Effects of 192 IgG-saporin on acetylcholinesterase histochemistry in male and female rats

Rodrigue Galani,* Hélène Jeltsch, Olivia Lehmann, Fabrice Bertrand and Jean-Christophe Cassel

Laboratoire de Neurosciences Comportementales et Cognitives, UMR 7521 ULP/CNRS–IFR 37, Strasbourg, France

ABSTRACT: Sex hormones may exert neuroprotective effects in various models of brain lesions. Male and female Long–Evans rats were subjected to intracerebroventricular injections of 2 μg 192 IgG-saporin or vehicle. Starting 2 days before surgery, half the male rats were treated with estradiol for 7 days. Three weeks after surgery, they were sacrificed for histochemical staining of acetylcholinesterase (AChE) and densitometric evaluations. The lesion induced a substantial to dramatic decrease of the AChE-positive fiber density in the cingulate, somatosensory, piriform, retrosplenial and perirhinal cortices, and in the hippocampus. Weak effects were found in the striatum. There was no significant decrease in the dorsal thalamus. Sex had no significant effect on AChE-positive staining in any brain area. In males, estradiol treatment did not alter the effects of 192 IgG-saporin. These results show that sex or estradiol treatment in male rats does not interfere with the immunotoxic effects of intracerebroventricular injections of 192 IgG-saporin on cholinergic projections from the basal forebrain. © 2002 Elsevier Science Inc. All rights reserved.

KEY WORDS: 192 IgG-saporin, AChE, Basal forebrain, Sex, Immunotoxin, Rat, Estrogen.

INTRODUCTION

Until recently, the functions of the cholinergic neurons in the basal forebrain have been investigated using various lesion techniques [4,5,37]. With 192 IgG-saporin, a monoclonal antibody (192 IgG) raised against the low affinity nerve growth factor receptor (p75^{NGF} receptor) coupled to the ribosome-inactivating toxin saporin (from *Saponaria officinalis*), it became possible to damage cholinergic neurons in the basal forebrain more selectively [38]. This immunotoxin can be injected into the ventricular system (i.c.v.), cholinergic nuclei (nucleus basalis magnocellularis, medial septum, diagonal band of Broca) or cholinergic target fields [17,23,27]. Such injections induce a more or less extensive cholinergic denervation of the hippocampus, the cortex, or of both, for instance in case of i.c.v. injections [23,26,36].

In a recent article in which 192 IgG-saporin was injected into the nucleus basalis magnocellularis, McGaughy and Sarter [25] mentioned that the basal forebrain cholinergic neurons of female rats might be more sensitive to 192 IgG-saporin than those of male

rats [24]. In our laboratory, male and female Long–Evans rats have been subjected to virtually identical lesions (2 μg 192 IgG-saporin into the lateral ventricles), but the greatest cholinergic damage and cognitive impairments were found in males (unpublished data vs. Lehmann et al. [23]).

Beyond the apparent discrepancy, such observations might indicate that the status of sex hormones in the experimental subjects might interfere with the efficiency of the immunotoxin. The role of hormones (e.g., estrogen or progesterone) in neuroprotection has been reviewed in many articles (for recent reviews: [2,7,8,15,30,32,40]) including those focused on aging (e.g., [9,11,12,31]) and special attention was paid to the cholinergic basal forebrain system (e.g., [1,10,22]). Most of the literature in that research field provide evidence for protective effects of estrogen after ischemia, axotomy or exposure to some neurotoxins [7].

However, it is not clear whether estrogen may exert its protective effects for all types of insults. For instance, Hörtnagl et al. [18] reported that female rats on proestrus exhibited increased sensitivity to the neurotoxic action of AF64A injected intracerebroventricularly, and Aggarwal and Gibbs [1] recently demonstrated that male and female rats showed comparable damage after injection of ibotenic acid into the nucleus basalis magnocellularis or after transection of the fimbria–fornix pathways.

Additionally, it is noticeable from the literature that, among various mechanisms thought to be involved in the neuroprotection induced by estrogen treatment, evidence supports the role of estrogen as a modulator of neuronal growth factor systems [3,13,19,33]. Interestingly, the p75^{NGF} receptor was shown to be colocalized with the estrogen receptor in cholinergic neurons of the basal forebrain [33,34]. Moreover, estrogen replacement resulted in significant decrease of p75^{NGF} mRNA and protein, attenuated the loss of p75^{NGF} immunoreactivity following transection of the fimbria–fornix [13,14], and decreased the expression of neurotrophins [19] in basal forebrain cholinergic nuclei. As 192 IgG-saporin exerts its effects through the p75^{NGF} receptor, it might be hypothesized that the hormonal status of rats may interact with the efficiency of the immunotoxin.

In the present experiment, we report on the effects of i.c.v. injections of 192 IgG-saporin on acetylcholinesterase (AChE) histochemistry in male and female rats, and in male rats treated with β-estradiol-3-benzoate for 2 days before and 5 days after surgery.

* Address for correspondence: Dr. Rodrigue Galani, Ph.D. LN2C UMR 7521 CNRS/ULP, 12 rue Goethe, 67000 Strasbourg, France. Fax: +33-390-24-19-58; E-mail: rodrigue.galani@psycho-ulp.u-strasbg.fr

MATERIALS AND METHODS

Subjects and Design

All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (council directive #87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animales; permission # 6212 to J.-C.C. and 6714-bis to H.J.; O.L., F.B. and R.G. under the former's responsibility) and international (NIH publication, No. 86-23, revised 1985) laws and policies.

