

## AGE-RELATED ATTENUATION OF STIMULATED CORTICAL ACETYLCHOLINE RELEASE IN BASAL FOREBRAIN-LESIONED RATS

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**Abstract**—*In vivo* microdialysis was used to measure the effects of partial deafferentation of cortical cholinergic inputs on acetylcholine efflux in young (four to seven months) and aged (24–28 months) male F344/BNNIA rats. Partial deafferentation was produced by bilateral infusions of the immunotoxin 192 immunoglobulin G-saporin (0.56 µg/1.0 µl) or its vehicle solution into the ventral pallidum/substantia innominata region of the basal forebrain. The lesion produced comparable (65%) decreases in basal cortical acetylcholine efflux in young and aged rats. Presentation of a complex environmental stimulus (exposure to darkness/palatable food), in conjunction with the systemic administration of the benzodiazepine receptor weak inverse agonist ZK 93 426, increased cortical acetylcholine efflux in young shams, aged shams and young lesioned rats, but not in aged lesioned rats. Administration of the benzodiazepine receptor partial inverse agonist FG 7142, in the absence of the environmental stimulus, comparably stimulated cortical acetylcholine efflux in young and aged sham rats. FG 7142-induced increases in acetylcholine efflux were attenuated by approximately 50% following partial deafferentation in both young and aged rats.

These results suggests that, under certain conditions, ageing potently interacts with the integrity of the cortical cholinergic afferent system. The effects of ageing on cortical cholinergic function may be most potentially revealed by experiments assessing age-related limitations in the responsiveness of a partially deafferented cholinergic system to certain behavioral and/or pharmacological stimuli. © 1999 IBRO. Published by Elsevier Science Ltd.

**Key words:** ageing, cortex, acetylcholine, microdialysis, 192 immunoglobulin G-saporin, basal forebrain.

Normal human ageing and dementia are associated with decreased cholinergic inputs to telencephalic areas.<sup>3,27,28,38</sup> The number of cholinergic cell bodies in the basal forebrain<sup>2,6,15</sup> and various markers of cortical cholinergic transmission, such as choline acetyltransferase activity or hemicholinium binding,<sup>7,8,11,23</sup> decrease significantly during ageing and in senile dementia. Moreover, these markers of cortical acetylcholine (ACh) correlate with the severity of the dementia,<sup>5,24,25</sup> and this relationship, along with studies on the cognitive effects of acutely administered cholinergic antagonists in humans and animals, and of basal forebrain lesions in animals, has formed the basis of the “cholinergic hypothesis” of cognition (for a discussion of criticisms surrounding this hypothesis, see Refs 32 and 33).

As a result of the findings in aged and demented humans, research on the relationships between age-related changes in cognitive functions and the cholinergic system in rodents has intensified in recent years. This research is guided by the presumption that declines in cortical cholinergic function and related cognitive abilities, similar to

those described above in humans, occur in aged rodents. While this reasoning is intuitively appealing, a review of the effects of age, as a singular independent variable, on the integrity of basal forebrain cholinergic neurons and on markers of cholinergic transmission has not revealed reliable or robust effects.<sup>34</sup> Normal ageing in rodents may subtly affect cortical cholinergic transmission, yet the robust manifestation of functional consequences requires an additional provocation or insult to the system. Such an ageing-related vulnerability to subsequent trauma (i.e. age acting as an “intervening experimental variable”) may underlie the relatively late expression of cognitive dysfunctions associated with insidious progressive neurodegenerative diseases.

An assessment of the significance of age as an intervening variable first necessitates an understanding of the neuronal effects of an insult to the forebrain cholinergic system in young adults. Recently, we characterized, in young adults, the trans-synaptic modulation of frontoparietal ACh release after partial cortical cholinergic deafferentation with the selective cholinotoxin 192 immunoglobulin G (IgG)-saporin.<sup>9</sup> Local intracortical infusions of 192 IgG-saporin produced a 47% reduction in basal cortical ACh efflux, consistent with reductions in

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**Abbreviations:** ACh, acetylcholine; AChE, acetylcholinesterase; BZR, benzodiazepine receptor; IgG, immunoglobulin G.

the density of acetylcholinesterase (AChE)-containing fibers. In spite of this lesion, partially deafferented rats exhibited a normal stimulation of ACh efflux following presentation of an environmental (onset of darkness paired with palatable food) or pharmacological [systemic injection of the benzodiazepine receptor (BZR) partial inverse agonist FG 7142] stimulus. In addition, the ability of darkness/food to stimulate cortical ACh efflux was similarly potentiated by systemic administration of the BZR weak inverse agonist ZK 93 426 in lesioned and sham-lesioned animals.

The above findings on partial deafferentation in young adults suggest that the capacity to respond to certain environmental and pharmacological stimuli is preserved following the lesion. The present experiment determined whether such capacities for stimulated cortical ACh release are maintained when the deafferentation occurs in aged rats. Specifically, young and aged adult rats received intra-basalis infusions of 192 IgG-saporin or its vehicle solution, and the effects on basal and stimulated frontoparietal ACh efflux were determined using a repeated microdialysis perfusion design. The capacity for stimulated cortical ACh release was determined following exposure to darkness coupled with food (with and without administration of the BZR weak inverse agonist ZK 93 426) or administration of the BZR partial inverse agonist FG 7142.

## EXPERIMENTAL PROCEDURES

### *Animals*

Young adult (four to seven months of age) or aged (24–28 months) male F344/BNNIA rats (National Institute of Aging Colony, Charles River, MA) were maintained in a temperature- and humidity-controlled environment, with food and water freely available. All efforts were made to minimize animal suffering and to reduce the number of animals used. Animal care and experimentation were performed in accordance with protocols approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee. All animals were extensively handled for several days prior to surgery.

