ELSEVIER

Brain Research Reviews 27 (1998) 143–156

BRAIN RESEARCH REVIEWS

Full-length review

Age-related changes in rodent cortical acetylcholine and cognition: main effects of age versus age as an intervening variable

Martin Sarter *, John P. Bruno

Department of Psychology and Neuroscience Program, The Ohio State University, 27 Townshend Hall, Columbus, OH 43210, USA

Accepted 16 December 1997

Abstract

Evidence from aged and demented humans has stimulated research on the effects of age on the integrity of cortical cholinergic afferents in rodents. However, a comprehensive review of the available data does not consistently support the hypothesis that normal aging in rodents robustly affects the function of basal forebrain cholinergic projections to the cortex. These data indicate the limited significance of age as an independent experimental variable in research on age-related changes in cortical acetylcholine and associated behavioral or cognitive functions. Alternatively, recent studies demonstrated that normal aging in rodents potently interacts with the consequences of experimental manipulations of this system. Thus, aging acts as an intervening variable in experiments designed to elucidate age-related changes in the vulnerability and restorative capacity of this neuronal system after injury and degenerative processes. Investigations of the interactions between the effects of age and the capacity of the cholinergic systems to respond to detrimental processes reveal robust consequences of aging on cortical acetylcholine and the cognitive functions mediated by this neuronal system.

Keywords: Aging; Acetylcholine; Cortex; Basal forebrain; Rodent

Contents

* Corresponding author. Fax: +1-614-688-4733; E-mail: msarter@magnus.acs.ohio-state.edu

0165-0173/98/$19.00

1. Introduction

Evidence supporting the hypothesis that the integrity of the basal forebrain cholinergic neurons that project to cortical areas decline during normal human aging and dementia has been frequently reviewed [10,36,80]. Markers of cortical acetylcholine (ACh) such as choline acetyltransferase (ChAT) activity or hemicholinium binding decrease robustly in senile dementia and during normal aging in humans [27,28,38,75,82,107]. Likewise, counts of cholinergic cell bodies in the basal forebrain reveal a loss ranging from 25–70% in elderly, healthy people and greater losses in demented patients [14,26,55,62,102,121]. The strongest support for a critical role of cortical ACh in age- and dementia-associated cognitive decline has been provided by studies demonstrating correlations between decreases in markers of cortical ACh, but not of other cortical transmitter systems, and the severity of dementia [24,76–78,81,83]. Additional lines of mostly indirect evidence, including the effects of anticholinergic drugs in intact, aged, or demented humans and the behavioral effects of basal forebrain lesions in animals support the hypothesis that the cognitive decline in normal aging and in dementia, at least to some extent, reflects the loss of basal forebrain cholinergic neurons (for review, see Refs. [19,23,32,35,36,51,72]). The resulting 'cholinergic hypothesis' received considerable criticism in recent years, largely because of the limited effects of cholinomimetic drugs to alleviate the cognitive impairments associated with age or dementia. This and other criticisms have been addressed previously (see Ref. [92]) and are likely related to insufficiently explored complexities in the pharmacology of cholinergic transmission and the functions of cortical ACh [39,91,95].

As a result of the findings in aged and demented humans, research on the relationship between age-related changes in cognitive functions and the cholinergic system in rodents has intensified in recent years. This focus on aged animals has been, at least in part, motivated by the belief that 'natural' animal analogues of the age-related decline in cortical cholinergic functions and in cognitive abilities are necessary to advance research on the 'cholinergic hypothesis'. While this assumption appears intuitively correct, the commentary below discusses its limitations as well as research strategies that better utilize age as an independent variable in animal experiments in this area. First, the evidence supporting a decline in cortical cholinergic function in aged rodents is discussed. Second, the usefulness of normal aging as an independent experimental variable will be evaluated. Third, we will present the position that a focus on interactions between normal aging and the responses of the brain to insult and degeneration may provide a more valid approach toward understanding the role of cortical ACh in age- and degeneration-related cognitive disorders. Recent studies revealing the potential of age as an intervening variable will be explored.

It is important to note that the review of the available data and the discussions below are restricted to the cholinergic system innervating cortical areas, and that conclusions may not be readily generalized to other transmitter systems or cholinergic target systems (e.g., [47,48]). The focus of this discussion on cholinergic inputs to the cortex is based on the massive innervation of the cortex by ACh and the hypotheses about the role of this input in age- and dementia-related impairments in cognition (see above; see also Ref. [93]). Normal aging, rather than representing a singular revealing variable concerning the functions of cortical ACh in aged subjects, may play a more significant role as an intervening variable. Experimental approaches to test this general hypothesis focus on the age-related capacities of the cholinergic system to respond to damage and degenerative developments.

2. Cortical acetylcholine in normal aged rodents

2.1. Basal forebrain cholinergic neurons

The available evidence from studies on the number of cholinergic neurons in the area of the nucleus basalis of Meynert/substantia innominata reveals possible species-, strain-, age-, and method-specific differences in the effects of age on neuron number (see Table 1; see Ref. [16] for anatomical terminology). As summarized in Table 1, the available data on age-related differences in the number of basal forebrain cholinergic neurons in rats are inconclusive. Notably, data on the effects of age on the number of neurons in other brain areas of rodents (e.g., see Ref. [37]) and in the basal forebrain of aged monkeys are equally complex [63,88,117], suggesting the possibility of fundamental differences between the effects of age in humans and animals on the number of neurons in the basal forebrain and possibly in other brain areas. In addition to the absence of robust effects of age on neuronal number, the available data do not support the view that there is a greater degree of cholinergic cell loss in behaviorally impaired animals [8,33]. Moreover, correlations between, for example, escape latency in the water maze [8,33] or response latencies in passive avoidance tasks [85] and cholinergic cell loss in the basal forebrain generally are not expected as these behavioral measures may not depend on the integrity of the basal forebrain cholinergic system [11,112,119].

The available studies, however, suggest changes in the morphological appearance of basal forebrain cholinergic neurons, including increases and decreases in the size of neurons and in the overall size of the region of cholinergic cell bodies [3,8,33,44,64]. The functional implications of such changes in morphological appearance are unclear. Interpretations in terms of age-related vulnerability and

Table 1
Age-related changes in the number of cholinergic neurons in the basal forebrain

Strain	Ages (months)	Behavioral paradigm impaired/unimpaired was based on:	Counting method	Age (months): % change from young	Study
Kuo–Wistar	3, 26	passive avoidance	ChAT-IR; not further specified	UI: −9.4 (ns); I: −29.2 (s)	Riekkinen et al., 1992 [85]
Sprague–Dawley	3, 12, 18, 30	Morris water maze	ChAT-IR; various morphological criteria and Abercrombie correction	12 UI: −19 (ns); 18 UI: −19 (ns); 18 I: −15.8 (ns); 30 I: −45 (s)	Fischer et al., 1991 [33]
Sprague–Dawley	6, 24–33	n/a	ChAT-IR; $p75^{NTR}$; AChE-stain; Abercrombie correction	24–33: ChAT: −26 (s); p75: −33.5 (s)	De Lacalle et al., 1996 [25]
Fisher-344	3, 23	n/a	AChE-stain; automated image analysis	23: −3.9 (ns)	Luine et al., 1986 [59]
Fisher-344	6, 27, 33	Morris water maze	ChAT-IR; morphometric analysis	27 UI: −4.7 (ns); 33 UI: −15.8 (ns); 27 I: +7 (ns); 33 I: +1.4 (ns)	Armstrong et al., 1993[a] [8]
Wistar	3, 24	n/a	AChE-stain	24: −56.3 (s)	Altavista et al., 1990 [3]
CD-1 mice	3, 24	n/a	ChAT-IR; automated image analysis	24: −23.5 (ns)	Mesulam et al., 1987 [64]
C57Bl/BNNIA mice	7, 15, 53	n/a	AChE-stain; grid counting method	15: +0.13 (ns); 53: +2.23 (ns)	Hornberger et al., 1985 [44]

Basal forebrain = restricted here to basal nucleus of Meynert and substantia innominata.
I = behaviorally impaired.
UI = behaviorally unimpaired.
ChAT-IR = choline acetyltransferase immunoreactivity.
$p75^{NTR}$ = antibody against the low-affinity neurotrophin receptor.
% change = depicts the % change from the mean number of neurons in young animals; if such values were not provided in the original studies, they were calculated on the basis of group means.
n.s. = not significant as indicated in the study; s = significant as indicated in the study.
[a] Based on their data on the substantia innominata, pars ventralis, their data on pars dorsalis similarly did not differ between groups (see table 3 in Ref. [8]).

associated decline in cortical cholinergic transmission, while intuitively attractive, require confirmation by more direct measures of presynaptic cholinergic function.