Our study used 20 male and 12 female Long–Evans rats (R. Janvier, France) aged about 90 days at the time of surgery (males: 321 ± 2.9 g; females: 201 ± 5.1 g). Two days before surgery, eight male rats were treated with subcutaneous (s.c.) injections of β-estradiol-3-benzoate (groups ML+E, $n = 4$ and MS+E, $n = 4$) whilst all other rats received injections of the vehicle (peanut oil). During surgery, all rats from the group ML + E, six other male rats (ML) and six female rats (FL) received bilateral infusions of 192 IgG-saporin. The other rats were Sham-operated (FS, $n = 6$; MS, $n = 5$). All rats were housed in transparent Makrolon cages (42 cm × 26 cm × 15 cm) under a 12:12 h dark–light cycle (lights on at 0700 h), with *ad lib.* access to food and water throughout the experiment. The colony and testing rooms were under controlled temperature (21°C).

Surgeries and Estradiol Treatment

All surgical procedures were conducted under aseptic conditions. A mixture of ketamine (6.75 mg/kg, i.p.) and xylazine (0.85 mg/kg, i.p.) was used for anaesthesia. Injections of 192 IgG-saporin (ATS, San Diego, USA; 1 μg/μl per lateral ventricle, concentration 1 μg/μl of PBS) or PBS into the lateral ventricles were performed stereotaxically through a 2-μl Hamilton syringe at the following coordinates (in mm): A −0.8 (from Bregma), L ±1.4 (from the midline), V −4.3 (from Bregma) for males, and A −0.8 (from Bregma), L ±1.3 (from the midline), V −4.1 (from Bregma)

for females, with the incisor bar set at the interaural line [28]. After each injection, the needle was left *in situ* for 9 min before being slowly retracted. In all Sham-operated rats of both sexes, a volume of 1 μl of PBS was injected into each lateral ventricle.

In rats, from groups MS+E and ML+E, the estradiol treatment (β-estradiol-3-benzoate, 20 μg/ml kg in peanut oil injected (s.c.); Sigma, St. Louis, USA) was started 2 days before surgery and continued over 7 consecutive days. The rats from the other groups were injected daily with 1 ml/kg of peanut oil (s.c.) over the same period of time.

Histochemical Verifications

After 21 days, all rats were sacrificed for histochemical verifications.

The rats were injected with an overdose of pentobarbital (100 mg/kg, i.p.; Sanofi, France) and transcardially perfused with 60 ml of saline followed by 60 ml of 0.1 M phosphate-buffered paraformaldehyde (4°C, pH 8). After extraction and post-fixation for about 4 h, the brains were placed in a cryoprotective solution (0.1 M phosphate-buffered 20% sucrose solution) for about 36 h before being frozen and stored at −80°C. Using a cryostat (−23°C), the brains were cut into 30-μm thick coronal sections which were collected on gelatine-coated slides. The sections were dried at room temperature for at least 36 h and stained for AChE according to a method similar to that described by Koelle [20]. Ethopropazine (0.3 mM; Sigma, St. Louis, USA) was used to block non-specific cholinesterases and acetylthiocholine iodide (4 mM; Sigma, St. Louis, USA) was used as the substrate.

Evaluation of AChE-Density

The quantification of AChE-positive reaction products was assessed by an adaptation of a method described by Turchi and Sarter [35]. The various locations in which the measurements were made are shown in Fig. 1. Briefly, an AChE staining index was obtained by measuring the exposure time (in seconds) indicated by a Vanox Olympus Microscope (model AHBT) when the apparatus
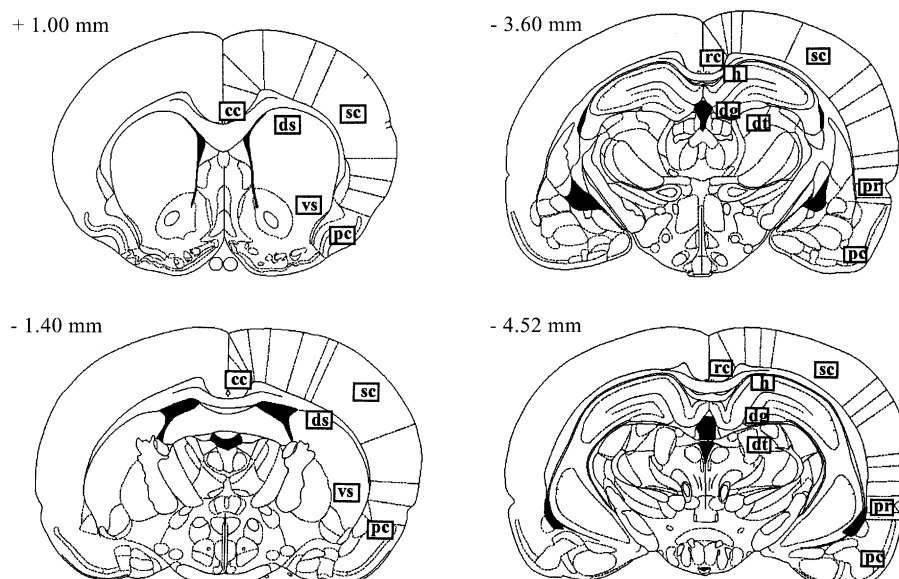