### *Surgery and behavioral training*

Animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The immunotoxin 192 IgG-saporin (0.56 µg/1.0 µl/hemisphere; Batch No. 31695031, Chemicon, Temecula, CA) or its vehicle solution (Dulbecco's saline) was slowly infused bilaterally (1.0 µl/min) into the ventral pallidum/substantia innominata region of the basal forebrain of young and aged adult rats according to the following coordinates: AP -0.5 mm, L ± 2.9 mm and DV - 6.7 mm (from dural surface).<sup>26</sup> The concentration and volume of the immunotoxin were based on previous experiments from our laboratory<sup>9,16</sup> and other laboratories,<sup>37</sup> and were adjusted to the relatively lower activity of this particular batch of the toxin. In a previous experiment, infusions of comparable concentrations of the toxin produced some limited non-selective loss of neurons in an area within a radius of 200–250 µm from the tip of the infusion needle.<sup>16</sup> Animals

were given injections of penicillin (30,000 U, i.m.) and saline (2–3 ml, i.p.) at the conclusion of surgery.

Animals were handled and habituated to the microdialysis testing chambers (parabolic clear plastic bowls; 35.0 cm height, 38.0 cm diameter; Carnegie Medicin, Stockholm, Sweden) and to injections of saline (i.p.) during the two-week period between intra-basalis infusions of 192 IgG-saporin or its vehicle solution and the implantation of a microdialysis guide cannula (below). During the second week, animals were trained to associate the onset of darkness in the testing room with the presentation of palatable food (a piece of fruit-flavored cereal), a manipulation that reliably increases cortical ACh efflux.<sup>9,18–20</sup> Four to five hours after placing the rats in the dialysis bowls, the lights in the testing room were extinguished, followed by the presentation of one piece of cereal. After seven consecutive days of this training, the latency of the animals to consume the cereal was less than 30 s. No consistent differences in latency related to age or lesion condition were observed.

Two weeks following the initial surgery, each animal was implanted with a microdialysis guide cannula (0.65 mm o.d.) with stainless steel stylet (Bioanalytical Systems, W. Lafayette, IN) at a 45° angle into the right frontoparietal cortex. The cannula tip was lowered 1.0 mm below the dural surface and permanently fixed to the skull with stainless steel screws and dental cement. Anesthesia, surgical conditions and post-surgical care were identical to those described above for the initial surgery.

### *Microdialysis*

After guide cannula implantation, animals were allowed a three-day recovery period, during which habituation and training continued. The first microdialysis session was performed on the fourth day after surgery. Each animal received four microdialysis sessions, with a non-dialysis day between sessions. We have previously demonstrated the validity of this repeated dialysis design for studying the modulation of cortical<sup>21</sup> and striatal<sup>12</sup> ACh efflux.

On each test day, subjects were habituated to the testing chamber for 30 min before insertion of the concentric microdialysis probe (0.5 mm o.d., 2.0 mm membrane length; Bioanalytical Systems). Probes were perfused at a flow rate of 2.0 µl/min with an artificial cerebrospinal fluid containing (in mM): NaCl 126.5, NaHCO<sub>3</sub> 27.5, KCl 2.4, Na<sub>2</sub>SO<sub>4</sub> 0.5, KH<sub>2</sub>PO<sub>4</sub> 0.5, CaCl<sub>2</sub> 1.1, MgCl<sub>2</sub> 0.8, D-glucose 4.9. The reversible AChE inhibitor neostigmine bromide (0.5 µM; Sigma Chemical, St Louis, MO) was added to the perfusion medium to promote recovery of detectable levels of cortical ACh with concentric dialysis probes. Collection of dialysates began 3 h after probe insertion, a time-point at which basal cortical ACh efflux is stable and dependent on axonal depolarization in the region surrounding the probe.<sup>18</sup> Four consecutive 15-min baseline dialysate samples were then collected.

After the last baseline collection, each animal received an injection of the BZR weak inverse agonist ZK 93 426 (1.0 or 5.0 mg/kg, i.p.; Schering AG, Berlin, Germany) or its vehicle solution, 10% Cremephor EL (CEL; BASF, Ludwigshafen, Germany) in saline. Only one dose was administered per microdialysis session, and each animal received all three doses, counterbalanced across the first three sessions. Fifteen minutes after injection of drug or vehicle solution, the lights in the testing room were extinguished and the animal was presented with a piece of fruit-flavored cereal. Four additional 15-min dialysate samples were then collected while the lights remained off.

The BZR partial inverse agonist FG 7142 (8.0 mg/kg, i.p.; Research Biochemicals International, Natick, MA) was always administered during the fourth microdialysis session, again following the fourth baseline collection. FG 7142 administration was not followed by the darkness/cereal stimulus.

### Acetylcholine analysis

ACh in each dialysate was quantified by high-performance liquid chromatography with electrochemical detection.<sup>29</sup> ACh and choline were separated by a C18 carbon polymer column (530 mm × 1 mm; Bioanalytical Systems) using a mobile phase (pH 8.5) containing 50 mM NaH<sub>2</sub>PO<sub>4</sub> and 10 mM NaCl. Post-column derivitization of ACh and choline was achieved by an immobilized enzyme reactor column (Bioanalytical Systems) containing covalently bound AChE and choline oxidase. The hydrogen peroxide generated by the enzymatic degradation of ACh and choline was further broken down and detected by a “peroxidase-wired” glassy carbon electrode (Bioanalytical Systems) coupled to an LC-4C electrochemical detector (Bioanalytical Systems). Detector output was recorded and analysed using CHROMGRAPH software (Bioanalytical Systems). The peak corresponding to ACh was quantitated by integration of the peak area and comparison with a four-point external standard curve bounding the expected range of ACh values. The detection limit for ACh by this method was 20 fmol/injection.