2.2. Cortical ChAT activity

ChAT activity does not represent a critical rate-limiting step in the synthesis of ACh [15,22,96] and, as such, does not provide a particularly sensitive measure of cholinergic transmission. Measures of cortical ChAT activity, however, have proven useful for estimating the number of cholinergic cells and cholinergic terminals in histochemical studies and neurochemical studies [31,34,113]. As would be expected from the absence of an effect of age on the number of cholinergic neurons in the basal forebrain (see Section 2.1), the results from studies measuring cortical ChAT activity also do not reveal consistent changes in the cortex of normal aged rodents (see Table 2). As mentioned above, variability in the strains of animals and methods (e.g., histochemical vs. neurochemical analyses) used to measure ChAT activity may contribute to some inconsistencies in the data. Furthermore, differences in the age-related overall pathological status of animals of different strains likely increase the variability in the findings [84,101]. Collectively, however, the available evidence does not allow rejection of the hypothesis that the number of cortical cholinergic afferents are unchanged in aged rodents.

2.3. Sodium-dependent high affinity choline uptake (HACU)

HACU represents the rate-limiting step in the synthesis of ACh and may provide a more sensitive measure of cholinergic activity than ChAT activity [15,22]. While the number of studies measuring cortical HACU in aged rodents has remained small, the available data indicate that cortical HACU in aged rodents is not decreased. For example, Lebrun et al. [54] did not find differences in basal HACU in the frontal cortex of C57BL/6 mice aged 9 and 96 weeks. Moreover, they observed that radial arm maze testing increased cortical HACU to a similar degree in young and aged animals. Meyer et al. [66] compared cortical choline uptake between 5- to 6- and 23- to 25-month-old Fischer 344 rats and found no difference (see also the study by Sirviö et al. [99] who measured cortical choline uptake in Kuo–Wistar rats aged 10–11 or 24–25 months). Thus, the ability of cortical cholinergic terminals to incorporate choline via this sodium-dependent carrier appears unaffected by age, suggesting a normal capacity for ACh synthesis.

2.4. Cortical ACh release

The most dynamic measure of the functional integrity of cortical cholinergic afferents involves the measurement of ACh release. With the exception of the results from two studies, both by Pepev and co-workers [17,123], one in Wistar rats and one in an undefined strain of rats obtained from Charles River [17], the available data suggest that basal or resting cortical ACh efflux does not differ between young and aged rats (see Table 3). These two studies [17,103] used transversal dialysis probes and extremely high concentrations of physostigmine in the perfusion fluid (7 μM), rendering the data difficult to interpret because of the extensive autoreceptor stimulation resulting from the high concentrations of extracellular ACh under these conditions (e.g., [116]). The findings listed in Table 3 suggest that normal aging does not affect basal cortical ACh release in rodents.

However, age-related differences in the capacity of cortical cholinergic terminals to respond to particular types of stimulation have been found (Table 4). While most types of stimulation used in these experiments (electrical stimulation, stimulation by muscarinic autoreceptor blockade, trans-synaptic stimulation with benzodiazepine receptor antagonists and inverse agonists) revealed no deficits in ACh release in aged animals, stimulation with high potassium/calcium concentrations showed a reduced potency for increasing ACh release in vitro from cortical synaptosomes of aged rats [65]. Similarly, ACh release measured in vivo from the frontoparietal cortex of aged rats was found to be less sensitive to the stimulating effects of high K^+/Ca^{2+} than that of young rats [70]. Interestingly, administration of atropine in this study (through reversed microdialysis) did not reveal age-related differences in ACh efflux although atropine-induced increases in efflux were approximately 3 times higher than those produced by high K^+/Ca^{2+} [70] (see Table 4).

As discussed in Moore et al. [70], the absence of age-related differences in ACh efflux following the administration of atropine prohibits the speculation that the differences in potassium-stimulated ACh efflux were due to age-related limitations in the overall capacity of cortical cholinergic inputs to release ACh. As potassium indiscriminately increases the release of other transmitters in the perfused area, differences in the sensitivity of other cortical neurons to depolarization may have contributed to this age-related finding. For example, Abdulla et al. [1] demonstrated that iontophoretical application of the $GABA_A$ receptor antagonist bicuculline, but not of the muscarinic agonist carbachol, revealed age-related differences in the excitability of cortical neurons. The speculation that age-related changes in the responsivity of noncholinergic cortical neurons contributed to the diminished stimulatory effects of high K^+/Ca^{2+} is supported by Meyer et al. [65] who demonstrated that the *maximum* ACh release from synaptosomes was not affected by aging.

2.5. Cortical ACh in aged animals: conclusions

The available data favor the hypothesis that normal aging in rodents does *not* robustly or consistently affect

Table 2
Age-related changes in cortical ChAT activity

Strain	Ages (months)	Cortical area(s)	Age (months): % change from young	Study
Fisher	3, 24	frontal cortex	24: −9.2 (s)	Haba et al., 1988 [40]
Fischer 344	4, 12, 24	cerebral cortex	12: +6.25 (ns); 24: +13.3 (ns)	Waller and London, 1989 [118]
Fischer 344	3–2, 23–24	frontal cortex	23: +6.6 (ns)	Nakamura and Ishihara, 1989 [73]
Fischer 344	3, 24	frontal cortex enthorinal cortex	24: +1.2 (ns); 24: 0 (ns)	Luine and Hearns, 1990 [58]
Fischer 344	3–4, 24–26	cerebral cortex	24: −15 (s)	Michalek et al., 1989 [67]
Fischer 344	4, 26	cortex	26: +9 (ns)	Schwartz et al., 1990 [98]
Fischer 344	3, 21	frontal cortex	21: −13.6 (ns)	Tandon et al., 1991 [109]
C57BL/6J mice	6, 12, 30	cerebral cortex	12: −22.2 (ns); 30: +22.2 (ns)	Strong et al., 1980 [106]
Sprague–Dawley	3, 22–24	cortex	22 I: [a](ns); 22 SI: [a](ns)	Hellweg et al., 1990 [41]
Sprague–Dawley	3, 21	frontal cortex	21 I[b]: −6.9 (ns); 21 UI[b]: −6.9 (ns)	Abdulla et al., 1995 [1]
Sprague–Dawley	10, 18, 26	cerebral cortex	18: −28.6 (s); 26: −53.6 (s)	Strong et al., 1980 [106]
Sprague–Dawley	6–7, 15–16, 23–26	frontal cortex	15: −8.7 (ns); 23: −14.4 (s)	Stone et al., 1989 [105]
Sprague–Dawley	1, 23	frontal cortex	23: −31.8[c] (s)	Thal et al., 1991 [111]
Wistar	8, 26	cerebral cortex	26: 0 (ns)	Ingram et al., 1981 [45]
Wistar	4, 31	anterior cingulate posterior cingulate	31: −15.9 (s); 31: −7.6 (ns)	Biegon et al., 1986 [13]
Wistar	3–4, 24–25, 32–33	cerebral cortex	24: [a](ns); 33: [a](ns)	Michalek et al., 1989 [67]
Long–Evans	3, 9, 27	frontal cortex	9: [a](ns); 27: −37 (s)	Araujo et al., 1990 [4]

For abbreviations see Table 1. SI: severely impaired.
Note that the spelling of the names of the strains varies and follows the spelling in the individual studies.
[a]Value could not be reliably determined.
[b]I/UI/SI: based on Morris water maze.
[c]Calculated from their figure 1C.