FIG. 1. Different areas in which the density of the AChE-positive reaction products were measured. All counts were taken bilaterally. Abbreviations: cc: cingulate cortex, dg: dentate gyrus, ds: dorsal striatum, dt: dorsal thalamus, h: hippocampus CA1, pc: piriform cortex, pr: perirhinal cortex, rc: retrosplenial cortex, sc: somatosensory cortex, vs: ventral striatum.
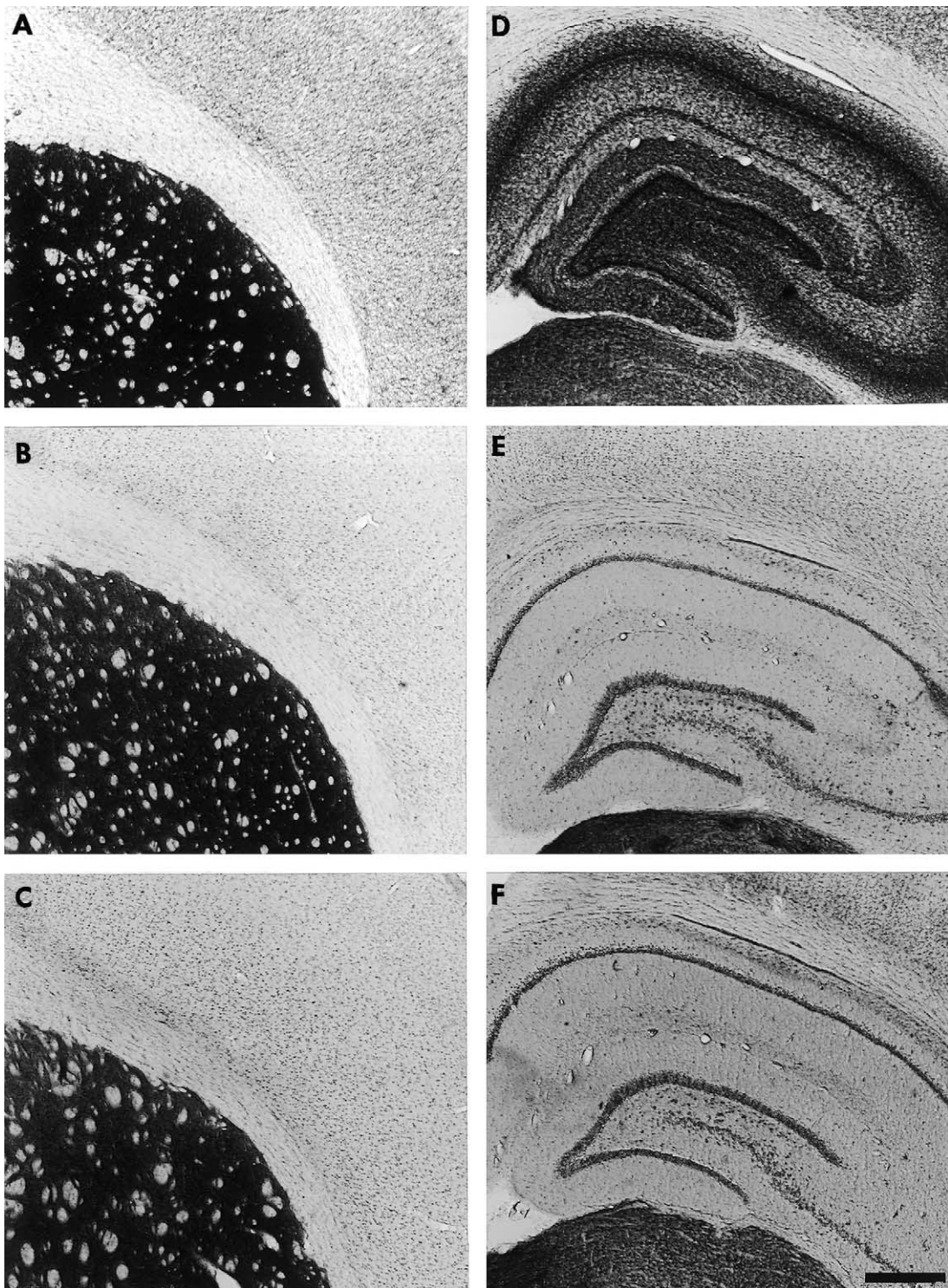
FIG. 2. Examples of AChE-density in the frontal cortex and the striatum (A–C), the hippocampus and the dorsal thalamus (D–F) in Sham-operated male rats (A, D), in male rats given 192 IgG-saporin (B, E) and in female rats given 192 IgG-saporin (C, F). As there was no significant effect of estrogen treatment, these results are not illustrated. Scale bar = 500 μm.