### Histology

Three days after the last microdialysis session, animals were given a sublethal dose of sodium pentobarbital and transcardially perfused with 0.2% heparin in saline followed by 10% formalin. The brains were removed, blocked rostral to the cerebellum and stored in 10% formalin at 4°C, with transfer to 30% sucrose phosphate buffer at least three days before sectioning. Sections from each brain were processed for AChE staining in order to confirm appropriate probe placement.<sup>35</sup>

### Statistical analysis

Basal efflux values (pmol ACh/min) for each animal in each session were defined as the median of the four baseline collections for that session. Comparisons of basal efflux among the four age/lesion groups, as a function of microdialysis session, were conducted using ANOVA.

Responsivity to the darkness/cereal stimulus and to ZK 93 426 was assessed by an overall four-way ANOVA, with Collection Interval (five levels) and Dose (three levels) as within-subjects factors, and Lesion Condition (two levels) and Age (two levels) as between-subjects factors. Collection Interval analysis included the last baseline collection, post-injection, and three post-darkness/cereal collections. Efflux values for this analysis were expressed as percentage change from the median baseline for each animal in each dialysis session to control for inter-animal and inter-session variability in basal cortical ACh efflux. Planned comparisons, consisting of paired *t*-tests using a modified Bonferroni correction to minimize Type I errors,<sup>13</sup> were conducted where indicated by significant main or interaction effects in the ANOVA.

Effects of systemic FG 7142 on cortical ACh efflux were similarly assessed by a three-way ANOVA with five levels of the within-subjects factor Collection Interval (last baseline and four post-drug collections), and Lesion Condition and Age as between-subjects factors (same levels as above). Again, paired *t*-tests with Bonferroni corrections were conducted where appropriate. Unless stated otherwise (i.e. Bonferroni corrected  $\alpha$ ), statistical significance was defined as  $P < 0.05$ .

## RESULTS

### Microdialysis probe placement

Inspection of AChE-positive tissue sections revealed that all dialysis probes were located within

the frontoparietal cortex. There were no systematic differences between the two ages or two lesion conditions with respect to probe placement.

### Basal cortical acetylcholine efflux

Figure 1 summarizes the effects of age and lesion on basal cortical ACh efflux. The data are presented for each of the four microdialysis sessions, as well as an overall mean across the multiple sessions. Overall, basal efflux did not differ between the two ages ( $F_{1,19} = 1.64$ ,  $P = 0.216$ ). As expected, administration of 192 IgG-saporin significantly reduced cortical ACh efflux ( $F_{1,19} = 140.40$ ,  $P = 0.001$ ), and this reduction was comparable in young (62%) and aged rats (63%) ( $F_{1,19} = 0.16$ ,  $P = 0.693$ ). The ANOVA did reveal a significant effect of Session ( $F_{3,57} = 4.86$ ,  $P = 0.004$ ) and a Lesion × Session interaction ( $F_{3,57} = 3.60$ ,  $P = 0.019$ ). An analysis of simple main effects revealed that the source of this interaction was an effect of Session in sham-treated rats ( $F_{3,33} = 5.44$ ,  $P = 0.004$ ). Paired *t*-tests indicated significant differences between sessions 1 and 2 and between sessions 2 and 4 (both  $P < 0.025$ , Bonferroni corrected  $\alpha$ ). However, there was no systematic decline in basal ACh efflux with repeated perfusions, as there was no significant difference between efflux in sessions 1 and 4 ( $P = 0.390$ ). Importantly, and relevant to the significant interactions seen following stimulated ACh efflux (see below), the effects of Lesion, Age and Session on basal ACh efflux did not interact ( $F_{3,57} = 0.97$ ,  $P = 0.414$ ).

### Stimulated cortical acetylcholine efflux

*Effects of the darkness/palatable food stimulus.* The effects of exposure to darkness/palatable cereal, and the ability of the BZR inverse agonist ZK 93 426 to potentiate this stimulus, on cortical ACh efflux in young and aged, sham and lesioned rats are summarized in Figs 2–5. Overall, exposure to this complex stimulus markedly stimulated cortical ACh efflux as evidenced by a significant effect of Collection Interval (see the summary of ANOVA results in Table 1). Planned comparisons revealed that the main effect of Collection Interval reflected numerous differences (all  $P < 0.02$ , Bonferroni corrected  $\alpha$ ), including the last baseline versus each subsequent collection interval, the post-injection interval versus the 0–15 min interval, and the 0–15 versus the 30–45 min intervals (illustrating the transient nature of the stimulated efflux).

There were also significant differences between the effects of the two doses of ZK 93 426 and its vehicle to modulate the effects of darkness/cereal on ACh efflux (see effects of Dose in Table 1). The main effect of Dose was due to higher elevations in ACh efflux, relative to the vehicle injection, following each dose of ZK 93 426 (both  $P < 0.033$ ,