Table 3
Age-related changes in basal cortical ACh release

Strain	Ages (months)	Method	Cortical area(s)	Aged rodents: % change from young	Study
Sprague–Dawley	3, 24	slices; guinea pig ileum bioassay	cerebral cortex	24: 0 (ns)[a]	Pedata et al. 1983 [79]
Long–Evans	3, 9, 27	slices; radioenzymatic assay	frontal cortex	9: −12.5 (ns); 27: −25 (ns)	Araujo et al. 1990 [4]
Fischer 344	6, 24	from synaptosomes; radioenzymatic assay	cerebral cortex	24: −5.6 (ns)	Meyer et al. 1984 [66]
Fischer 344	6, 24	from synaptosomes	cerebral cortex	24: −28 (ns)[b]	Meyer et al. 1986 [65]
Wistar	2, 18	in vivo microdialysis; transversal probe; 7 μM physostigmine; radioenzymatic assay;	frontal cortex	18: −35 (s)	Wu et al. 1988 [123]
Charles River[d]	3, 19	in vivo microdialysis; transversal probe; 7 μM physostigmine; HPLC	parietal cortex	19: −39 (s)	Casamenti et al. 1991 [17]
Fischer 344	6–8, 27–28	in vivo microdialysis in anesthetized rats; probe type not defined; 100 μM physostigmine; HPLC	parietal cortex	27: −4.5 (ns)	Kurosawa et al. 1989 [53]
Fischer 344	4–9, 18–23	in vivo microdialysis in awake animals; concentric probe; 5 μM neostigmine; HPLC	frontoparietal cortex	18: −12.5 (ns)	Moore et al. 1992 [69]
BNNia/F344	4, 22	in vivo microdialysis in awake animals; concentric probe; 0.5 μM neostigmine; HPLC	frontoparietal cortex	22: +50 (ns)[c]	Moore et al. 1996 [70]
Wistar–Imamichi	3–4, 23–34	in vivo microdialysis in awake animals; concentric probe; 10 μM eserine; HPLC	prefrontal cortex	light phase: +55 (ns); dark phase: −12 (ns)	Mitsushima et al. 1996 [68]

For abbreviations, see Table 1.
[a] Estimation based on their figure 2.
[b] Estimation based on their figure 1.
[c] Baseline ACh efflux was 0.22 ± 0.04 pmol/min in 4 month-old rats and 0.33 ± 0.15 pmol/min in aged rats. Note that, while the variability of ACh efflux in aged rats was considerable, the trend was for higher efflux.
[d] Strain not defined.

Table 4
Age-related changes in stimulated cortical ACh release

Strain	Ages (months)	Method	Cortical area(s)	Stimulus, aged rodents: % change from young	Study
Sprague–Dawley	3, 24	slices; electrical stimulation (1–10 Hz); guinea pig ileum bioassay	cerebral cortex	10 Hz, 24: −32 (s)[a]	Pedata et al. 1983 [79]
Long–Evans	3, 9, 27	Slices; K^+-stimulated; radioenzymatic assay	frontal cortex	K^+, 9: +6.6 (ns); K^+, 27: −26.6 (s)	Araujo et al. 1990 [4]
Fischer 344	6, 24	Synaptosomes; radioenzymatic assay; K^+-stimulated	cerebral cortex	K^+, 24: −12.9 (s)[b]	Meyer et al. 1984 [66]
Fischer 344	6, 24	Synaptosomes; K^+ and calcium ionophore (CI)-stimulated	cerebral cortex	K^+, 24: −28.6 (s)[c]; CI, 24: −33 (s)[c,d]	Meyer et al. 1986 [65]
Fischer 344	6–8, 27–28	in vivo microdialysis in anesthetized rats; probe not defined; 100 μM physostigmine; HPLC; electrical stimulation of nucleus basalis (ES)	parietal cortex	ES, 24: +37 (ns)[e,f]	Kurosawa et al. 1989 [53]
Fischer 344	4–9, 18–23	in vivo microdialysis in awake animals; concentric probe; 5 μM neostigmine; HPLC; stimulation by ZK 93426 (ZK)	frontoparietal cortex	ZK, 18: +31.8 (s)[e]	Moore et al. 1992 [69]
BNNia/F344	4, 22	in vivo microdialysis in awake animals; concentric probe; 0.5 μM neostigmine; HPLC; stimulation by intracortical atropine (A) and K^+/Ca^{2+} (KC)	frontoparietal cortex	A, 22: +41.3 (ns)[e,g]; KC, 22: −40 (s)[e]	Moore et al. 1996 [70]

For abbreviations, see Table 1.
[a]Estimation based on their figure 2.
[b]Estimation based on their figure 1 at 0 μM oxotremorine.
[c]Estimation based on their figure 1.
[d]The potency, but not the efficacy of the calcium ionophore A 23187 was reduced in aged animals, suggesting that the releasable pool was unaffected by age.
[e]Calculated by computing the mean absolute release based on their % change numbers and absolute baseline release numbers, followed by a calculation of the % group difference.
[f]At 500 μA.
[g]At 1 μM atropine.

the presynaptic function of cholinergic neurons terminating in the cortex. This conclusion does not reject the possibility that age affects postsynaptic cholinergic receptor mechanisms in rodents, although this hypothesis is not well supported by the available evidence on the effects of age on cortical muscarinic M1 or nicotinic receptors [4,9,12,40,52,56,57,67,98,100,108,109,118]. More importantly, however, age-related changes in postsynaptic receptor mechanisms represent a research subject separate from attempts to utilize aged animals to investigate aspects of the 'cholinergic hypothesis', specifically because cortical postsynaptic muscarinic receptors appear largely spared in aged and demented humans [24,50,60,86,97]. Finally, as it is concluded that the function of cortical cholinergic afferents is not reliably impaired in aged rodents, it is important to reiterate the view that the sporadically reported correlations between cholinergic markers and behavioral performance are difficult to reconcile because the behavioral measures employed most frequently to establish these correlations (Morris water maze performance, passive avoidance) recently were found not to be sensitive to cholinospecific lesions of the basal forebrain in rats [11,21,112,119].

3. Normal aging as an independent experimental variable

Experiments involving aged rodents typically intend to reveal age-related differences in biological and/or behavioral measures, i.e., age per se represents the independent variable in such experiments. Traditionally, in research on the role of age-related changes in the cholinergic system on behavioral or cognitive abilities, variation of age is presumed, often implicitly, to equate with variation in the integrity of the cholinergic system. As discussed above, the basis for this assumption is not well developed and age as an independent variable may be of limited significance with respect to the integrity of cortical cholinergic afferents in rodents.

Additional considerations support this view. As the goal of traditionally designed experiments is to relate age-related changes in neuronal mechanisms to changes in behavioral or cognitive variables, and as the systematic variation of the independent variable represents the minimum requirement for statistical inference, the usefulness of 'age' as an independent variable depends on the determination of a defined relationship (linear, quadratic, etc.) between chronological age and changes in biological mechanisms. Testing multiple time points in animal aging studies clearly represents an elementary condition to establish such relationships [18,87]. In reality, however, such relationships between aging and neurobiological mechanisms have been rarely established and, as discussed above, are not available with respect to the basal forebrain cholinergic projection to the cortex.

The basis for the lack of robust effects of normal aging in rodents on the basal forebrain cholinergic system remains a matter of speculation. Possible relevant explanations may range from the limited maximal life span of the inbred strains typically used for this research, to environmental and nutritional housing conditions of laboratory rodent strains.

4. Normal aging as an intervening experimental variable

The effects of normal aging in rodents on the integrity of the cholinergic basal forebrain system are hypothesized to be revealed by experiments testing the role of this system in the response to experimentally induced alterations in the function of cortical cholinergic afferents ([36,61,93]; see also the discussion in Ref. [20]). This approach generally assumes that normal aging in rodents affects the basal forebrain cholinergic system, the demonstration of which, however, requires an interacting, additional 'provocation' of this system. Such an additional manipulation is hypothesized to interact with an age-related increase in the vulnerability of the cholinergic system that reveals itself by an augmented effect of such a manipulation in aged animals, or by a reduced capacity of the aged cholinergic system to recover from, or compensate for, the detrimental effects of the experimental manipulation of this system. In terms of experimental design, such an alternative strategy is to test the effects of aging as an intervening variable.

Compromising the cholinergic system with acute experimental manipulations (e.g., brain lesions) may reveal interactions between age at the time of trauma and brain damage that indicate an enhanced vulnerability associated with aging. This age-related vulnerability in experimental models may underlie the relatively late expression of behavioral dysfunctions associated with insidious progressive neurodegenerative diseases [2,5–7,46].