TABLE 1

AVERAGE AChE-POSITIVITY INDEX (MEAN AND SEM) AND PERCENT DIFFERENCE FROM CONTROLS IN THE DIFFERENT REGIONS AND AT DIFFERENT LEVELS FROM BREGMA (PAXINOS AND WATSON [28]) IN MALE AND FEMALE RATS

Brain region	MS	ML	% Change	FS	FL	% Change	MS	ML	% Change	FS	FL	% Change
	+1.0 mm from Bregma						−1.4 mm from Bregma					
Cingulate cx	1.59 ± 0.21	0.11 ± 0.04*	−86.7	1.99 ± 0.22	0.20 ± 0.07*	−93.4	0.90 ± 0.12	0.12 ± 0.06*	−93.3	1.07 ± 0.11	0.08 ± 0.03*	−90.0
Somatosensory cx	0.67 ± 0.16	0.15 ± 0.04*	−78.1	0.77 ± 0.11	0.10 ± 0.03*	−86.4	0.64 ± 0.11	0.14 ± 0.05*	−77.7	0.59 ± 0.05	0.08 ± 0.02*	−86.8
Dorsal striatum	4.24 ± 0.45	2.48 ± 0.34	−41.5	4.98 ± 0.31	3.89 ± 0.68	−5.19	2.89 ± 0.36	1.69 ± 0.11	−41.5	2.89 ± 0.19	2.74 ± 0.52	−21.8
Ventral striatum	12.23 ± 0.35	5.18 ± 0.70*	−54.5	10.71 ± 0.8	7.35 ± 1.10	−19.6	11.71 ± 2.82	5.33 ± 1.24	−57.6	11.50 ± 1.39	9.23 ± 1.26	−31.3
Piriform cx	1.52 ± 0.09	0.18 ± 0.06*	−92.3	1.98 ± 0.28	0.14 ± 0.02*	−93.0	1.83 ± 0.37	0.14 ± 0.04*	−88.0	1.73 ± 0.14	0.13 ± 0.02*	−92.9
	−3.6 mm from Bregma						−4.5 mm from Bregma					
Retrosplenial cx	0.84 ± 0.17	0.25 ± 0.08*	−71.4	0.92 ± 0.04	0.09 ± 0.02*	−90.10	0.90 ± 0.18	0.25 ± 0.06*	−72.2	1.03 ± 0.10	0.10 ± 0.03*	−92.2
Somatosensory cx	0.57 ± 0.14	0.22 ± 0.08*	−61.0	0.55 ± 0.06	0.05 ± 0.02	−90.90	0.63 ± 0.14	0.25 ± 0.07*	−60.3	0.64 ± 0.08	0.08 ± 0.02*	−87.3
Hippocampus CA1	1.98 ± 0.33	0.25 ± 0.08*	−87.8	1.91 ± 0.15	0.13 ± 0.04*	−93.10	1.56 ± 0.23	0.27 ± 0.07*	−83.2	1.74 ± 0.18	0.14 ± 0.03*	−92.4
Dentate gyrus	1.75 ± 0.56	0.36 ± 0.06*	−79.3	2.02 ± 0.30	0.31 ± 0.05*	−84.60	1.49 ± 0.19	0.43 ± 0.08*	−71.1	1.82 ± 0.18	0.25 ± 0.04*	−86.7
Perirhinal cx	0.84 ± 0.19	0.17 ± 0.06*	−98.8	1.01 ± 0.09	0.13 ± 0.03*	−88.11	0.91 ± 0.17	0.20 ± 0.07*	−78.8	1.12 ± 0.08	0.26 ± 0.05*	−77.4
Piriform cx	1.58 ± 0.31	0.21 ± 0.04*	−87.3	1.82 ± 0.11	0.17 ± 0.02*	−91.20	1.39 ± 0.28	0.22 ± 0.04*	−84.1	1.59 ± 0.08	0.10 ± 0.02*	−93.7
Dorsal thalamus	1.51 ± 0.22	1.19 ± 0.12	−21.1	1.56 ± 0.11	1.26 ± 0.17	−19.20	1.26 ± 0.16	1.41 ± 0.17	+12.0	2.00 ± 0.28	1.46 ± 0.18	−27.0

Abbreviations: cx: cortex, M: male, F: female, S: Sham-operated, L: Lesion (192 IgG-saporin).
* Significant Lesion effect, $p < 0.05$.

TABLE 2

AVERAGE AChE-POSITIVITY INDEX (MEAN AND SEM) AND PERCENT DIFFERENCE FROM CONTROLS IN THE DIFFERENT REGIONS AND AT DIFFERENT LEVELS FROM BREGMA (PAXINOS AND WATSON [28]) IN MALE RATS TREATED OR NOT WITH ESTRADIOL