### BASAL CORTICAL ACh EFFLUX

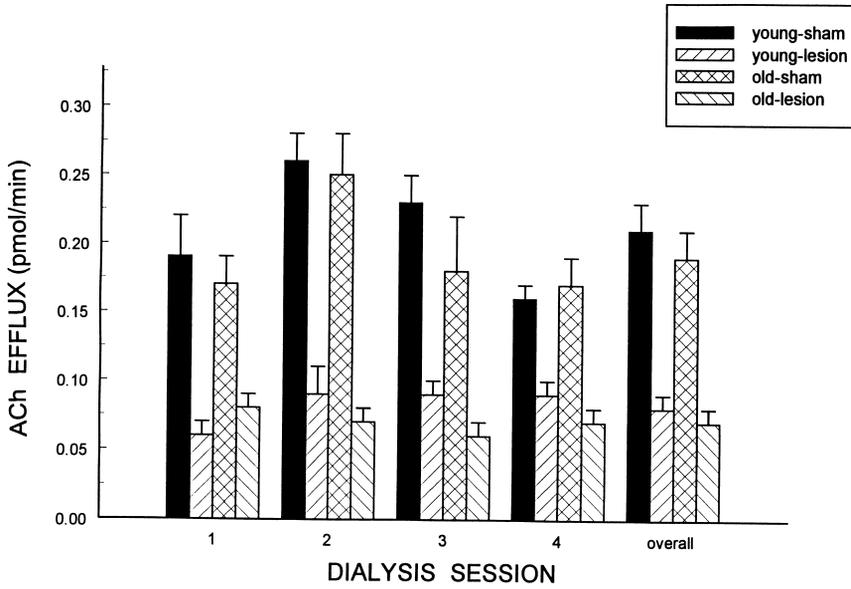


Fig. 1. Mean ( $\pm$  S.E.M.) basal cortical ACh efflux (pmol/min) in young (four to seven months) and old (24–28 months) rats as a function of microdialysis session in a repeated perfusion design. Animals, at each age range, were either sham-treated or 192 IgG-saporin-lesioned. As depicted in the overall means (collapsed across the four dialysis sessions), basal efflux was similar between the two ages and, relative to sham-treated controls, was comparably reduced in young (62%) and old (63%) lesioned rats ( $n = 6$  rats/condition).

### YOUNG - SHAMS

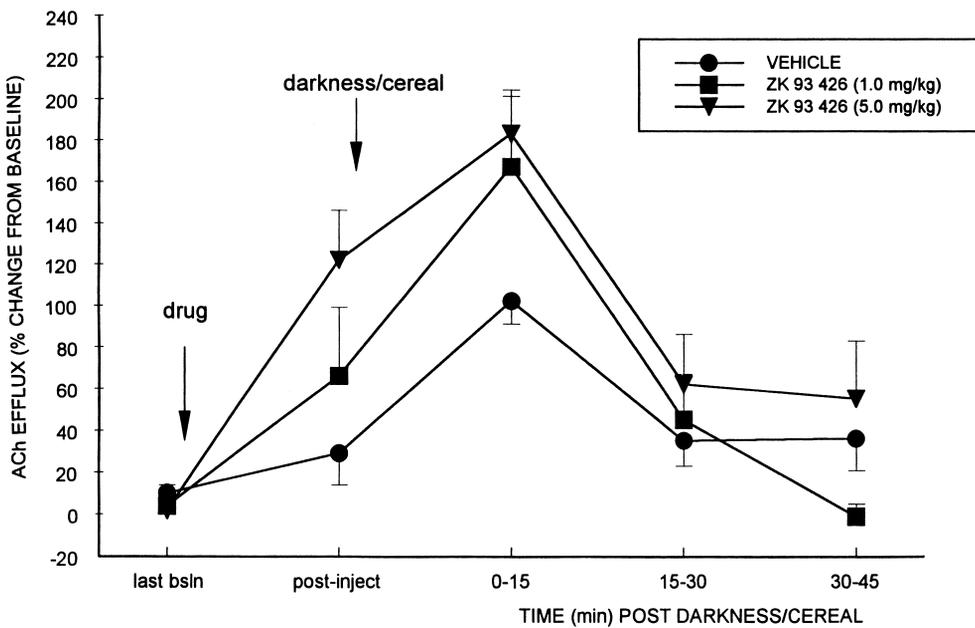


Fig. 2. Mean ( $\pm$  S.E.M.) stimulated cortical ACh efflux (percentage change from median baseline) in young sham-treated rats exposed to darkness/cereal 15 min after receiving vehicle or ZK 93 426 (1.0 or 5.0 mg/kg). Dialysates were collected at 15-min intervals. Animals received each of the stimuli, in counterbalanced order, during three microdialysis sessions ( $n = 6$  rats/condition).

