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Presynaptic regulation of neurotransmitter release in the cortex of aged rats with differential memory impairments

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Abstract

Cluster analysis of water-maze reference-memory performances of 25–27-month-old (compared to 3–5-month-old) rats distinguished subpopulations of young adult rats (YOUNG), aged rats with no significant impairment (AU), aged rats with moderate impairment (AMI), and aged rats with severe impairment (ASI). In the frontoparietal cortex, we subsequently assessed the electrically evoked release of tritium in slices preloaded with [^{3}H]choline, [^{3}H]noradrenaline (NA), or [^{3}H]serotonin (5-HT) and the effects of an agonist (oxotremorine, UK 14,304, and CP 93,129) of the respective autoreceptors. Cholinergic and monoaminergic markers were measured in homogenates. Overall, aged rats exhibited reduced accumulation of [^{3}H]choline (−25%) and weaker evoked transmitter release (in % of accumulated tritium: −44%, −20%, and −34%, for [^{3}H]acetylcholine, [^{3}H]NA, and [^{3}H]5-HT, respectively). In all rats, the inhibitory effects of the autoreceptor agonists on the evoked release of [^{3}H] were comparable. Acetylcholinesterase (AChE), not choline acetyltransferase (ChAT), activity was reduced. The results suggest age-related modifications in the cholinergic, noradrenergic, and serotonergic innervation of the frontoparietal cortex, alterations of evoked transmitter release, but no interference with presynaptic autoinhibition of the release. Neither of these alterations seemed to account for the cognitive impairment assessed.

Keywords: Aging; CP 93,129; Oxotremorine; Spatial memory; UK 14,304; Water maze

1. Introduction

Over the past three decades, an exponentially growing number of investigations focused on the cognitive impairments due to aging and on their possible neuropathological substrates (e.g., Grady and Craik, 2000). Part of this research concentrated on the relationship between memory deficits and alterations of basal forebrain cholinergic functions (e.g., Bartus et al., 1982; Bartus, 2000). Aged rodents, like aged humans, also exhibit memory dysfunctions, as demonstrated in a variety of learning tasks, particularly in those placing emphasis on spatial memory (Barnes et al., 1980; Ingram et al., 1981, 1994; Gage et al., 1984; Rapp et al., 1987; Gallagher and Pelleymounter, 1988). Two major components of the basal forebrain cholinergic system comprise the septo-hippocampal and basalo-cortical projection neurons. In the hippocampus of aged rats, there may be several changes in receptor functions, as substantiated by studies showing a reduced number or a functional alteration of binding sites for galanin (Planas et al., 1998), serotonin (5-HT; Gozlan et al., 1990; Nyakas et al., 1997), acetylcholine (ACh; Sastry et al., 1983; Consolo et al., 1986; Amenta et al., 1995; Aubert et al., 1995; Narang, 1995), and noradrenaline (NA; e.g., Kalaria et al., 1989; Burnett et al., 1990; Huguet and Tarrade, 1992). Such observations also apply to cortical regions of aged rodents (e.g., Araujo et al., 1990; Robson et al., 1993; Krzywkowski et al., 1994). Part of the aforementioned binding sites may contribute to the local regulation of synaptic and nonsynaptic neurotransmitter release in the hippocampus and the cortex, either as autoreceptors (e.g., 5-HT$_{1B}$ receptors at serotonergic terminals or M$_2$ receptors at the cholinergic ones; Millan et al., 2000) or as heteroreceptors (e.g., 5-HT$_{1B}$ receptors at the cholinergic terminals; Vizi and Kiss, 1998).

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In a recent study, we investigated the presynaptic status of cholinergic, noradrenergic, and serotonergic functions in the hippocampus of aged rats exhibiting various degrees of spatial reference-memory impairments in a Morris water maze (Birthelmer et al., in press). The results suggested that aging induced some modifications in the cholinergic and serotonergic innervation of the hippocampus, altered the evoked release of the corresponding transmitters, but did not seem to interfere with presynaptic mechanisms involved in the modulation of this release. Furthermore, when significant, these changes were most often comparable among all aged rats, regardless of their level of performances in the memory task (good, average, bad).