Brain region	MS	MS + E	% Change	ML	ML + E	% Change	MS	MS + E	% Change	ML	ML + E	% Change
	+1.0 mm from Bregma						−1.4 mm from Bregma					
Cingulate cx	1.59 ± 0.21	1.52 ± 0.15	−4.4	0.11 ± 0.04	0.22 ± 0.08	+100	0.9 ± 0.12	1.03 ± 0.03	+14.4	0.12 ± 0.06	0.17 ± 0.05	+41.6
Somatosensory cx	0.67 ± 0.16	0.63 ± 0.09	−5.9	0.15 ± 0.04	0.21 ± 0.07	+40	0.64 ± 0.11	0.64 ± 0.08	0.0	0.14 ± 0.05	0.18 ± 0.05	+28.5
Dorsal striatum	4.24 ± 0.45	4.38 ± 1.60	+3.3	2.48 ± 0.34	3.28 ± 2.03	+32.2	2.89 ± 0.36	3.24 ± 0.96	+12.1	1.69 ± 0.11	2.52 ± 0.53	+49.1
Ventral striatum	12.23 ± 0.35	9.56 ± 2.43	+21.8	5.18 ± 0.70	8.22 ± 1.52	+58.6	11.71 ± 2.82	8.44 ± 1.15	−27.9	5.33 ± 1.24	11.71 ± 2.82	+119.6
Piriform cx	1.52 ± 0.09	1.52 ± 0.13	0.0	0.18 ± 0.06	0.18 ± 0.11	0.0	1.83 ± 0.37	1.74 ± 0.15	−4.9	0.14 ± 0.04	0.34 ± 0.17	+142.0
	−3.6 mm from Bregma						−4.5 mm from Bregma					
Retrosplenial cx	0.84 ± 0.17	0.98 ± 0.09	+16.6	0.25 ± 0.08	0.21 ± 0.05	−16.0	0.90 ± 0.18	0.96 ± 0.02	+6.6	0.25 ± 0.06	0.17 ± 0.06	−32.0
Somatosensory cx	0.57 ± 0.14	0.64 ± 0.12	+12.3	0.22 ± 0.08	0.17 ± 0.05	−22.7	0.63 ± 0.14	0.63 ± 0.04	0.0	0.25 ± 0.07	0.21 ± 0.08	−16.0
Hippocampus CA1	1.98 ± 0.33	1.99 ± 0.18	+0.5	0.25 ± 0.08	0.24 ± 0.06	−4.0	1.56 ± 0.23	1.63 ± 0.20	+4.4	0.27 ± 0.07	0.24 ± 0.08	−11.1
Dentate gyrus	1.75 ± 0.56	2.01 ± 0.26	+14.8	0.36 ± 0.06	0.40 ± 0.09	+11.1	1.49 ± 0.19	1.64 ± 0.25	+10.0	0.43 ± 0.08	0.36 ± 0.08	−25.0
Perirhinal cx	0.84 ± 0.19	0.93 ± 0.10	+10.7	0.17 ± 0.06	0.26 ± 0.08	+52.9	0.91 ± 0.17	0.93 ± 0.27	+2.19	0.20 ± 0.07	0.31 ± 0.19	+55.0
Piriform cx	1.58 ± 0.31	1.76 ± 0.29	+11.4	0.21 ± 0.04	0.34 ± 0.27	+61.9	1.39 ± 0.28	1.73 ± 0.21	+24.4	0.22 ± 0.04	0.32 ± 0.13	+45.4
Dorsal thalamus	1.51 ± 0.22	1.48 ± 0.29	+2.0	1.19 ± 0.12	1.24 ± 0.09	+4.2	1.26 ± 0.16	1.76 ± 0.30	+39.6	1.41 ± 0.17	1.21 ± 0.21	−14.1

Some of the values given in percentage may seem high, but the difference from controls was not significant. Abbreviations: cx: cortex, M: male, S: Sham-operated, L: Lesion (192 IgG-saporin), +E: male rats treated with β-estradiol-3-benzoate s.c.

was switched into the photograph mode (ISO 100, reciprocity: 0, exposure adjustment: 1, magnification: 10×5) and the objective was focused on different areas of each section (see Fig. 1). A total of 22 measures for each hemisphere were taken from four sections of each brain. Four measures were taken at 1.00 mm anterior and 1.40 mm posterior to Bregma (cingulate, somatosensory, piriform cortices and striatum), and seven measures were taken at 3.60 and 4.52 mm posterior to Bregma (retrosplenial, somatosensory, piriform and perirhinal cortices, hippocampal CA1, dentate gyrus and dorsal thalamus). A minimal and constant exposure time of 1.12 s was found in regions where there were no AChE-positive reaction products at all (i.e., in a totally denervated cortical region in rats given 192 IgG-saporin). This 1.12-s value was considered a "background" (section without AChE-positive staining, gelatine, slide and cover-glass) and subtracted from all measures before analysis. All measurements were made by the same experimenter who was unaware of the sex, the surgical or the pharmacological treatment of the rats.

Statistical Analysis

The AChE-positive fiber density from the left and right hemispheres of each measurement area were first averaged. These average values were then analyzed by analyses of variance (ANOVA), followed where appropriate, by 2×2 comparisons based on the Newman–Keuls multiple range test [39]. A first analysis was made according to a 2 (Male, Female) $\times$ 2 (Sham, Lesion) design in order to assess the effects of the sex and the lesion (male rats given estradiol were not considered). A second analysis was made according to a 2 (Sham, Lesion) $\times$ 2 (Oil, Estradiol) design on all male rats in order to assess the effects of the lesion and of estradiol. One ML rat died from an anesthetic shock and another one was subjected to euthanasia a few days prior to the scheduled sacrifice date because of respiratory disease. The results from these rats were not considered for statistical analysis.

RESULTS

Overall, the i.c.v. injections of the toxin induced a profound depletion of AChE-positive reaction products in almost all cortical areas, as well as over the entire hippocampus (Cornu Ammonis + dentate gyrus). These observations, illustrated in Figs. 2A–F, were confirmed by the measurements shown in Tables 1 and 2.