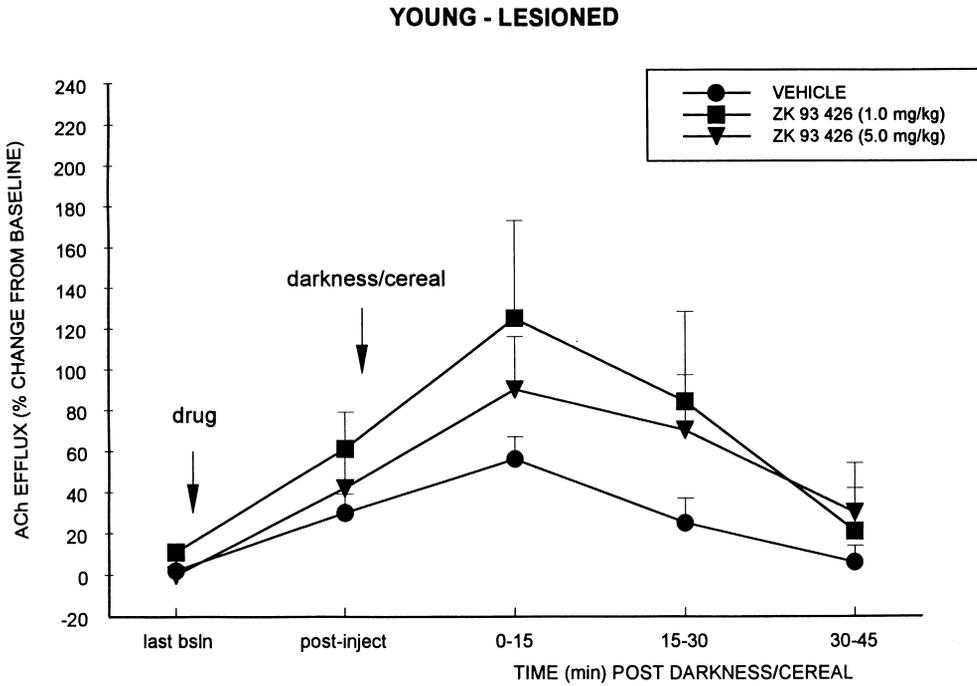


Fig. 3. Mean ( $\pm$  S.E.M.) stimulated cortical ACh efflux (percentage change from median baseline) in young lesioned rats exposed to darkness/cereal 15 min after receiving vehicle or ZK 93 426 (1.0 or 5.0 mg/kg). Animals were lesioned with intra-basalis injections of the cholinotoxin 192 IgG-saporin. Dialysates were collected at 15-min intervals. Animals received each of the stimuli, in counterbalanced order, during three microdialysis sessions ( $n = 6$  rats/condition).

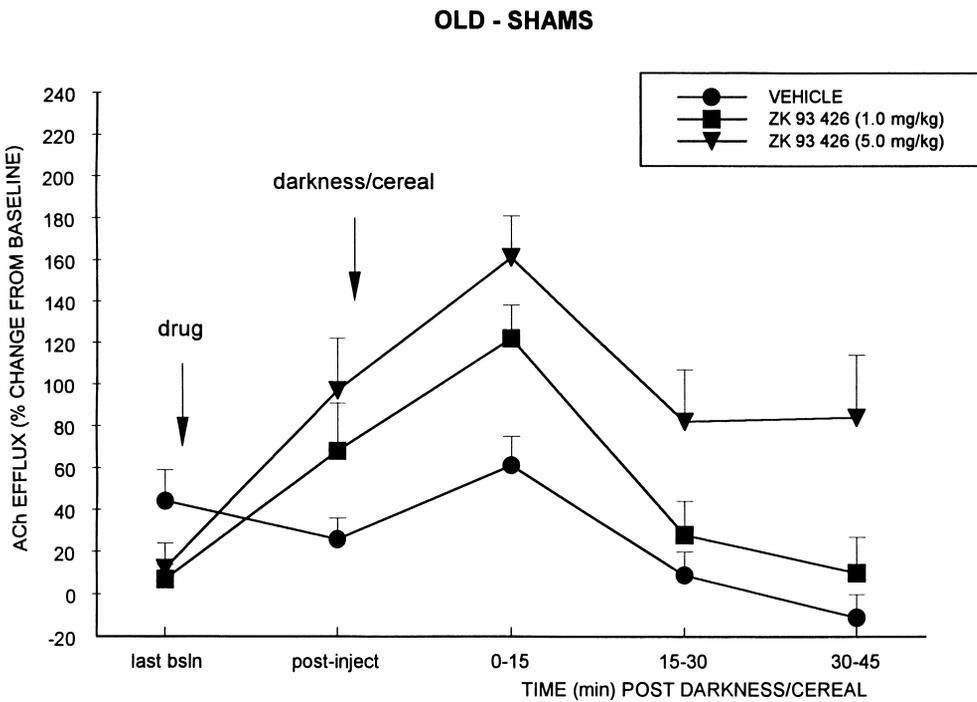


Fig. 4. Mean ( $\pm$  S.E.M.) stimulated cortical ACh efflux (percentage change from median baseline) in aged sham-treated rats exposed to darkness/cereal 15 min after receiving vehicle or ZK 93 426 (1.0 or 5.0 mg/kg). Dialysates were collected at 15-min intervals. Animals received each of the stimuli, in counterbalanced order, during three microdialysis sessions ( $n = 6$  rats/condition).

## OLD - LESIONED

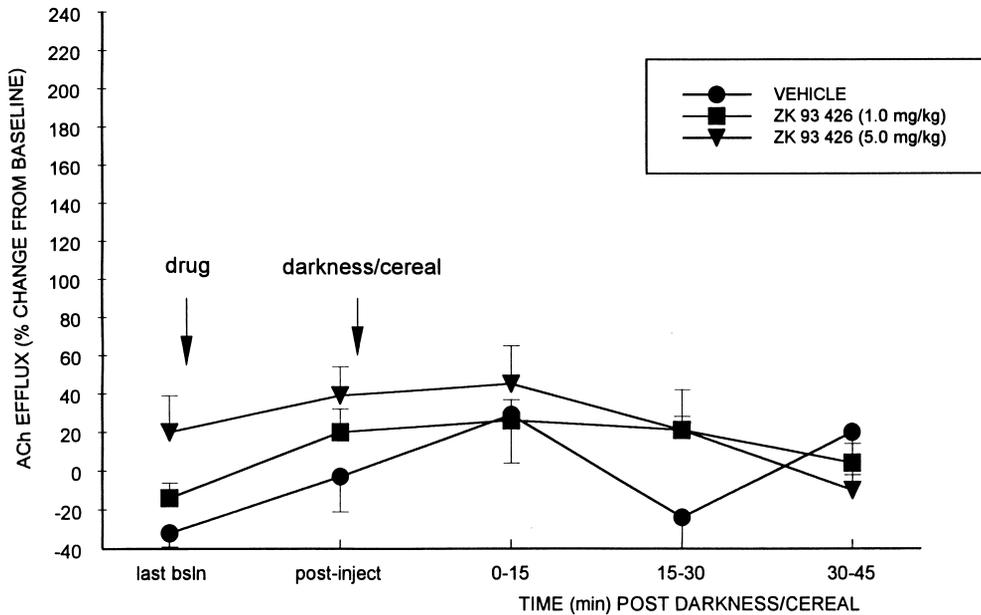


Fig. 5. Mean ( $\pm$  S.E.M.) stimulated cortical ACh efflux (percentage change from median baseline) in aged lesioned rats exposed to darkness/cereal 15 min after receiving vehicle or ZK 93 426 (1.0 or 5.0 mg/kg). Animals were lesioned with intra-basalis injections of the cholinotoxin 192 IgG-saporin. Dialysates were collected at 15-min intervals. Animals received each of the stimuli, in counterbalanced order, during three microdialysis sessions ( $n = 6$  rats/condition).