Several issues concerning the optimization of studies focusing on the effects of aging as an intervening variable in relations between cortical cholinergic transmission and cognitive behavior deserve consideration. First, studies examining the neurobehavioral effects of some acute trauma at various ages should demonstrate a significant interaction between age (at the time of insult) and the brain damage itself. This 'age by trauma' interaction is necessary to conclude that the aging process is associated with an altered vulnerability to the neurobehavioral effects of brain damage. Second, it is important to complement the trauma studies in aged animals with studies in which the effects of early brain damage are assessed at different stages of the aging process. Given our lack of understanding of the etiology of various progressive neurodegenerative diseases it is overly restrictive to limit the age at

which the damage is sustained. Aging may reveal a vulnerability for the unmasking of brain damage that occurred significantly earlier in development. Third, the neurobiological measure of cholinergic transmission should be sensitive enough to reveal functional impairments. While no individual measure is necessarily sufficient to reveal deficits in cholinergic transmission, caution should be taken not to overinterpret the functional effects of aging or age–trauma interactions on traditional measures such as ChAT or AChE activity (see above). Such changes may, under certain circumstances, be sufficient to speculate on the degree of cholinergic deafferentation. However, without a more thorough knowledge of the compensatory mechanisms following subtotal destruction of cholinergic inputs and the manner in which this plasticity may change with age, measures of ChAT or AChE activity may have limited potential in reflecting posttraumatic recovery processes (see also Refs. [103,114]). It will be important to generate more in vivo neurochemical studies and ideally couple these studies with information about the postsynaptic effects of acetylcholine (i.e., electrophysiology; e.g., [94]). Finally, the behavioral paradigms selected should clearly be sensitive to manipulations of the basal forebrain–cortical cholinergic system. As stressed above, several studies focused on the putative relationship between age-related changes in cortical ACh and variables of behavioral performance which are not selectively sensitive to disruptions of cholinergic transmission. A corollary to this issue is that the traumatic manipulation used to compromise cholinergic transmission should be as selective as possible for cholinergic neurons. While these issues may appear obvious, and while there is a striking paucity of studies in which age is used as an intervening variable in studying the neurobehavioral effects of compromised cholinergic transmission, a limited number of available experiments indicate that such research approaches and experimental designs are productive [74,103,104,110,115, 120,124].

A recent study on the interactions between the effects of age and detrimental manipulations of the cortical cholinergic afferent system confirmed the general assumption that age-related effects in cortical ACh are potently revealed in experiments treating age as an intervening variable. A partial loss of cortical cholinergic inputs was previously shown to reduce, as expected, basal cortical ACh release measured in vivo using microdialysis in young adult animals ([29]; it should be noted that loss of cortical cholinergic inputs in this study was produced by multiple intracortical infusions of 192 IgG-saporin; see also Ref. [43]). Moreover, various behavioral and pharmacological manipulations designed to increase cortical ACh release demonstrated that, in terms of ACh release relative to baseline, partial loss of cortical cholinergic inputs did not affect the capacity of the residual system to respond to such challenges (for details see Ref. [29]). In a more recent study [30], partial loss of cortical cholinergic inputs was produced by infusions of 192 IgG-saporin into the basal forebrain of young-adult (4–7 months of age) and aged (24–28 months of age) F344/Brown–Norway rats. Similar to the results of previous experiments (see Table 3), baseline ACh release did not differ in young and aged sham-lesioned animals. Furthermore, the partial loss of cortical cholinergic inputs resulted in identical decreases in cortical ACh release in young and aged animals (in terms of absolute values, see Fig. 1)

In order to test the capacity of the residual cholinergic system to respond to stimulation, rats were exposed to a previously entrained complex stimulus of exposure to darkness coupled with palatable food reward (‘lights-out’ in Fig. 2). We have previously demonstrated that this stimulus leads to enhanced cortical ACh efflux in intact rats [29]. Moreover, the stimulus also produces elevated cortical ACh efflux in young rats with partial cortical cholinergic deafferentations and this stimulation can be potentiated by administering the BZR weak or selective inverse agonist ZK 93426 ([29]; see also Refs. [69,90]). Similar to previous experiments assessing the effects of such drugs on cortical ACh in aged animals [69], drug-induced cortical ACh efflux in young and old sham-lesioned animals were identical and, similar to the findings reported in Fadel et al. [29], drug-induced cortical ACh efflux in young, lesioned animals was similar, relative to baseline, to the increase observed in young and old sham-lesioned rats. However, in old, lesioned rats, increases in ACh efflux were severely attenuated following either treatment. Fig. 2 illustrates the interactions between age and partial cortical cholinergic deafferentation on activated cortical ACh release.

It is important to reiterate that the baseline ACh release in young-lesioned and old-lesioned animals was identical. Furthermore, inspection of AChE-stained section indicated comparable residual cholinergic fiber densities in the area surrounding the dialysis probe in the frontoparietal cortex. Thus, the limited ability of residual cortical cholinergic afferents in old-lesioned rats to respond to stimulation could not be attributed to an overt increase in the vulnerability of aged animals to this neurotoxin. Rather, these data suggest, in general terms, that the regulation of cortical ACh release in aged, lesioned animals was fundamentally altered compared to young, lesioned animals. It is unclear whether this was a result of a recovery process (not revealed by the residual AChE-positive fiber density or by basal ACh release) following the lesion in young animals that was limited by age (e.g., [5,6]), or whether the lesion accelerated an age-related detrimental process in the regulation of cortical ACh release. In either case, the potential mechanisms mediating the interactions between the effects of age and lesion range from the presynaptic regulation of synthesis and release, the afferent innervation of cortical cholinergic synapses or basal forebrain somas [33], to changes in cellular mechanisms of cholinergic neurons (e.g., [71]).

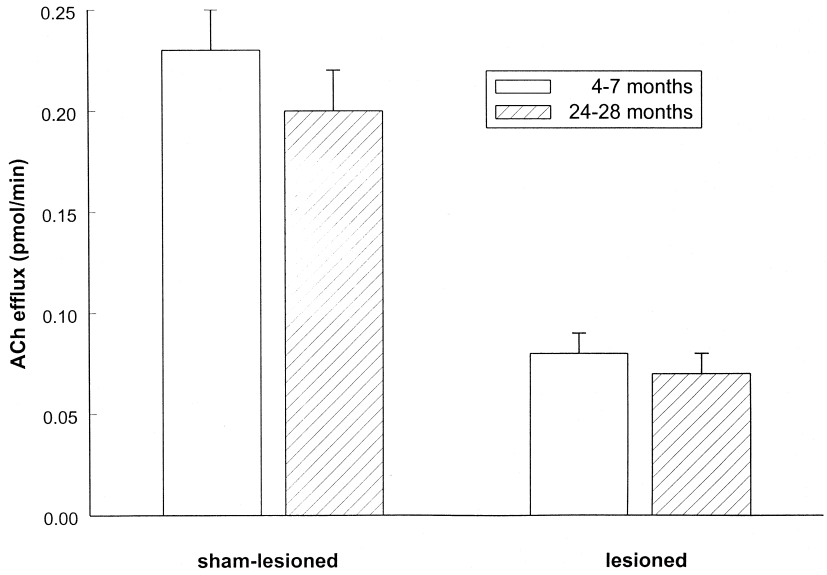

Fig. 1. Baseline frontoparietal ACh release in F344/BNNia rats aged 4–7 or 24–28 months ($n = 6$ per group). The data shown represent the averages over three different dialysis sessions [30]. Animals were dialyzed 2–3 weeks after the infusion of vehicle or 192 IgG-saporin into the basal forebrain (0.56 μg/1.0 μl/hemisphere; for details about the microdialysis method see Ref. [29]). Similar to our previous studies (e.g., [69]), basal ACh release in the frontoparietal cortex was identical in young and old, sham-lesioned animals. The lesion resulted in a similar decrease in ACh release in young and aged animals. Inspection of AChE-fiber-stained sections also indicated a comparable loss in cholinergic inputs to the frontoparietal cortex of young and aged rats (not shown).