In the present study, we focused on the functional status of the major target of the nucleus basalis magnocellularis, namely, the frontoparietal cortex. This region was chosen because (i) it is one of the loci where important age-related alterations occur in rodents and humans, (ii) under some circumstances (e.g., nonselective lesions) experimental damage to the nucleus basalis magnocellularis induce cognitive deficits in rodents (but see Galani et al., 2002 for detailed discussion), (iii) age-related changes in autoreceptor-mediated mechanisms modulating ACh, NA, and 5-HT release have not been subject to numerous investigations yet. Moreover, in the few studies in which such mechanisms were studied (e.g., Araujo et al., 1990; Zsilla et al., 1994; Hersi et al., 1995), the findings on receptor functions were not always brought back to the cognitive performances of the rats and, as was the case for the hippocampus, most often, only one transmitter system was considered. Therefore, using essentially the same approach as in our recent study (Birthelmer et al., in press), we investigated and compared the release, and its modulation by autoreceptors, of ACh, NA, and 5-HT in slices prepared from the frontoparietal cortex of aged rats (25–27 months). The aged rats were from a larger population in which good, average, and bad performers could be distinguished according to their performances in a Morris water-maze reference-memory task. Rats aged of 3 months were used as controls. We examined whether age altered (i) the uptake of [^{3}H]choline, [^{3}H]NA, and [^{3}H]5-HT, (ii) the electrically evoked release of [^{3}H]ACh, [^{3}H]NA, and [^{3}H]5-HT, (iii) the modulation of the electrically evoked release of [^{3}H]ACh by the muscarinic agonist oxotremorine, of [^{3}H]NA by the α_2 receptor agonist UK 14,304, and of [^{3}H]5-HT by the 5-HT$_{1B}$ agonist CP 93,129, and (iv) other neuronal markers such as choline acetyltransferase (ChAT) or acetylcholinesterase (AChE) activity, or the concentration of monoamines (NA, 5-HT, and dopamine [DA]).

2. Experimental procedures

2.1. Subjects

Eleven young adults (3–5 months) and 39 aged (25–27 months) Long–Evans female rats (R. Janvier, France) were used (ages at the time of behavioral testing). The aged rats were purchased when they were 3 months old. They were housed in the laboratory in Makrolon cages (59 × 38 × 20 cm) in groups of six until the age of 24 months. Before water-maze testing was started, they were placed three per cage (42 × 26 × 15 cm; one adult rat with two aged rats, or three aged rats). Food and water were available ad libitum. The colony and the testing rooms were maintained on a 12-h light/12-h dark cycle (lights on at 0700 h) under controlled temperature (22 °C). The young rats arrived at the laboratory at the age of 2.5 months. They were housed in groups of six during 2 weeks until being placed for water-maze testing as described above. All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (council directive # 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animales; permissions # 6212 to J.-C.C.; A.L. under the responsibility of the former) and international (NIH publication no. 80-23, revised 1996) laws and policies. All efforts were made to reduce the number of subjects used to a minimum regarding statistical constraints and to minimize suffering throughout the experiments.

2.2. Morris water-maze test

The water maze consisted of a circular pool (diameter 160 cm; height 60 cm) filled to half its height with water (20 °C) made opaque by addition of powdered milk. The experimental room contained different extra-maze cues, and the illumination was provided by a neon light placed 1.80 m above the center of the pool. Each rat was tested for 5 days and given four consecutive trials per day. For each trial, the rat was placed in the pool, facing the wall at a randomly designed starting point from where it was released. A maximum of 60 s was given to reach a transparent circular platform (diameter 11 cm) submerged 1 cm underneath the water surface at a constant position. When the rat had climbed onto the platform, it was left there for 10 s before being removed and placed on the next starting point. If the rat did not find the platform on time, it was placed on the platform for 10 s by the experimenter. Swim paths were recorded using a video camera connected to a tracking system (Ethovision, Noldus, The Netherlands). The time needed by the rat to reach the platform (latency) as well as the distance swum during each trial were recorded and taken into account for statistical analysis. Twenty-four hours after the last testing day, the platform was removed, and all rats were given a probe trial for 60 s. The time spent in the quadrant where the platform was located over the acquisition trials was recorded.