Table 1. Summary of ANOVA results for darkness/cereal/ZK 93 426-stimulated acetylcholine efflux

Effect	<i>F</i>	d.f.	<i>P</i>
Lesion	16.96	1,20	0.001
Age	7.49	1,20	0.013
Lesion $\times$ Age	2.83	1,20	0.108
Dose	8.95	2,40	0.001
Lesion $\times$ Dose	2.20	2,40	0.124
Age $\times$ Dose	0.55	2,40	0.579
Lesion $\times$ Age $\times$ Dose	0.38	2,40	0.687
Collection Interval	48.38	4,80	< 0.001
Lesion $\times$ Collection Interval	6.08	4,80	< 0.001
Age $\times$ Collection Interval	3.46	4,80	0.012
Lesion $\times$ Age $\times$ Collection Interval	0.42	4,80	0.794
Dose $\times$ Collection Interval	3.76	8,160	< 0.001
Lesion $\times$ Dose $\times$ Collection Interval	3.01	8,160	0.004
Age $\times$ Dose $\times$ Collection Interval	0.57	8,160	0.804
Lesion $\times$ Age $\times$ Dose $\times$ Collection Interval	2.20	8,160	0.030

Statistical summary of main effects and interactions following the ANOVA on data from the stimulation of cortical ACh efflux following exposure to darkness/cereal/ZK 93 426. The factors included: Condition (sham, lesion); Age (young, old); Dose (vehicle, 1.0 mg/kg ZK 93 426, 5.0 mg/kg ZK 93 426); and Collection Interval (last baseline, post-injection, 0–15, 15–30, 30–45 min following exposure to darkness/cereal); d.f., degrees of freedom.

Bonferroni corrected  $\alpha$ ). There was no overall significant difference between the 1.0 and 5.0 mg/kg doses of ZK 93 426 ( $t_{23} = 1.54$ ,  $P = 0.138$ ).

While the overall ANOVA also revealed main effects of Lesion (sham-treated rats were more responsive than lesioned rats to stimulation), Age (young rats were more responsive than aged rats), and several interactions involving these factors and Collection Interval (see Table 1), the critical statistical result given the hypothesis being tested is the significant interaction of Lesion  $\times$  Age  $\times$  Dose  $\times$  Collection Interval. Thus, the ability to stimulate cortical ACh efflux varied as a function of the rats' age, lesion status and the nature of the stimulus. A series of planned comparisons were conducted to reveal the sources of this complex interaction. Separate ANOVAs on the interaction between Lesion and Dose were conducted at each time-point (Collection Interval) for each of the two ages. With respect to the young rats, these analyses revealed no time-points in which there was a significant interaction between lesion status and dose (all  $P > 0.05$ ). These findings are supportive of the conclusion that partial deafferentations of cholinergic inputs in young rats did not influence the ability of these three complex stimuli to stimulate cortical ACh efflux.

However, similar analyses revealed significant Lesion  $\times$  Dose interactions in aged rats during the last baseline ( $F_{2,20} = 5.738$ ,  $P = 0.011$ ) and the

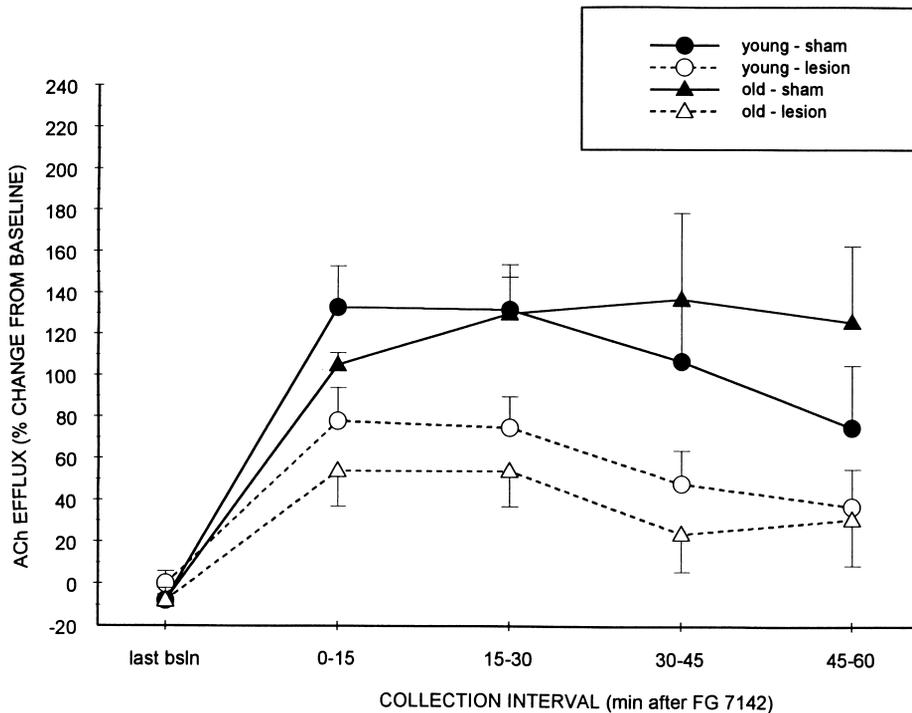


Fig. 6. Mean ( $\pm$  S.E.M.) stimulated cortical ACh efflux in young and aged, sham and lesioned rats following administration of FG 7142 (8.0 mg/kg, i.p.). This test was conducted on the fourth microdialysis session. Dialysates were collected at 15-min intervals ( $n = 6$  rats/condition).

