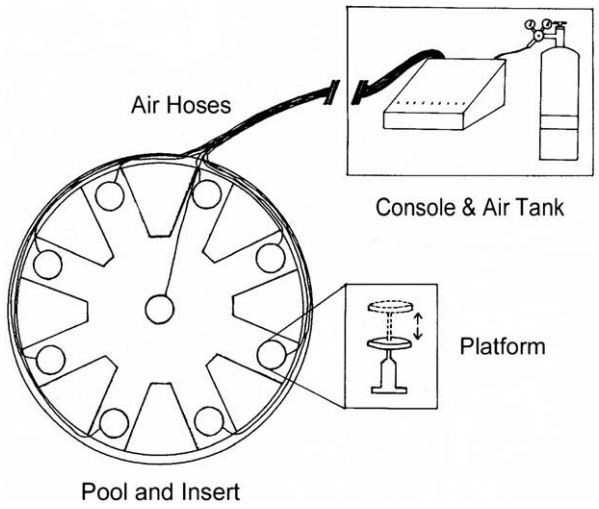

Fig. 1. Schematic drawing (not to scale) of the WRAM. The console and air tank were located about 1.5 m from the pool. Toggle switches on the console allowed the experimenter, who monitored the rats by a camera over the pool, to control the raising/lowering of the individual platforms. The console contained the solenoids that controlled air flow to each platform. Not shown are the guillotine doors that could be lowered into slots at the opening of each arm, thus permitting the maze to be configured with one to eight radiating arms.

To start a session, all platforms were in the up position and the rats were placed on the center platform. This was lowered after 20 s. The rat could escape to any of the platforms in the five arms. After 20 s, this and all other arm platforms submerged while the center platform simultaneously rose and provided the escape from the water. After 20 s, the center platform again submerged while all of the arm platforms except the one(s) that were previously visited, was (were) raised. This procedure continued until the rat located the one remaining arm platform. The rationale for requiring that the rat visit the center platform was to reduce the likelihood of non-spatial arm entry strategies e.g. successive entries of left (or right) adjacent arms. In our previous study of 192 IgG-S rats on a land based RAM (Pappas et al., 2000), the rats were also required to revisit the central hub area between arm entries.

Two types of errors were recorded: (1) center errors where the rat upon exiting an arm, entered another arm instead of going to the center platform and (2) arm errors where the rat entered a previously visited arm after leaving the center platform. Center errors reflect the rat's ability to perform an invariant component of each test. Arm errors reflect working memory, as in the land-based RAM.

Visual attention task

The testing apparatus consisted of a dodecagon hub (Coulbourn Instruments, Allentown, PA, USA, Model H10-35R-12) that was