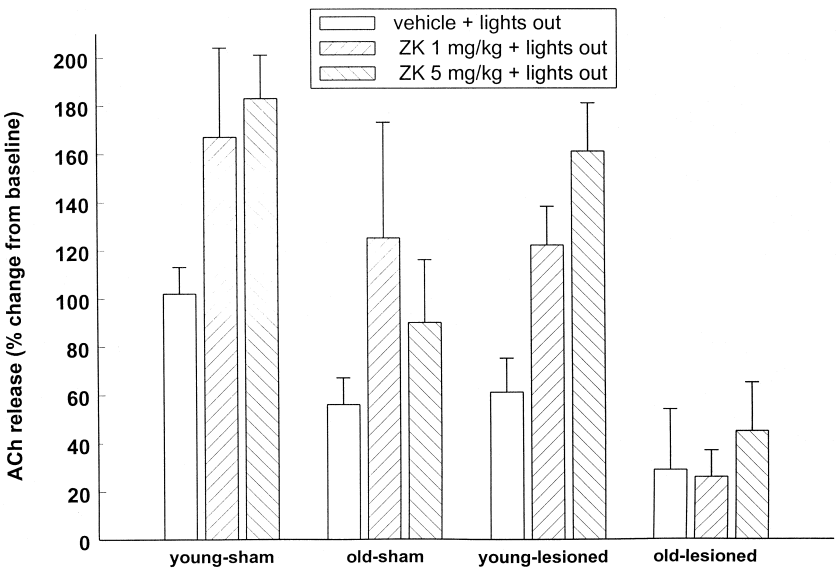

Fig. 2. Effects of the administration of the benzodiazepine receptor selective inverse agonist ZK 93426, in interaction with the presentation of a darkness–palatable food stimulus on cortical ACh release. As discussed previously (e.g., [91]), the stimulatory effect of ZK 93426 on cortical ACh depends on the level of activity in the cholinergic system. Therefore, animals were pretrained to associate darkness in the room with the presentation of palatable food for 7 days (1 trial/day). This stimulus results in a reliable, 60–100% increase in cortical ACh release (see open bars which depict the effects of vehicle and darkness/palatable food). The effect of this stimulus interacts with the ability of ZK 93426 to further increase cortical ACh release (see also Ref. [90]). The data shown in this figure are expressed as % change from baseline for each group of animals (ordinate). The figure depicts ACh release measured during the first collection interval (15 min duration) following the presentation of the darkness/fruitloop-stimulus, the latter occurred 15 min after the treatment with vehicle or drug (data taken from Ref. [30]). Statistical analyses revealed an interaction between the effects of age and lesion on stimulated cortical ACh release. This interaction was due to an attenuated increase in ACh release in aged, lesioned animals when compared with the other three groups. Compared to previous studies on the effects of age on cortical ACh release (see Tables 3 and 4), these data illustrate the significance of age as an intervening variable in experiments intended to reveal age-related changes in cortical ACh and, when compared to the data shown in Fig. 1, the critical importance of challenging manipulations to reveal the interactions between the effects of age and loss of cortical cholinergic inputs.

Several avenues will assist in revealing the nature of this interaction. For example, young-lesioned animals may exhibit an attenuation of activated ACh release when they reach the age of 24–28 months, demonstrating that the interaction between the effects of age and the lesion does not depend on the time of the lesion. If this is the case, age-related increases in the acute vulnerability to the toxin can be excluded as a confounding factor. The extent to which the above described interaction depends on the particular toxin (i.e., 192 IgG-saporin) used in this experiment, or whether it is generalizable to a large variety of insults to this system, also needs to be assessed. Furthermore, it will be important to test hypotheses about the generalizability of the age-related attenuated increase in cortical ACh release in lesioned animals by assessing the effects of other pharmacological and behavioral stimuli. Pharmacological stimuli may include presynaptic manipulations (such as cholinergic autoreceptor blockade) or local depolarization of afferents of cholinergic terminals in the cortex (see Refs. [69,70]). Behavioral stimuli previously used to activate cortical ACh release range from rather simple manipulations such as the presentation of a conditioned stimulus for palatable food [29,69,90] to performance in attentional tasks known to depend critically on the integrity of the basal forebrain cholinergic system [42,61,93,94,114]. As cellular changes observed in the nucleus basalis and in the terminal areas of cholinergic projections in Alzheimer's disease raise the possibility that a pathological process precedes the manifestation of symptoms [49,89], i.e., that the loss of cholinergic neurons represents a developmental process that escalates and manifests during aging, the investigation of interactions between the effects of age and other detrimental manipulations of this system may provide important insights in the mechanisms mediating the effects of age on this neuronal system.

5. Conclusions

The view that chronological age is a poor indicator of biological age has been previously discussed (see Ref. [20]). As reviewed above, the chronological age of rodents per se does not predict robust biological aging of the cortical cholinergic afferents. The reasons for the limited sensitivity of this system to the effects of age in rodents are a matter of speculation and include the possibility that laboratory rodent strains do not age sufficiently to develop significant age-determined changes in cortical cholinergic inputs. However, accumulating data indicate that an age-related process is progressing in this system and in this species. While the effects of this process remain difficult to reveal in studies employing age as the independent, sole variable, they manifest dramatically in interaction with experimental insults, and possibly with degenerative processes. It is increasingly speculated that age-related detrimental processes in this neuronal system in humans commence earlier in life, and that the onset of behavioral or cognitive symptoms may depend on a sufficient degree of decline in the integrity of this system. Thus, acceleration of the pathological process or limitations in the ability of this system to recover from and compensate for detrimental processes may emerge as the dominating variables in the effects of age on cortical cholinergic inputs (e.g., [122]). Experiments aimed at the determination of such mechanisms and the nature of the interactions with the effects of age are expected to reveal that chronological aging as a sole, independent variable is of minor significance [20] but that age permits converging pathological processes to manifest their effects on cortical cholinergic inputs. The experiments discussed above [29,30] provide an initial, fruitful paradigm to explore the mechanisms mediating the increased vulnerability of the aged cholinergic system to the effects of partial deafferentation.

Acknowledgements

The authors' research was supported in part by PHS Grants AG10173 (MS and JPB) and NS32938 (MS). MS was supported by a Research Scientist Award (MH01072). We are grateful to Jim Fadel and Anne Marie Himmelheber for their help in preparing this manuscript.

References

[1] F.A. Abdulla, M.A.J. Abu-Bakra, M.-R. Calaminici, J.-D. Stephenson, J.D. Sinden, Importance of forebrain cholinergic and GABAergic systems to the age-related deficits in water maze performance of rats, Neurobiol. Aging 16 (1995) 41–52.

[2] J.E. Alberch, E. Perez-Navarro, E. Arenas, J. Marsal, Involvement of nerve growth factor and its receptor in the regulation of the cholinergic function in aged rats, J. Neurochem. 57 (1991) 1483–1487.

[3] M.C. Altavista, P. Rossi, A.R. Bentivoglio, P. Crociani, A. Albanese, Aging is associated with a diffuse impairment of forebrain cholinergic neurons, Brain Res. 508 (1990) 51–59.

[4] D.M. Araujo, P.A. Lapchak, M.J. Meaney, B. Collier, R. Quirion, Effects of aging on nicotinic and muscarinic autoreceptor function in the rat brain: relationship to presynaptic cholinergic markers and binding sites, J. Neurosci. 10 (1990) 3069–3078.

[5] T. Arendt, L. Marcova, V. Bigl, M.K. Brückner, Dendritic reorganization in the basal forebrain under degenerative conditions and its defects in Alzheimer's disease: I. Dendritic organization of the normal human basal forebrain, J. Comp. Neurol. 151 (1995) 169–188.

[6] T. Arendt, M.K. Brückner, V. Bigl, L. Marcova, Dendritic reorganization in the basal forebrain under degenerative conditions and its defects in Alzheimer's disease: II. Ageing, Korsakoff's disease, Parkinson's disease, and Alzheimer's disease, J. Comp. Neurol. 151 (1995) 189–222.

[7] T. Arendt, M.K. Brückner, V. Bigl, L. Marcova, Dendritic reorganization in the basal forebrain under degenerative conditions and its defects in Alzheimer's disease: III. The basal forebrain compared with other subcortical areas, J. Comp. Neurol. 151 (1995) 169–188.

[8] D.M. Armstrong, R. Sheffield, G. Buzsaki, K. Chen, L.B. Hersh, B. Nearing, F.H. Gage, Morphologic alterations of choline acetyl-