2.3. Dissection of the cortex

Two to eight weeks after completion of water-maze testing, the rats were sacrificed by decapitation, their brains quickly removed, and two bands of frontoparietal cortex

were dissected free from the other proximal brain regions (anterior limits were at about +2.2 mm, posterior limits at about −0.8 mm, and lateral limits at about 6 mm depth; coordinates from Bregma according to Paxinos and Watson, 1986). A small tissue band (about 0.5 mm thickness in the antero-posterior plan) was separated on the rostral border of the cortex pieces and homogenized in 1 ml 0.32 M sucrose (in 2.5 mM HEPES, pH 7.4) in a Potter Elvehjem glass/Teflon homogenizer (eight strokes at 500 rpm). Homogenates were also prepared from the dorsal hippocampus and from the striatum. From these crude homogenates, the following aliquots were prepared and stored at −80 °C until neurochemical determinations (see below): a 20-μl sample (diluted with 180 μl 0.1N NaOH) for measurement of protein according to Lowry et al. (1951), two 100-μl aliquots for determination of ChAT and AChE activity, respectively, and a mixture of 300 μl crude homogenate with 300 μl 0.2N $HClO_4$ (containing 250 mg Na_2SO_3 and 200 mg Na_2EDTA per liter) for determination of NA, dihydroxyphenylacetic acid (DOPAC), DA, 5-hydroxyindole acetic acid (5-HIAA), and 5-HT concentration by HPLC.

2.4. Accumulation of [^{3}H]choline, [^{3}H]noradrenaline, and [^{3}H]serotonin and evoked release of tritium in cortical slices

The cortical tissue pieces from each brain side were cut in 350-μm slices (perpendicular to the medio-lateral axis) using a McIlwain tissue chopper. The slices were distributed to three petri dishes containing 2 ml Krebs–Henseleit (KH) buffer each with either [^{3}H]choline (0.1 μM for ACh release experiments), [^{3}H]NA (0.1 μM for NA release experiments), or [^{3}H]5-HT (0.1 μM for 5-HT release experiments) and incubated for 45 min at 37 °C under carbogen. The KH solution had the following composition (in mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 10; ascorbic acid, 0.6; Na_2EDTA, 0.03; saturated with carbogen, pH adjusted to 7.4.

After incubation and several washing steps, slices preloaded with [^{3}H]choline, [^{3}H]NA, or [^{3}H]5-HT were transferred into superfusion chambers (12 chambers per superfusion apparatus, one slice per chamber) and superfused with oxygenated KH buffer (37 °C) at a rate of 1.2 ml/min. The superfusion medium was supplemented routinely with hemicholinium-3 (10 μM) for [^{3}H]ACh release experiments, desipramine (1 μM) for [^{3}H]NA release experiments, and 6-nitroquipazine (1 μM) for [^{3}H]5-HT release experiments. An electrical field stimulation (rectangular, 18 pulses, 3 Hz, 4 V/chamber, 26–30 mA) was applied 15 min after beginning of the superfusion. Fractions (2 min) to be measured were collected from 30 min of superfusion onwards. The release of [^{3}H]ACh, [^{3}H]NA, and [^{3}H]5-HT, respectively, was induced by three periods of electrical field stimulations (90 rectangular pulses at 3 Hz, 2 ms, 8 V/chamber, 52–60 mA) after 34 (S_1), 50 (S_2), and 66 min (S_3) of superfusion. Drugs to be tested were added to the superfusion medium of some chambers from 8 min before the second and the third stimulation onwards. Oxotremorine (0.1 and 1 μM), UK 14,304 (0.01 and 0.1 μM), or CP 93,129 (0.0316 and 0.316 μM) were applied to slices preloaded with [^{3}H]choline, [^{3}H]NA, or [^{3}H]5-HT, respectively. At the end of the experiment (after 74 min of superfusion), the radioactivity of superfusate samples and slices (dissolved in 250 μl Solvable, Packard, Frankfurt, Germany) was determined by liquid scintillation counting. The 'fractional rate of tritium outflow' (in percent of tissue tritium per 2 min) was calculated as: (picomoles tritium outflow per 2 min) × 100/(picomoles tritium in the slice at the start of the corresponding 2-min period). The 'baseline tritium outflow' in the fraction preceding S_1 is given either in absolute terms (nCi [^{3}H]outflow) or in relative terms ('fractional rate of tritium outflow per 2 min'). The 'stimulation-evoked overflow of tritium' was calculated by subtraction of the baseline outflow and is shown either in absolute terms (nCi [^{3}H]overflow) or in relative terms (in percent of the tritium content of the slice at the onset of the respective stimulation period). Effects of drugs added before S_2 and S_3 were determined as the ratio of the overflow evoked by the corresponding stimulation period (S_2/S_1 or S_3/S_1) and compared to the appropriate control ratio (no drug addition before S_2 and S_3).