The first analysis was performed on the rats not treated with estradiol, that is MS, ML, FS and FL rats. It showed a significant overall Lesion effect at all four levels from Bregma [$F(1, 14) = 6.95$ at least and 128.26 at most, $0.05 > p > 0.001$] and in all cortical and hippocampal regions assessed. This effect was due to a 50% decrease at least of the AChE-positivity in all lesioned rats as compared to their Sham-operated counterparts. In the dorsal thalamus, there was no significant overall Lesion effect, whether at -3.6 or -4.5 mm from Bregma (see Table 1). In the striatum, the effects were not very consistent and, when significant, they were much less pronounced than in the other brain regions. In the dorsal striatum, there was a significant effect of the lesion at $+1.0$ mm from Bregma. In the ventral striatum, the Lesion effect was significant at both anteriority levels [$F(1, 14) = 6.56$, at least, $p < 0.05$]. Whatever level or brain region was considered, we could not find any statistical evidence for a significant overall effect of Sex, nor a significant effect of the Lesion $\times$ Sex interaction.

The analysis of the data from all male rats, that is rats from MS + E, MS, ML + E and ML groups, showed a significant Lesion effect at all four anteroposterior levels [$F(1, 14) = 6.57$ at least and 172.37 at most, $0.05 > p > 0.001$] and in all cortical and hippocampal regions assessed. This effect was due to a decrease of the AChE-positivity in all lesioned rats as compared to

their Sham-operated counterparts (-50% at least). In the dorsal thalamus, there was no significant Lesion effect, whether at -3.6 or -4.5 mm from Bregma (see Table 2). In the striatum, the effects were not as consistent and, when significant, they were much less pronounced than in the other brain regions. In the dorsal striatum, there was no significant effect of the lesion at both anteriority levels. In the ventral striatum, the Lesion effect was significant at both anteriority levels [$F(1, 14) = 6.56$, at least, $p < 0.05$]. Whatever anteroposterior level or brain region was considered, we could not find any statistical evidence for a significant overall effect of Estrogen treatment, nor for a significant effect of the Lesion $\times$ Estrogen interaction.

DISCUSSION

The present results show that bilateral i.c.v. injections of 192 IgG-saporin induced a consistent and significant decrease of the AChE-positive fiber density in various cortical regions (cingulate, somatosensory, piriform, retrosplenial and perirhinal), as well as in the Cornu Ammonis and the dentate gyrus of the hippocampus. Conversely, the immunotoxin produced much less pronounced cholinergic damage in the striatum and no significant effect in the dorsal thalamus. Otherwise, considering all measures of AChE-positivity, there was no significant difference between male and female rats, and estradiol treatment did not alter the effects of 192 IgG-saporin in male rats.

When injected into the cerebral ventricles, 192 IgG-saporin kills cholinergic neurons of the basal forebrain and Purkinje cells of the cerebellum. This latter cell type is not only cholinergic but it also bears p75^{NGF} receptors [41]. Damage to the cholinergic neurons of the basal forebrain accounts for the extensive denervation of different cortical regions and of the hippocampus. In the present study, the cortical and hippocampal denervations produced by 192 IgG-saporin confirm previous results from the literature [41].

In adult rats, it is generally considered that cholinergic interneurons of the striatum bear few p75^{NGF} receptors (although present at birth and re-expressed in adults after striatal lesions [6,21]) and, therefore, should not be very sensitive to the immunotoxin. This is also the case with the cholinergic innervation of other structures such as the thalamus [41]. Although there was no significant evidence for thalamic denervation in the present study, our data indicate a reduction of AChE-positivity in the striatum. In a recent article, Perry et al. [29] presented brain sections from rats given intraseptal infusions of 192 IgG-saporin. On one of these sections, the striatum was almost completely devoid of AChE-staining. In an earlier report, Heckers et al. [16] also reported reduced cholinergic markers in the striatum after injections of 192 IgG-saporin into the basal forebrain. Altogether, these data suggest that cholinergic striatal interneurons may indeed be vulnerable to 192 IgG-saporin injected into the basal forebrain or into the cerebral ventricles.

In 1999, McGaughy and Sarter [25] stated that cholinergic neurons of the basal forebrain might be more sensitive to 192 IgG-saporin in female than in male rats. In our previous experiments, rats had been subjected to virtually identical lesion techniques (2 μg 192 IgG-saporin into the lateral ventricles), and we found cholinergic damage and cognitive impairments which were larger in male than in female rats (unpublished data on males vs. Lehmann et al. [23]). Beyond the possibility that these differences could be due to a variable potency from one batch to another, one cannot exclude *a priori* that the differential effects of 192 IgG-saporin might also be explained by a factor related to the sex of rats (i.e., sex hormones). The effects of estrogen on neuroprotection have been widely studied and these effects were shown to be mediated by the classical nuclear estrogen receptors, but also by other membrane receptors, by the modulation of neurotransmitter