30–45 min time-point ( $F_{2,20} = 10.705$ ,  $P = 0.001$ ), as well as a strong trend toward a significant interaction at the 0–15 min time-point ( $F_{2,20} = 3.255$ ,  $P = 0.06$ ). Planned comparisons revealed that these interactions reflected significant differences between basal efflux in sham and lesioned rats during the vehicle session ( $P < 0.033$ , Bonferroni  $\alpha$ ), efflux from 0 to 15 min in sham versus lesioned rats during both ZK 93 426 sessions ( $P < 0.033$ , Bonferroni  $\alpha$ ), and efflux from 30 to 45 min in sham versus lesioned rats during the ZK 93 426 (5.0 mg/kg) session ( $P < 0.033$ , Bonferroni  $\alpha$ ). Thus, in contrast to young rats, the partial deafferentation attenuated the stimulated release following certain conditions in aged rats.

**Effects of FG 7142.** The effects of age and lesion condition on FG 7142-induced cortical ACh efflux are summarized in Fig. 6. Administration of FG 7142 stimulated ACh efflux ( $F_{4,68} = 19.88$ ,  $P = 0.001$ ). Overall, each of the four collection intervals after administration of the drug differed significantly from the last baseline (all  $P < 0.04$ , Bonferroni corrected  $\alpha$ ). As was observed in the analysis of the effects of darkness/cereal/ZK 93 426, there was no main effect of Age ( $F_{1,17} = 0.02$ ,  $P = 0.894$ ). However, lesioned rats were less responsive than sham-treated controls to the effects of FG 7142 (Lesion:  $F_{1,17} = 10.61$ ,  $P = 0.005$ ; Lesion  $\times$  Collection Interval:  $F_{4,68} = 3.35$ ,  $P = 0.015$ ). Planned comparisons revealed that ACh efflux in lesioned

rats was reduced compared to that in sham animals at each of the four collection intervals after FG 7142 (all  $P < 0.04$ , Bonferroni corrected  $\alpha$ ). However, unlike the case with darkness/cereal/ZK 93 426, the partial deafferentation was no more effective in attenuating stimulated efflux in aged rats than in young rats (Lesion  $\times$  Age  $\times$  Collection Interval:  $F_{4,68} = 0.55$ ,  $P = 0.696$ ).

## DISCUSSION

These experiments revealed several important findings about the interactive effects of ageing and partial deafferentation on cortical ACh release. First, natural ageing *per se* does not appear to affect cortical ACh efflux under basal conditions. However, the effect of ageing on stimulated release depends upon the nature of the stimulus. There was an overall effect of age in response to the darkness/cereal/ZK 93 426 stimulus complex, but not in response to FG 7142. Second, the attenuating effect of partial deafferentation of corticofugal cholinergic neurons on cortical ACh release depends upon age at the time of the lesion (although future studies will need to dissociate the relative contributions of age at the time of lesion versus age at the time of testing). Rats lesioned as young adults maintained the capacity to enhance ACh efflux following the darkness/cereal/ZK 93 426 stimulus, whereas rats comparably lesioned as aged animals exhibited no such response. Third, the ability to detect Age  $\times$  Lesion

interactions in the regulation of cortical ACh release depended upon the nature of the stimulus. While aged lesioned rats were far less responsive than young lesioned rats to the stimulating effects of darkness/cereal/ZK 93 426, the two groups of animals exhibited similar changes in ACh efflux following administration of the BZR partial inverse agonist FG 7142. Each of these observations is discussed in detail below. Finally, there appears to be a synergism between the effects of age and deafferentation in response to certain types of stimulation. The marked inability of aged lesioned rats to increase cortical ACh efflux following the darkness/cereal/ZK 93 426 stimulus could not be predicted on the basis of the effects of age or lesion alone.

#### *Effects of age on cortical acetylcholine release*

**Basal conditions.** Our data demonstrate that basal ACh efflux in the frontoparietal cortex was comparable in young (four to seven months) and aged (24–28 months) sham-treated rats. These data replicate our previous demonstrations of a non-significant effect of age on basal cortical ACh efflux,<sup>18,22</sup> and are consistent with numerous *in vivo* microdialysis studies.<sup>10,14,17,30,34</sup> A smaller number of studies, however, have suggested age-related declines in basal cortical ACh efflux.<sup>4,39</sup> Several experimental factors may have contributed to the different results obtained in these studies, including differences among the aged rats in terms of strain, age and behavioral status, composition of the perfusion medium, use of transversal versus concentric probes, and the degree of habituation of the animals prior to testing.