transferase-positive neurons in the basal forebrain of aged behaviorally characterized Fisher 344 rats, Neurobiol. Aging 14 (1993) 457–470.
[9] I. Aubert, W. Rowe, M.J. Meaney, S. Gauthier, R. Quirion, Cholinergic markers in aged cognitively impaired Long–Evans rats, Neuroscience 67 (1995) 277–292.
[10] R.T. Bartus, R.L. Dean III, B. Beer, A.S. Lippa, The cholinergic hypothesis of geriatric memory dysfunction, Science 217 (1982) 408–417.
[11] M.G. Baxter, D.J. Bucci, L. Gorman, R.G. Wiley, M. Gallagher, Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats, Behav. Neurosci. 109 (1995) 714–722.
[12] A. Biegon, R. Duvdevani, V. Greenberger, M. Segal, Aging and brain cholinergic muscarinic receptors: an autoradiographic study in the rat, J. Neurochem. 51 (1988) 1381–1385.
[13] A. Biegon, V. Greenberger, M. Segal, Quantitative histochemistry of brain acetylcholinesterase and learning rate in the aged rat, Neurobiol. Aging 7 (1986) 215–217.
[14] V. Bigl, T. Arendt, S. Fischer, S. Fischer, M. Werner, A. Arendt, The cholinergic system in aging, Gerontology 33 (1987) 172–180.
[15] J.K. Blusztajn, R.J. Wurtman, Choline and cholinergic neurons, Science 221 (1983) 614–620.
[16] L.L. Butcher, K. Semba, Reassessing the cholinergic basal forebrain: nomenclature schemata and concepts, Trends Neurosci. 12 (1989) 483–485.
[17] F. Casamenti, C. Scali, G. Pepeu, Phosphatidylserine reverses the age-dependent decrease in cortical acetylcholine release: a microdialysis study, Eur. J. Pharmacol. 194 (1991) 11–16.
[18] P. Coleman, C. Finch, J. Joseph, The need for multiple time points in aging studies, Neurobiol. Aging 11 (1990) 1–2.
[19] D. Collerton, Cholinergic function and intellectual decline in Alzheimer's disease, Neuroscience 19 (1986) 1–28.
[20] T.J. Collier, P.D. Coleman, Divergence of biological and chronological aging: evidence from rodent studies, Neurobiol. Aging 12 (1991) 685–693.
[21] D.J. Connor, L.J. Thal, R.J. Mandel, P.J. Langlais, E. Masliah, Independent effects of age and nucleus basalis magnocellularis lesion: maze learning, cortical neurochemistry, and morphometry, Behav. Neurosci. 106 (1992) 776–788.
[22] J.R. Cooper, Unsolved problems in the cholinergic nervous system, J. Neurochem. 63 (1994) 395–399.
[23] J.A.M. Dekker, D.J. Connor, L.J. Thal, The role of cholinergic projections from the nucleus basalis in memory, Neurosci. Biobehav. Rev. 15 (1991) 299–317.
[24] Intraventricular Bethanecol Study Group, S.T. DeKosky, R.E. Harbaugh, F.A. Schmitt, R.A. Bakay, H.C. Chiu, D.S. Knopman, T.M. Reeder, A.G. Shetter, H.J. Senter, W.R. Markesberry, Cortical biopsy in Alzheimer's disease: diagnostic accuracy and neurochemical, neuropathological, and cognitive correlations, Ann. Neurol. 32 (1992) 625–632.
[25] S. De Lacalle, J.D. Cooper, C.N. Svendsen, S.B. Dunnett, M.V. Sofroniew, Reduced retrograde labelling with fluorescent tracer accompanies neuronal atrophy of basal forebrain cholinergic neurons in aged rats, Neuroscience 75 (1996) 19–27.
[26] S. De Lacalle, I. Iraizoz, L. MaGonzalo, Differential changes in cell size and number in topographic subdivisions of human basal nucleus in normal aging, Neuroscience 43 (1991) 445–456.
[27] S.L. Dewey, N.D. Volkow, J. Logan, R.R. MacGregor, J.S. Fowler, D.J. Schlyer, B. Bendriem, Age-related decreases in muscarinic cholinergic receptor binding in the human brain measured with positron emission tomography (PET), J. Neurosci. Res. 27 (1990) 569–575.
[28] P. Etienne, Y. Robitaille, P. Wood, S. Gauthier, N.P.V. Nair, R. Quirion, Nucleus basalis neuronal loss, neuritic plaques and choline acetyltransferase activity in advanced Alzheimer's disease, Neuroscience 19 (1986) 1279–1291.
[29] J. Fadel, H. Moore, M. Sarter, J.P. Bruno, Trans-synaptic stimulation of cortical acetylcholine release after partial 192 IgG-saporin-induced loss of cortical cholinergic afferents, J. Neurosci. 15 (1996) 6592–6600.
[30] J. Fadel, M. Sarter, J.P. Bruno, Interactions between the effects of age and cholinergic lesions on cortical acetylcholine release, Soc. Neurosci. Abstr. 22 (1996) 173.13.
[31] H.C. Fibiger, The organization and some projections of cholinergic neurons of the mammalian forebrain, Brain Res. Rev. 4 (1982) 327–388.
[32] H.C. Fibiger, Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence, Trends Neurosci. (1991) 220–223.
[33] W. Fischer, K. Chen, F.H. Gage, A. Björklund, Progressive decline in spatial learning and integrity of forebrain cholinergic neurons in rats during aging, Neurobiol. Aging 13 (1991) 9–23.
[34] F. Fonnum, Topographical and subcellular localisation of choline acetyl transferase in rat hippocampal region, J. Neurochem. 17 (1970) 1029–1037.
[35] M. Gallagher, Issues in the development of models for cognitive aging across primate and nonprimate species, Neurobiol. Aging 14 (1993) 631–633.
[36] M. Gallagher, P.J. Colombo, Ageing: the cholinergic hypothesis of cognitive decline, Curr. Opin. Neurobiol. 5 (1995) 161–168.
[37] M. Gallagher, P.W. Landfield, B. McEwen, M.J. Meaney, P.R. Rapp, R. Sapolsky, M. West, Hippocampal neurodegeneration in aging, Science 274 (1996) 484–485.
[38] C. Geula, M.-M. Mesulam, Systematic regional variations in the loss of cortical cholinergic fibers in Alzheimer's disease, Cereb. Cortex 6 (1996) 165–177.
[39] B.S. Givens, M. Sarter, Modulation of cognitive processes by transsynaptic activation of the basal forebrain, Behav. Brain Res. 84 (1997) 1–22.
[40] K. Haba, N. Ogawa, M. Kawata, A. Mori, A method for parallel determination of choline acetyltransferase and muscarinic cholinergic receptors: application in aged-rat brain, Neurochem. Res. 13 (1988) 951–955.
[41] R. Hellweg, W. Fischer, C. Hock, F.H. Gage, A. Björklund, H. Thoenen, Nerve growth factor levels and choline acetyltransferase activity in the brain of aged rats with spatial memory impairments, Brain Res. 537 (1990) 123–130.
[42] A.M. Himmelheber, M. Sarter, J.P. Bruno, Operant performance and cortical acetylcholine release: role of response rate, reward density, and non-contingent stimuli, Cogn. Brain Res. 6 (1997) 23–36.
[43] L.A. Holley, R.G. Wiley, D.A. Lappi, M. Sarter, Cortical cholinergic deafferentation following intracortical infusion of 192 IgG-saporin: a quantitative histochemical study, Brain Res. 663 (1994) 277–286.
[44] J.C. Hornberger, S.J. Buell, D.G. Flood, T.H. McNeill, P.D. Coleman, Stability of numbers but not size of mouse forebrain cholinergic neurons to 53 months, Neurobiol. Aging 6 (1985) 269–275.
[45] D.K. Ingram, E.D. London, C.L. Goodrick, Age and neurochemical correlates of radial maze performance in rats, Neurobiol. Aging 2 (1981) 41–47.
[46] I. Iraizoz, S. De Lacalle, L.M. Gonzalo, Cell loss and nuclear hypertrophy in topographical subdivisions of the nucleus basalis of Meynert in Alzheimer's disease, Neuroscience 41 (1991) 33–40.
[47] J.A. Joseph, T.K. Dalton, W.A. Hunt, Age-related decrements in the muscarinic enhancement of K^+-evoked release of endogenous striatal dopamine: an indicator of altered cholinergic–dopaminergic reciprocal inhibitory control in senescence, Brain Res. 454 (1988) 140–148.
[48] J.A. Joseph, G.S. Roth, Loss of muscarinic regulation of striatal dopamine function in senescence, Neurochem. Int. 20 (1992) 237S–240S.
[49] J.A. Kaye, T. Swihart, D. Howieson, A. Dame, M.M. Moore, T.