receptor function or by its anti-oxidant effects [7]. The effects of estrogen have also been attributed to interactions between neurotrophin (e.g., p75^{NGF}) and estrogen receptors [13,14,19]. Thus, we expected that the cholinergic effect of the immunotoxin would be different, i.e., be larger or smaller, depending on a differential regulation of p75 receptors between male and female rats and/or between males treated with estradiol and males not subjected to this treatment. Our results showed that the male rats not treated with estradiol and the female rats presented a comparable reduction of AChE-positive reaction products in the cortex and the hippocampus. The results also showed that treatment with estradiol at a daily dose of 20 μg/ml kg during 7 days did not alter the effect of the immunotoxin. It is, however, noteworthy that the effects of estrogen on cholinergic neurons may depend upon the dose and the duration of the treatment [10,12]. For instance, Gibbs [10] reported that 25 or 100 μg estradiol repeatedly administered over 4 weeks to ovariectomized rats resulted in a significant decrease of the number of p75^{NGF} immunoreactive cells in the medial septum. Gibbs [10] also found that such a decrease was not observed after a 1- or 2-week treatment period at the same doses, or after a longer treatment period at lower doses. Thus, even though it remains possible that the variability of batch potency may account for the different effects of 192 IgG-saporin between males and females in previous experiments, we cannot exclude that, in the present experiment, the dose and the duration of estradiol treatment in male rats may have been too short to alter the effects of 192 IgG-saporin. This hypothesis needs to be tested in further experiments using i.c.v. or intraparenchymal injections of various doses of the cholinergic immunotoxin in combination with a control of the estrous cycle at the time of injection.

REFERENCES

1. Aggarwal, P.; Gibbs, R. B. Estrogen replacement does not prevent the loss of choline acetyltransferase-positive cells in the basal forebrain following either neurochemical or mechanical lesions. Brain Res. 882:75–85; 2000.
2. Brinton, R. D. Cellular and molecular mechanisms of estrogen regulation of memory function and neuroprotection against Alzheimer's disease: Recent insights and remaining challenges. Learn. Mem. 8:121–133; 2001.
3. Blurton-Jones, M. M.; Roberts, J. A.; Tuszynski, M. H. Estrogen receptor immunoreactivity in the adult primate brain: Neuronal distribution and association with p75, trkA, and choline acetyltransferase. J. Comp. Neurol. 405:529–542; 1999.
4. Cassel, J.-C.; Duconseille, E.; Jeltsch, H.; Will, B. The fimbria–fornix/cingular bundle pathways: A review of neurochemical and behavioral approaches using lesions and transplantation techniques. Prog. Neurobiol. 51:663–716; 1997.
5. Collerton, D. Cholinergic function and intellectual decline in Alzheimer's disease. Neuroscience 19:1–28; 1986.
6. Gage, F. H.; Batchelor, P.; Chen, K. S.; Chin, D.; Higgins, G. A.; Koh, S.; Deputy, S.; Rosenberg, M. B.; Fischer, W.; Björklund, A. NGF receptor re-expression and NGF-mediated cholinergic neuronal hypertrophy in the damaged adult neostriatum. Neuron 2:1177–1184; 1989.
7. Garcia-Segura, L. M.; Azcoitia, I.; DonCarlos, L. L. Neuroprotection by estradiol. Prog. Neurobiol. 63:29–60; 2001.
8. Garcia-Segura, L. M.; Cardona-Gomez, G. P.; Chowen, J. A.; Azcoitia, I. Insulin-like growth factors-I and estrogen receptors interact in the promotion of neuronal survival and neuroprotection. J. Neurocytol. 29:425–437; 2000.
9. Gibbs, R. B. Estrogen and nerve growth factor-related systems in brain. Effects on basal forebrain cholinergic neurons and implications for learning and memory processes and aging. Ann. NY Acad. Sci. 743:165–196; 1994.
10. Gibbs, R. B. Effects of estrogen on basal forebrain cholinergic neurons vary as a function of dose and duration of treatment. Brain Res. 757:10–16; 1997.
11. Gibbs, R. B. Impairment of basal forebrain cholinergic neurons associated with aging and long-term loss of ovarian function. Exp. Neurol. 151:289–302; 1998.
12. Gibbs, R. B. Effects of gonadal hormone replacement on measures of basal forebrain cholinergic function. Neuroscience 101:931–938; 2000.
13. Gibbs, R. B.; Pfaff, D. W. Effects of estrogen and fimbria/fornix transection on p75NGFR and ChAT expression in the medial septum and diagonal band of Broca. Exp. Neurol. 116:23–39; 1992.
14. Gibbs, R. B.; Wu, D.; Hersh, L. B.; Pfaff, D. W. Effects of estrogen replacement on the relative levels of choline acetyltransferase, trkA, and nerve growth factor messenger RNAs in the basal forebrain and hippocampal formation of adult rats. Exp. Neurol. 129:70–80; 1994.
15. Green, P. S.; Simpkins, J. W. Neuroprotective effects of estrogens: Potential mechanisms of action. Int. J. Dev. Neurosci. 18:347–358; 2000.
16. Heckers, S.; Ohtake, T.; Wiley, R. G.; Lappi, D. A.; Geula, C.; Mesulam, M.-M. Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. J. Neurosci. 14:1271–1289; 1994.
17. Holley, L. A.; Wiley, R. G.; Lappi, D. A.; Sarter, M. Cortical cholinergic deafferentation following the intracortical infusion of 192 IgG-saporin: A quantitative histochemical study. Brain Res. 663:277–286; 1994.
18. Hörtnagl, H.; Hansen, H.; Kindel, G.; Schneider, B.; el Tamer, A.; Hanin, I. Sex differences and estrous cycle-variations in the AF64A-induced cholinergic deficit in the rat hippocampus. Brain Res. Bull. 31:129–134; 1993.
19. Jezierski, M. K.; Sohrabji, F. Neurotrophin expression in the reproductively senescent forebrain is refractory to estrogen stimulation. Neurobiol. Aging 22:311–321; 2001.
20. Koelle, G. B. The histochemical localization of cholinesterases in the central nervous system of the rat. J. Comp. Neurol. 100:211–235; 1954.
21. Koh, S.; Oyler, G. A.; Higgins, G. A. Localization of nerve growth factor receptor messenger RNA and protein in the adult rat brain. Exp. Neurol. 106:209–221; 1989.
22. Lapchak, P. A.; Araujo, D. M.; Quirion, R.; Beaudet, A. Chronic estradiol treatment alters central cholinergic function in the female rat: Effect on choline acetyltransferase activity, acetylcholine content, and nicotinic autoreceptor function. Brain Res. 525:249–255; 1990.
23. Lehmann, O.; Jeltsch, H.; Lehnhardt, O.; Pain, L.; Lazarus, C.; Cassel, J.-C. Combined lesions of cholinergic and serotonergic neurons in the rat brain using 192 IgG-saporin and 5,7-dihydroxytryptamine: Neurochemical and behavioral characterization. Eur. J. Neurosci. 12:67–79; 2000.
24. McGaughy, J.; Kaiser, T.; Sarter, M. Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: Selectivity of the behavioral impairment and relation to AChE-positive fiber density. Behav. Neurosci. 110:247–265; 1996.
25. McGaughy, J.; Sarter, M. Effects of ovariectomy, 192 IgG-saporin-induced cortical cholinergic deafferentation, and administration of estradiol on sustained attention performance in rats. Behav. Neurosci. 113:1216–1232; 1999.
26. Nilsson, O. G.; Leanza, G.; Rosenblad, C.; Lappi, D. A.; Wiley, R. G.; Björklund, A. Spatial learning impairments in rats with selective immunolesion of the forebrain cholinergic system. Neuroreport 3:1005–1008; 1992.
27. Ohtake, T.; Heckers, S.; Wiley, R. G.; Lappi, D. A.; Mesulam, M. M.; Geula, C. Retrograde degeneration and colchicine protection of basal forebrain cholinergic neurons following hippocampal injections

