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TIME COURSE OF BEHAVIORAL CHANGES FOLLOWING BASAL FOREBRAIN CHOLINERGIC DAMAGE IN RATS: ENVIRONMENTAL ENRICHMENT AS A THERAPEUTIC INTERVENTION

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Abstract—The present experiment was designed to study changes in behavior following immunolesioning of the basal forebrain cholinergic system. Rats were lesioned at 3 months of age by injection of the 192 IgG-saporin immunotoxin into the medial septum area and the nucleus basalis magnocellularis, and then tested at different times after surgery (from days 7–500) on a range of behavioral tests, administered in the following order: a nonmatching-to-position task in a T-maze, an object-recognition task, an object-location task, and an open-field activity test. The results revealed a two-way interaction between post-lesion behavioral testing time and memory demands. In the nonmatching-to-position task, memory deficits appeared quite rapidly after surgery, i.e. at a post-lesion time as short as 1 month. In the object-recognition test, memory impairments appeared only when rats were tested at late post-lesion times (starting at 15 months), whereas in the object-location task deficits were apparent at early post-lesion times (starting from 2 months). Taking the post-operative time into account, one can hypothesize that at the shortest post-lesion times, behavioral deficits are due to pure cholinergic depletion, while as the post-lesion time increases, one can speculate the occurrence of a non-cholinergic system decompensation process and/or a gradual degeneration process affecting other neuronal systems that may contribute to mnemonic impairments. Interestingly, when middle-aged rats were housed in an enriched environment, 192 IgG-saporin-lesioned rats performed better than standard-lesioned rats on both the nonmatching-to-position and the object-recognition tests. Environment enrichment had significant beneficial effects in 192 IgG-saporin-lesioned rats, suggesting that lesioned rats at late post-lesion times (over 1 year) still have appreciable cognitive plasticity.

Key words: 192 IgG-saporin, memory, nonmatching-to-position, object-recognition, object-location.

The cholinergic basal forebrain consists of aggregates of cholinergic neurons that innervate various limbic and neocortical areas. Cholinergic projections emanating from basal forebrain structures including the medial septum (MS), nucleus basalis magnocellularis (NBM), and the diagonal band of Broca (DBB), have been identified as playing an important role in cognitive functions such as attention, working memory, and learning (Bartus et al., 1982; Dekker et al., 1990; Collerton, 1986; Olton, 1990; Everitt and Robbins, 1997; Sarter and Bruno, 1997; Ridley et al., 1999). There is a large body of data indicating that the loss of cholinergic input to the hippocampus and cortex is one of the major neuropathological components of the cognitive deficits characteristic of Alzheimer's disease (Gaykema et al., 1992; Rossner, 1997; Wrenn and Wiley, 1998; Whitehouse et al., 1982). Various animal models have been developed to stimulate these cholinergic dysfunctions. The introduction of the immunotoxin 192 IgG-saporin (SAP) appears to allow for more selective cholinergic lesions than classical methods that rely on mechanical lesions or the injection of cholinotoxins (Walsh and Opello, 1992) or excitotoxins (AMPA, QUIS; Waite et al., 1994). 192 IgG-SAP is a monoclonal antibody to the rat low-affinity neurotrophin receptor, p75, located on cholinergic nerve terminals in the cortex and hippocampus and on cholinergic cell bodies in the basal forebrain, i.e. the NBM and the MS/DBB (Cuello et al., 1990). When this antibody is coupled with the ribosomal-inactivating protein SAP, it is able to successfully destroy cholinergic neurons by inhibition of protein synthesis (Wiley, 1992; Wenk et al., 1994). At present, the 192 IgG-SAP immunotoxin is considered an effective tool for selectively eliminating cholinergic neurons from the basal forebrain, and it has proven to be very useful for investigating the role of the cholinergic system in cognition (for a review, see Wrenn and Wiley, 1998; McGaughy et al., 2000; Wenk, 1997; Rossner, 1997). When injected i.c.v. 192 IgG-SAP induces an 80–90% reduction in choline acetyltransferase (ChAT) activity in the cortex and hippocampus within 7 days, while having minimal effects on other neurotransmitters. However, receptor p75 is also found on a limited population of noncholinergic cell bodies primarily located in the brain stem and cerebellum, and there is evidence that 192 IgG-SAP acts upon these cells if injected i.c.v. (Waite et al., 1994, 1995; Heckers et al., 1994). Site-specific injection of 192 IgG-SAP produces a long-lasting loss of cholinergic neurons around the injection site (Torres et al., 1994; Wenk et al., 1994; Perry et al., 2001). The functional consequences of 192 IgG-SAP-induced lesions have been widely studied over the last decade and have been the topic of a number of useful reviews (Rossner, 1997; Wenk, 1997; Wrenn and Wiley, 1998; McGaughy et al., 2000). One of the objectives of the present study was to assess the effects of cholin-

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Abbreviations: ChAT, choline acetyltransferase; DBB, diagonal band of Broca; HDB, horizontal diagonal band of Broca; MS, medial septum; NBM, nucleus basalis magnocellularis; PBS, phosphate-buffered saline; 192 IgG-SAP, 192 IgG-saporin; TBS, Tris-buffered saline; VDB, vertical diagonal band of Broca.