Karnos, R. Camicioli, M. Ball, B. Oken, G. Sexton, Volume loss of hippocampus and temporal lobe in healthy elderly persons destined to develop dementia, Neurology 48 (1997) 1297–1304.
[50] K.J. Kellar, P.J. Whitehouse, A.M. Martino-Barrows, K. Marcus, D.L. Price, Muscarinic and nicotinic cholinergic binding sites in Alzheimer's disease cerebral cortex, Brain Res. 436 (1987) 62–68.
[51] R.P. Kesner, Reevaluation of the contribution of the basal forebrain cholinergic system to memory, Neurobiol. Aging 9 (1988) 609–616.
[52] P. Kubanis, S.F. Zornetzer, G. Freund, Memory and postsynaptic cholinergic receptors in aging mice, Pharmacol. Biochem. Behav. 17 (1982) 313–322.
[53] M. Kurosawa, A. Sato, Y. Sato, Well-maintained responses of acetylcholine release and blood flow in the cerebral cortex to focal electrical stimulation of the nucleus basalis of Meynert in aged rats, Neurosci. Lett. 100 (1989) 198–202.
[54] C. Lebrun, T.P. Durkin, A. Marighetto, R. Jaffard, A comparison of the working memory performances of young and aged mice combined with parallel measures of testing and drug-induced activations of septo–hippocampal and nbm-cortical cholinergic neurones, Neurobiol. Aging 11 (1990) 515–521.
[55] S. Lehericy, E. Hirsch, P. Cervera-Pierot, L.B. Hersh, S. Bakchine, F. Piette, C. Duyckaerts, J.J. Hauw, F. Javoy-Agid, Y. Agid, Heterogeneity and selectivity of the degeneration of cholinergic neurons in the basal forebrain of patients with Alzheimer's disease, J. Comp. Neurol. 330 (1993) 15–31.
[56] A.S. Lippa, C.C. Loullis, J. Rotrosen, D.M. Cordasco, D.J. Critchett, J.A. Joseph, Conformational changes in muscarinic receptors may produce diminished cholinergic neurotransmission and memory deficits in aged rats, Neurobiol. Aging 6 (1985) 317–323.
[57] A.S. Lippa, R.W. Pelham, B. Beer, D.J. Critchett, R.L. Dean, R.T. Bartus, Brain cholinergic dysfunction and memory in aged rats, Neurobiol. Aging 1 (1980) 13–19.
[58] V. Luine, M. Hearns, Spatial memory deficits in aged rats: contributions of the cholinergic system assessed by ChAT, Brain Res. 523 (1990) 321–324.
[59] V.N. Luine, K.J. Renner, S. Heady, K.J. Jones, Age- and sex-dependent decreases in ChAT in basal forebrain nuclei, Neurobiol. Aging 7 (1986) 193–198.
[60] D.C. Mash, D.D. Flynn, L.T. Potter, Loss of M2 muscarinic receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation, Science 228 (1985) 1115–1117.
[61] J. McGaughy, T. Kaiser, M. Sarter, Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical ACh-positive fiber density, Behav. Neurosci. 110 (1996) 247–265.
[62] P.L. McGeer, E.G. McGeer, J. Suzuki, C.E. Dolman, T. Nagai, Aging, Alzheimer's disease, and the cholinergic system of the basal forebrain, Neurology 34 (1984) 741–745.
[63] N. Mestre, N. Bons, Age-related cytological changes and neuronal loss in basal forebrain cholinergic neurons in *Micorcebus murinus* (lemurian, primate), Neurodegeneration 2, 25–32.
[64] M.-M. Mesulam, E.J. Mufson, J. Rogers, Age-related shrinkage of cortically projecting cholinergic neurons: a selective effect, Ann. Neurol. 22 (1987) 31–36.
[65] E.M. Meyer, F.T. Crews, D.H. Otero, K. Larsen, Aging decreases the sensitivity of rat cortical synaptosomes to calcium ionophore-induced acetylcholine release, J. Neurochem. 47 (1986) 1244–1246.
[66] E.M. Meyer, E. St. Onge, F.T. Crews, Effects of aging on rat cortical presynaptic cholinergic processes, Neurobiol. Aging 5 (1984) 315–317.
[67] H. Michalek, S. Fortuna, A. Pintor, Age-related differences in brain choline acetyltransferase, cholinesterases and muscarinic receptor sites in two strains of rats, Neurobiol. Aging 10 (1989) 143–148.
[68] D. Mitsushima, T. Mizuno, F. Kimura, Age-related changes in diurnal acetylcholine release in the prefrontal cortex of male rats as measured by microdialysis, Neuroscience 72 (1996) 429–434.
[69] H. Moore, M. Sarter, J.P. Bruno, Age-dependent modulation of in vivo cortical acetylcholine release by benzodiazepine receptor ligands, Brain Res. 596 (1992) 17–29.
[70] H. Moore, S. Stuckman, M. Sarter, J.P. Bruno, Potassium- but not atropine-stimulated cortical acetylcholine efflux is reduced in aged rats, Neurobiol. Aging 17 (1996) 571–656.
[71] E.J. Mufson, J.M. Li, T. Sobreviela, J.H. Kordower, Decreased *trkA* gene expression within basal forebrain neurons in Alzheimer's disease, NeuroReport 8 (1996) 25–29.
[72] J.L. Muir, Acetylcholine, aging, and Alzheimer's disease, Pharmacol. Biochem. Behav. 56 (1997) 687–696.
[73] S. Nakamura, T. Ishihara, Region of selective increase in activities of CNS cholinergic marker enzymes during learning of memory tasks in aged rats, Pharmacol. Biochem. Behav. 34 (1989) 805–810.
[74] S. Nakamura, T. Ohno, Spatial learning impairment in aged rats: comparing between aged basal forebrain lesioned and normal aged rats, Behav. Brain Res. 70 (1995) 69–76.
[75] A. Nordberg, I. Alafuzoff, B. Winblad, Nicotinic and muscarinic subtypes in the human brain: changes with aging and dementia, J. Neurosci. Res. 31 (1992) 102–111.
[76] A.M. Palmer, Neurochemical studies of Alzheimer's disease, Neurodegeneration 5 (1996) 381–391.
[77] A.M. Palmer, P.T. Francis, J.S. Benton, N.R. Sims, D.M.A. Mann, D. Neary, J.S. Snowdon, D.M. Bowen, Presynaptic serotonergic dysfunction in patients with Alzheimer's disease, J. Neurochem. 48 (1987) 8–15.
[78] A.M. Palmer, P.T. Francis, D.M. Bowen, J.S. Benton, D. Neary, D.M.A. Mann, J.S. Snowdon, Catecholaminergic neurons assessed antemortem in Alzheimer's disease, Brain Res. 414 (1987) 365–375.
[79] F. Pedata, J. Slavikova, A. Kotas, G. Pepeu, Acetylcholine release from rat cortical slices during postnatal development and aging, Neurobiol. Aging 4 (1983) 31–35.
[80] E.K. Perry, The cholinergic hypothesis—ten years on, Br. Med. Bull. 42 (1986) 63–65.
[81] E.K. Perry, G. Blessed, B.E. Tomlinson, R.H. Perry, T.J. Crow, A.J. Cross, G.J. Dockray, R. Dimaline, A. Arregui, Neurochemical activities in human temporal lobe related to aging and Alzheimer-type changes, Neurobiol. Aging 2 (1981) 251–256.
[82] E.K. Perry, M. Johnson, J.M. Kerwin, M.A. Piggott, J.A. Court, P.J. Shaw, P.G. Ince, A. Brown, R.H. Perry, Convergent cholinergic activities in aging and Alzheimer's disease, Neurobiol. Aging 13 (1992) 393–400.
[83] E.K. Perry, B.E. Tomlinson, G. Blessed, K. Bergmann, P.H. Gibson, R.H. Perry, Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia, Br. Med. J. 2 (1987) 1457–1459.
[84] J.P. Phelan, S.N. Austad, Selecting animal models of human aging: inbred strains often exhibit less biological uniformity than F_1 hybrids, J. Gerontol. 49 (1994) B1–B11.
[85] P. Riekkinen Jr., M. Riekkinen, J. Sirviö, R. Miettinen, P. Riekkinen, Loss of cholinergic neurons in the nucleus basalis induces neocortical electroencephalographic and passive avoidance deficits, Neuroscience 47 (1992) 823–831.
[86] J.O. Rinne, P. Lönnberg, P. Marjamäki, U.K. Rinne, Brain muscarinic receptor subtypes are differently affected in Alzheimer's disease and Parkinson's disease, Brain Res. 483 (1989) 402–406.
[87] J. Rogers, Efficient experimental design in aging studies, Neurobiol. Aging 12 (1991) 695–697.
[88] D.L. Rosene, Comparing age-related changes in the basal forebrain and hippocampus of the rhesus monkey, Neurobiol. Aging 14 (1993) 669–670.
[89] A. Saleh, J. Verhaagen, P.A. Dijkhuizen, D.F. Swaab, Co-localization of high-affinity neurotrophin receptors in nucleus basalis of Meynert neurons and their differential reduction in Alzheimer's disease, Neuroscience 75 (1996) 373–387.