2.5. HPLC determination of the concentration of monoamines

Tissue concentrations of DA, DOPAC, 5-HT, 5-HIAA, and NA were determined by HPLC with electrochemical detection. Following thawing, the samples (see above) were centrifuged (10 min at 17,000 × *g*; 4 °C) and the supernatants filtered using Millex-GV4 (0.22 μm; Millipore, Eschborn, Germany) filters. Twenty microliters of the filtrated supernatants was injected onto a reversed-phase HPLC column (Luna 5 μm C8; 250 × 4.6-mm column; Cat. No. OOG-4040-EO; Phenomenex, Aschaffenburg, Germany), and separation was performed at 30 °C at a flow of 1 ml/min (pump model 480, Gynkotek, München, Germany). The mobile phase had the following composition (per liter): 3.9 g sodium acetate, 4.0 g citric acid (monohydrate), 114 mg 1-octanesulfonic acid (sodium salt), 35.7 mg Na_2EDTA, 138.1 ml methanol, and 7.15 ml ethanol (pH adjusted to 4.2 with acetic acid). The amounts of the monoamines and some of their metabolites were determined using the electrochemical detector INTRO (Antec, Leyden, The Netherlands) set at 600 mV (at 30 °C) and a standard calibration curve. The detection limits were as follows (retention times in parenthesis): 1.0 pg for NA (5.4 min), 1.5 pg for DOPAC (8.0 min), 1.3 pg for DA (9.6 min), 1.5 pg for 5-HIAA (12.2 min), and 1.6 pg for 5-HT (19.2 min).

2.6. Determination of choline acetyltransferase activity

ChAT activity of the crude homogenate samples (see above) was determined according to Fonnum (1975) with

modifications. In brief, 100 μl of the crude homogenate was diluted with 100 μl (hippocampus and cortex) or 600 μl (striatum) of a freshly prepared medium containing 0.32 M sucrose, 129 mM NaCl, 88 mM NaH_2PO_4, 2.5 mM HEPES, 0.9 mM EGTA, 0.9 mM Na_2EDTA, 179 μM physostigmine, and 0.45% Triton-X100. Twelve microliters of this mixture (all samples in triplicates) was added to 6 μl of choline bromide (32 mM). The incubation was started by the addition of 6 μl of [^{14}C]acetyl-coenzyme A (50 nCi/assay; 0.227 mM final concentration) and vigorous mixing. After 20 min at 37 °C, 20 μl of the mixture was pipetted into a mixture of 5 ml sodium phosphate buffer (10 mM; pH 7.4) with 2 ml sodium tetraphenylborate in acetonitrile (5 mg/ml). From this mixture, the newly formed [^{14}C]ACh was extracted by careful shaking with 10 ml of toluene scintillator. Following separation of the aqueous from the organic phase, the samples were directly counted by LSC. In order to correct for nonspecific effects, in each case, two samples were run at 0 °C.

2.7. Determination of acetylcholinesterase activity

AChE activity of the crude homogenate samples (see above) was determined by the method of Ellman et al. (1961) using a 96-well microplate reader modification described by Ashour et al. (1987). In brief, 6 μl of the crude homogenate (all determinations in triplicates) per well was mixed with 290 μl of sodium phosphate buffer (50 mM; pH 7.4) containing 0.01% 5,5′-dithio-bis-(2-nitrobenzoic acid). The reaction was started by addition of 6 μl of acetylthiocholine iodide (500 μM, in 50 mM sodium phosphate buffer pH 7.4). The optical density of the wells was determined at 0, 10, 20, 30, and 60 min using a Dynex MRX microplate reader at 405 nm. The enzymatic formation of the yellow reaction product was always linear, at least up to 30 min. Moreover, the enzymatic activity of each sample was also determined in the presence of either 10 μM ethopropazine, 10 μM galanthamine, or 10 μM physostigmine, in order to differentiate between actylcholinesterase and butyrylcholinesterase activities. Cholinesterase activities (in nmoles/mg protein/min) were calculated from the absorption changes per minute according to the formula given by Ellman et al. (1961) using the molar absorption coefficient of the yellow reaction product at 412 nm.