of an immunotoxin against the p75 nerve growth factor receptor. Neuroscience 78:123–133; 1997.

28. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.

29. Perry, T.; Hodges, H.; Gray, J. A. Behavioural, histological and immunocytochemical consequences following 192 IgG-saporin immunolesions of the basal forebrain cholinergic system. Brain Res. Bull. 54:29–48; 2001.

30. Roof, R. L.; Hall, E. D. Gender differences in acute CNS trauma and stroke: Neuroprotective effects of estrogen and progesterone. J. Neurotrauma 17:367–388; 2000.

31. Singer, C. A.; McMillan, P. J.; Dobie, D. J.; Dorsa, D. M. Effects of estrogen replacement on choline acetyltransferase and trkA mRNA expression in the basal forebrain of aged rats. Brain Res. 789:343–346; 1998.

32. Stein, D. G. Brain damage, sex hormones and recovery: A new role for progesterone and estrogen. Trends Neurosci. 24:386–391; 2001.

33. Toran-Allerand, C. D. Mechanisms of estrogen action during neural development: Mediation by interactions with the neurotrophins and their receptors? J. Steroid Biochem. Mol. Biol. 56:169–178; 1996.

34. Toran-Allerand, C. D.; Miranda, R. C.; Bentham, W. D.; Sohrabji, F.; Brown, T. J.; Hochberg, R. B.; MacLusky, N. J. Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. Proc. Natl. Acad. Sci. USA 89:4668–4672; 1992.

35. Turchi, J.; Sarter, M. Cortical cholinergic inputs mediate processing capacity: Effects of 192 IgG-saporin-induced lesions on olfactory span performance. Eur. J. Neurosci. 12:4505–4514; 2000.

36. Walsh, T. J.; Kelly, R. M.; Dougherty, K. D.; Stackman, R. W.; Wiley, R. G.; Kutscher, C. L. Behavioral and neurobiological alterations induced by the immunotoxin 192 IgG-saporin: Cholinergic and non-cholinergic effects following i.c.v. injection. Brain Res. 702:233–245; 1995.

37. Wenk, G. L. The nucleus basalis magnocellularis cholinergic system: One hundred years of progress. Neurobiol. Learn. Mem. 67:85–95; 1997.

38. Wiley, R. G.; Oletmann, T. N.; Lappy, T. A. Immunolesioning: Selective destruction of neurons using immunotoxin to rat NGF receptor. Brain Res. 562:149–153; 1991.

39. Winer, B. J. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971.

40. Wise, P. M.; Dubal, D. B.; Wilson, M. E.; Rau, S. W. Estradiol is a neuroprotective factor in in vivo and in vitro models of brain injury. J. Neurocytol. 29:401–410; 2000.

41. Wrenn, C. C.; Wiley, R. G. The behavioral functions of the cholinergic basal forebrain: Lessons from 192 IgG-saporin. Int. J. Dev. Neurosci. 16:595–602; 1998.