**Stimulated conditions.** Similar to the results under baseline conditions, the present data suggest that aged sham-treated rats are as responsive to the stimulating effects of environmental and neuropharmacological manipulations as are young sham-treated animals. These data are consistent with several other reports, using *in vivo* methods, demonstrating a normal capacity for ACh release in aged rats following electrical stimulation of the basal forebrain,<sup>14</sup> behavioral activation,<sup>18</sup> systemic administration of muscarinic antagonists,<sup>18</sup> or local, intracortical perfusion of muscarinic antagonists.<sup>22</sup> There are, however, ageing-related deficits in the ability of local K<sup>+</sup> stimulation to enhance cortical<sup>22</sup> or hippocampal<sup>36</sup> ACh efflux. This attenuated response to K<sup>+</sup> stimulation in aged rats is not likely due to a general reduction in the capacity for synthesis/release of ACh, as the stimulated ACh efflux seen following local perfusion with atropine in aged rats far exceeded that seen even in young rats following local perfusion with K<sup>+</sup>.<sup>22</sup> There are obvious differences in the mechanisms underlying enhanced release following local depolarization and auto-receptor antagonism that might contribute to the

differential effects of ageing. Moreover, it is not clear whether the diminished responsivity to local K<sup>+</sup> reflects a compromised capacity of corticofugal cholinergic neurons in aged rats to respond to local depolarization or whether this effect reveals ageing-related changes in the sensitivity of local, non-cholinergic neurons modulating ACh release.<sup>1,22,34</sup> Collectively, these findings indicate that the detection of age-related impairments in stimulated cortical ACh release may depend upon the nature of the stimulus employed. This issue will reappear below in discussing the capacity for ACh release in aged rats following partial deafferentation.

#### *Stimulated release following partial deafferentation*

Rats that received an intra-basalis injection of the selective cholinotoxin 192 IgG-saporin as young adults exhibited a marked deafferentation of corticofugal neurons as evidenced by a significant reduction (i.e. 62%) in basal cortical ACh efflux. Despite this marked deafferentation, rats lesioned as young adults displayed a control-like enhancement of ACh efflux following exposure to the darkness/cereal/ZK 93 426 stimulus. Thus, the residual corticofugal neurons retained the ability to respond appropriately to this complex environmental/neuropharmacological stimulus. While the precise neuronal mechanisms underlying the ability of darkness/cereal/ZK 93 426 to stimulate cortical ACh efflux in intact rats have not been specified, it is likely that this stimulus reduces the degree of GABAergic inhibition on basal forebrain cholinergic neurons. Intra-basalis infusions of BZR ligands that positively modulate GABAergic transmission (i.e. chlor-diazepoxide) attenuate the effects of darkness/cereal on cortical ACh efflux, whereas BZR ligands that negatively modulate GABAergic transmission (i.e.  $\beta$ -methyl- $\beta$ -carboline-3-carboxylate) potentiate ACh efflux.<sup>20,31</sup>

Rats that received an intra-basalis injection of 192 IgG-saporin as aged adults failed to exhibit the increase in cortical ACh efflux following the darkness/cereal/ZK 93 426 stimulus observed in aged sham-treated controls or in animals partially deafferented as young adults. Decreases in basal efflux were comparable in rats lesioned at the two ages, and suggest that the interaction between age and lesion status was apparently not secondary to an enhanced vulnerability of aged animals to the neurotoxic effects of 192 IgG-saporin. It is important to determine whether rats sustaining a partial deafferentation of cortical cholinergic neurons as young adults would manifest similar deficits in stimulated ACh efflux if tested once reaching 24–28 months of age. Such an effect would further rule out the possibility that the Age  $\times$  Lesion interaction observed in the present experiment reflected an enhanced vulnerability to the effects of partial deafferentation on the regulation of cortical ACh release.

The neuronal mechanisms underlying the failure of aged lesioned animals to respond to an environmental/neuropharmacological stimulus that is sufficient to enhance release in aged sham-treated rats or rats lesioned as young adults are not clear. The lesion in the older rats apparently reveals a regulatory deficit in cholinergic transmission that is not evident in the intact, aged brain. The source(s) of this dysregulation could include limitations in the synthesis/release capacity of residual cholinergic neurons in the aged brain (an interpretation that is not supported by the ability of these same residual neurons to enhance their release following FG 7142), local, intracortical mechanisms regulating release of ACh at the level of the terminals, or multi-synaptic, cortical-subcortical loops.

Our assessment of the relative abilities of both darkness/cereal/ZK 93 426 and FG 7142 to stimulate cortical ACh release in young and old lesioned rats revealed the critical importance of the nature of the stimulus in highlighting the potency of age as an intervening variable in studying the regulation of cortical ACh release. While young lesioned rats retained a capacity for stimulated release following darkness/cereal/ZK 93 426 that was lost in aged lesioned rats, both groups exhibited a comparable (albeit reduced) increase in cortical ACh efflux following administration of FG 7142. The reasons for the differential sensitivities to the two stimuli are not clear. It is not simply the case that the injection of FG 7142 (8.0 mg/kg) is a more potent stimulus, capable of more effectively exciting a hyporesponsive residual cholinergic system, because the extent of the drug-induced increase in cortical ACh efflux in young and aged sham-treated rats is not greater

than the magnitude of the increase induced by darkness/cereal/ZK 93 426. The two stimuli may be affecting cortical ACh release via overlapping, but slightly different, neuronal systems or receptor subpopulations. In this regard, the present differences between ZK 93 426 and FG 7142 are consistent with our previous demonstration that administration of FG 7142 enhanced basal cortical ACh efflux,<sup>9,21</sup> whereas the BZR weak partial inverse agonist ZK 93 426 only potentiated stimulated efflux and did not affect basal levels.<sup>18,19,31</sup>

## CONCLUSIONS

The results of this experiment reveal the potency of using age as an intervening variable in studying the effects of ageing on cortical cholinergic transmission. Partial deafferentation of corticofugal neurons resulted in comparable depletions of basal cortical ACh. However, the residual cholinergic neurons in aged lesioned rats failed to exhibit the same degree of responsivity to an environmental/neuropharmacological stimulus as that seen in young lesioned rats. These data point to the limitations of age *per se* as a singular independent experimental variable in studies on the age-related declines in cortical cholinergic transmission, and suggest a fruitful experimental model for evaluating the effects of potential therapeutic agents.

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