[90] M. Sarter, J.P. Bruno, Cognitive functions of cortical acetylcholine: lesions from studies on the trans-synaptic modulation of activated efflux, Trends Neurosci. 17 (1994) 217–222.
[91] M. Sarter, J.P. Bruno, Trans-synaptic stimulation of cortical acetylcholine and attention: a rationale approach for the development of cognition enhancers, Behav. Brain Res. 83 (1997) 7–15.
[92] M. Sarter, J.P. Bruno, Cortical acetylcholine, attention, and neuropharmacological and cognitive principles directing treatment strategies for cognitive disorders, in: J.E. Brioni, M.W. Decker (Eds.), Pharmacological Treatment of Alzheimer's Disease: Molecular and Neurobiological Foundations, Wiley–Liss, New York, 1997, pp. 105–128.
[93] M. Sarter, J.P. Bruno, Cognitive functions of cortical acetylcholine: toward a unifying hypothesis, Brain Res. Rev. 23 (1997) 28–46.
[94] M. Sarter, J.P. Bruno, B.S. Givens, H. Moore, J. McGaughy, K. McMahon, Neuronal mechanisms of drug effects on cognition: cognitive activity as a necessary intervening variable, Cogn. Brain Res. 3 (1996) 329–343.
[95] M. Sarter, H.H. Schneider, D.N. Stephens, Treatment strategies for senile dementia: antagonist β-carbolines, Trends Neurosci. 11 (1988) 13–17.
[96] B.M. Schmidt, R.J. Rylett, Basal synthesis of acetylcholine in hippocampal synaptosomes is not dependent upon membrane-bound choline acetyltransferase activity, Neuroscience 54 (1993) 649–656.
[97] H. Schröder, E. Giacobini, R.G. Struble, P.G.M. Luiten, E.A. van der Zee, K. Zilles, A.D. Strosberg, Muscarinic cholinoceptive neurons in the frontal cortex in Alzheimer's disease, Brain Res. Bull. 27 (1991) 631–636.
[98] R.D. Schwartz, A.A. Bernabei, C.J. Spencer, T.A. Pugsley, Loss of muscarinic M1 receptors with aging in the cerebral cortex of Fisher 344 rats, Pharmacol. Biochem. Behav. 35 (1990) 589–593.
[99] J. Sirviö, A. Hervonen, P.J. Riekkinen, Sodium-dependent uptake of ^{3}H-choline in the cerebral cortex of ageing male rats, Pharmacol. Toxicol. 62 (1988) 227–229.
[100] T.D. Smith, M. Gallagher, F.M. Leslie, Cholinergic binding sites in rat brain: analysis by age and cognitive status, Neurobiol. Aging 16 (1995) 161–173.
[101] E.L. Spangler, K.S. Waggie, J. Hengemihle, D. Roberts, B. Hess, D.K. Ingram, Behavioral assessment of aging in male Fischer 355 and Brown–Norway rat strains and their F_1 hybrid, Neurobiol. Aging 15 (1994) 319–328.
[102] D.L. Sparks, J.C. Hunsaker, J.T. Slevin, S.T. DeKosky, R.J. Kryscio, W.R. Markesberry, Monoaminergic and cholinergic synaptic markers in the nucleus basalis of Meynert (nbM): normal age-related changes and the effect of heart disease and Alzheimer's disease, Ann. Neurol. 31 (1992) 611–620.
[103] P.H. Stephens, P.C. Tagari, A.C. Cuello, Age-related variations in plasticity of rat basal forebrain cholinergic neurons after cortical lesions, Brain Res. 448 (1988) 320–324.
[104] J.D. Stoehr, G.L. Wenk, Effects of age and lesions of the nucleus basalis on contextual fear conditioning, Psychobiology 23 (1995) 173–177.
[105] W.S. Stone, H.J. Altman, R.F. Berman, D.F. Caldwell, M.M. Kilbey, Association of sleep parameters and memory in intact old rats and young rats with lesions in the nucleus basalis magnocellularis, Behav. Neurosci. 103 (1989) 755–764.
[106] R. Strong, P. Hicks, L. Hsu, R.T. Bartus, J. Enna, Age-related alterations in the rodent brain cholinergic system and behavior, Neurobiol. Aging 1 (1980) 59–63.
[107] T. Suhura, O. Inoue, K. Kobayashi, K. Suzuki, Y. Tateno, Age-related changes in human muscarinic acetylcholine receptors measured by positron emission tomography, Neurosci. Lett. 149 (1993) 225–228.
[108] W. Surichamorn, O.N. Kim, N.H. Lee, W.S. Lai, E.E. El-Fakahany, Effects of aging on the interaction of quinuclidinyl benzilate, *N*-methylscopolamine, pirenzepine, and gallanine with brain muscarinic receptors, Neurochem. Res. 13 (1988) 1183–1191.
[109] P. Tandon, W.R. Mundy, S.F. Ali, K. Nanry, B.C. Rogers, H.A. Tilson, Age-dependent changes in receptor-stimulated phosphoinositide turnover in the rat hippocampus, Pharmacol. Biochem. Behav. 38 (1991) 861–867.
[110] A.E. Tamer, E. Wulfert, I. Hanin, Age-dependent effect of AF64A on cholinergic activity in the septo–hippocampal pathway of the rat brain: decreased responsiveness in aged rats, Neurosci. Lett. 203 (1996) 123–126.
[111] L.J. Thal, E. Gilbertson, D.M. Armstrong, F.H. Gage, Development of the basal forebrain cholinergic system: phenotype expression prior to target innervation, Neurobiol. Aging 13 (1991) 67–72.
[112] E.M. Torres, T.A. Perry, A. Blokland, L.S. Wilkinson, R.G. Wiley, D.A. Lappi, S.B. Dunnett, Behavioural, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system, Neuroscience 63 (1994) 95–122.
[113] S. Tucek, Observations on the subcellular distribution of choline acetyltransferase in the brain tissue of mammals and comparisons of acetylcholine synthesis from acetate and citrate in homogenates and nerve-ending fractions, J. Neurochem. 14 (1967) 519–529.
[114] J. Turchi, M. Sarter, Cortical acetylcholine and processing capacity: effects of cortical cholinergic deafferentation on crossmodal divided attention in rats, Cogn. Brain Res. 6 (1997) 147–158.
[115] J.W. Unger, Y. Schmidt, Quisqualic acid-induced lesion of the nucleus basalis of Meynert in young and aging rats: plasticity of surviving NGF receptor-positive cholinergic neurons, Exp. Neurol. 117 (1992) 269–277.
[116] P.N. Vinson, J.B. Justice Jr., Effect of neostigmine on concentration and extraction of acetylcholine using quantitative microdialysis, J. Neurosci. Methods 73 (1997) 61–67.
[117] M.L. Voytko, R.R. Suhkov, L.C. Walker, S.J. Breckler, D.L. Price, V.E. Koliatsos, Neuronal number and size are preserved in the nucleus basalis of aged rhesus Monkeys, Dementia 6 (1995) 131–141.
[118] S.B. Waller, E.D. London, Choline acetyltransferase activity and muscarinic binding in brain regions of aging Fischer-344 rats, Neurochem. Int. 14 (1989) 483–490.
[119] G.L. Wenk, J.D. Stoehr, G. Quintana, S. Mobley, R.G. Wiley, Behavioral, biochemical, histological, and electrophysiological effects of 192 IgG-saporin injections into the basal forebrain of rats, J. Neurosci. 14 (1994) 5986–5995.
[120] G.L. Wenk, J.D. Stoehr, S.L. Mobley, J. Gurney, R.J. Morris, Age-related decrease in vulnerability to excitatory amino acids in the nucleus basalis, Neurobiol. Aging 17 (1995) 1–7.
[121] P.J. Whitehouse, D.L. Price, R.G. Struble, A.W. Clark, J. Coyle, M.R. Delong, Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain, Science 215 (1981) 1237–1239.
[122] N.J. Woolf, L.L. Butcher, Dysdifferentiation of structurally plastic neurons initiates the pathologic cascade of Alzheimer's disease: toward a unifying hypothesis, in: M. Steriade, D. Biesold (Eds.), Brain Cholinergic Systems, Oxford Univ. Press, Oxford, 1990, pp. 386–438.
[123] C.F. Wu, R. Bertorelli, M. Sacconi, G. Pepeu, S. Consolo, Decrease of brain acetylcholine release in aging freely-moving rats detected by microdialysis, Neurobiol. Aging 9 (1988) 357–361.
[124] N. Zawia, G.W. Arendash, L. Wecker, Basal forebrain cholinergic neurons in aged rat brain are more susceptible to ibotenate-induced degeneration than neurons in young adult brain, Brain Res. 589 (1992) 333–337.