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Table 1. Time schedule for the series of tests, in days after surgery

Experimental step	Post-surgical time		
(A) Post-192 IgG-SAP-lesion-time effects on animal performance			
Nonmatching-to-position task	Day 7	Day 27	Day 365
Object-recognition task, 1-min retention interval	Day 26	Day 58	Day 425
Object-recognition task, 1-day retention interval	Days 33	Day 64	Day 434
Object-recognition task, 1-week retention interval	Day 47	Day 78	Day 443
Object-location task, 1-min retention interval	Day 56	Day 127	Day 456
Object-location task, 1-day retention interval	Day 67	Day 134	Day 463
Object-location task, 1-week retention interval	Day 84	Day 148	Day 476
Open-field task	Day 125	Day 205	Day 493
Kill	Day 133	Day 210	Day 500
(B) Therapeutic effects of an enriched environment			
Housed in an enriched environment	Day 275–500		
Nonmatching-to-position task	Day 365		
Object-recognition task, 1-min retention interval	Day 426		
Object-recognition task, 1-day retention interval	Day 435		
Object-recognition task, 1-week retention interval	Day 445		
Object-location task, 1-min retention interval	Day 458		
Object-location task, 1-day retention interval	Day 467		
Object-location task, 1-week retention interval	Day 479		
Open-field task	Day 494		
Kill	Day 500		

ergic damage following intraparenchymal injection of 192 IgG-SAP in rats on a range of behavioral tests reflecting different aspects of cognitive performance, including the nonmatching-to-position task, an object-recognition task, which provides a way of assessing the recognition-memory capacity by measuring an animal's tendency to explore a novel object (Ennaceur and Delacour, 1988) and an object-location task, which has proven useful for investigating the ability of animals to build a cognitive spatial map (O'Keef and Nadel, 1978). The behavioral performance of rats was examined at various post-operative times.

Another objective was to determine whether long-term environmental enrichment can improve memory abilities in lesioned and nonlesioned middle-aged rats. Environmental stimuli such those occurring in enriched environments have been shown to be very efficient at enhancing brain plasticity, affecting, for instance, neuron size and density, number of glial cells, dendritic branching, spine density, and number of synapses (Kolb et al., 1998; Van Praag et al., 2000), as well as the turnover of several neurotransmitters (Fulford et al., 1994; Escorihuela et al., 1995). Behavioral enrichment has been shown to exert beneficial effects on the cognitive functions of developing rats (Williams et al., 2001), and several studies have demonstrated that environmental enrichment also affects aged brains (Saito et al., 1994). In particular, Kempermann et al. (1998) reported that exposure to a challenging environment triggers a much larger up-regulation of adult hippocampal neurogenesis in old animals than in younger ones. Interestingly, recent studies suggest that exposure to an enriched environment after experimental traumatic brain injury improves cognitive recovery (Hamm et al., 1996; Passineau et al., 2001). All these data raise the question on the use of enriched environments for therapeutic purposes. Environmental stimulation may therefore have a beneficial effect on cognitive recovery in rats after cholinergic damage.

EXPERIMENTAL PROCEDURES

Subjects and design

Young adult male Wistar rats (Charles River, France) were used in the experiments. Animals were housed in standard conditions, i.e. in groups of two to three rats per cage under 12-h light/dark conditions with *ad libitum* access to food and water, except when food-deprived during behavioral training. Every effort was made to minimize animal suffering and to reduce the number of rats used. The principles of laboratory care and specific French recommendations from the Ministry of Agriculture were followed. All experiments conformed to named international guidelines on the ethical use of animals.

The aim of this study was two-fold. First, we investigated post-192 IgG-SAP lesion-time effects on animal's performance on four tests, administered in the following order: (i) a nonmatching-to-position task, (ii) an object-recognition test, (iii) an object-location test, and (iv) an open-field activity test. Fifty-four rats were used. Half of them were injected with the immunotoxin 192 IgG-SAP ($N=27$), and the other half with an equal volume of vehicle alone (phosphate-buffered saline, PBS; $N=27$). All the rats were operated on at 3 months of age. The rats (PBS and SAP) were then subdivided in the following way (Table 1A): the first group of rats was tested at post-lesion times starting 7 days after surgery (PBS, $N=9$; SAP, $N=9$), the second group was behaviorally tested at post-lesion times starting 1 month (day 27) after surgery (PBS, $N=9$; SAP, $N=8$), and the third group of rats was tested at post-lesion times starting 1 year (day 365) after surgery (PBS, $N=7$; SAP, $N=9$).

Second, we evaluated the therapeutic effects of an enriched environment. Eighteen rats were used. The rats were operated on at 3 months of age. Nine rats were injected with the immunotoxin 192 IgG-SAP and nine were injected with PBS. They were kept in