2.8. Statistical analysis

Reference-memory performances were compared among groups of young and aged rats, using a repeated-measures ANOVA on average distances corrected according to Lindner (1997). The corresponding effect is termed "Age effect" hereafter. To further analyze the data, a statistical distinction between different subpopulations of rats was made according to the cluster-analysis procedure analogous to that described by Ghirardi et al. (1992). The corresponding effect is termed the "Subpopulation effect" hereafter. The entire population of rats (young + aged) was subdivided into three clusters. Cluster 1 included all young adult rats (YOUNG) and 10 aged rats that showed the best performances (AU). Two of the aged and three of the young adult rats, respectively, were discarded on a random basis in order to reduce the sample sizes to eight. Cluster 2 ($n = 13$) included aged rats with moderate impairment (AMI). From this second cluster, five rats were discarded on a random basis in order to reduce the sample size from 13 to 8. Cluster 3 ($n = 16$) included aged rats with severe impairment (ASI); the group size was also reduced to eight. The discarded rats were not used in the present series of neuropharmacological experiments. Analysis of all other data also used ANOVA with consideration of factors Subpopulation, Drug, and Concentration that were combined or not according to the analysis required. Because young rats were also included in the cluster analysis, it is noteworthy that the aforementioned Subpopulation effect does not only refer to the three subgroups of aged rats, but to all subgroups, including that of young rats. Concerning the analysis of the drug effects, it is worth emphasizing that each stimulation in the presence of a given drug at a given concentration had its own control condition. Therefore, to analyze the Drug effects, we used an Age (YOUNG, Aged) or Subpopulation (YOUNG, AU, AMI, ASI) × Drug (Control, Active compound) × Concentration (e.g., 0.1 μM, 1 μM) design, the concentration being the independent variable (repeated measure, as the effects of both concentrations were tested on the same slice). It is also highlighted that before being analyzed, data obtained from different slices from the same rat were averaged to generate a single value per rat. When appropriate, 2 × 2 comparisons used a Newman–Keuls test. Where relevant, Spearman correlations or multiple regression analyses were computed without consideration of the data from the young rats.

2.9. Chemicals and drugs

Chemicals and drugs were obtained from the following sources, [1-^{14}C]-acetyl-coenzyme A (52 mCi/mmol) and [methyl-^{3}H]choline chloride (79 Ci/mmol) were from Amersham-Pharmacia, Braunschweig, Germany; 5-[1,2-^{3}H(*N*)]hydroxytryptamine creatinine sulfate ([^{3}H]5-HT, 25.5 Ci/mmol) and [^{3}H]norepinephrine (56.4 Ci/mmol) from NEN Life Sciences, Frankfurt, Germany; hemicholinium-3 from Chemcon, Freiburg, Germany; oxotremorine sesquifumarate from Sigma-Aldrich, Steinheim, Germany; 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrollo[3,2-b]pyrid-5-one (CP 93,129) from Dr. Pagani, Pfizer, Groton, USA; 6-nitro-2-(1-piperazinyl)-quinoline from RBI, Biotrend, Köln, Germany; 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline tartrate (UK 14;304) from Pfizer, Sandwich, Kent, UK.

3. Results

3.1. Morris water maze

Data are shown in Fig. 1. When all aged rats were considered a single population, ANOVA of distances swum showed significant Age [$F(1,30)=11.6$, $P<.002$] and Day [$F(4,120)=28.7$, $P<.001$] effects, and a significant Age × Day interaction [$F(4,120)=4.09$, $P<.01$]. The Age effect was due to distances that were significantly longer in all aged as compared to the young rats. The Day effect reflected a decrease of the path length, which was significant from 1 day to the next one ($P<.001$, for all comparisons). The interaction was due to the fact that in aged rats, the improvement of performances over days was progressive, as opposed to a sharper change in YOUNG rats from day 2 to day 3. ANOVA of the latencies (not illustrated) yielded a comparable picture, in which the same effects and the same differences were significant.

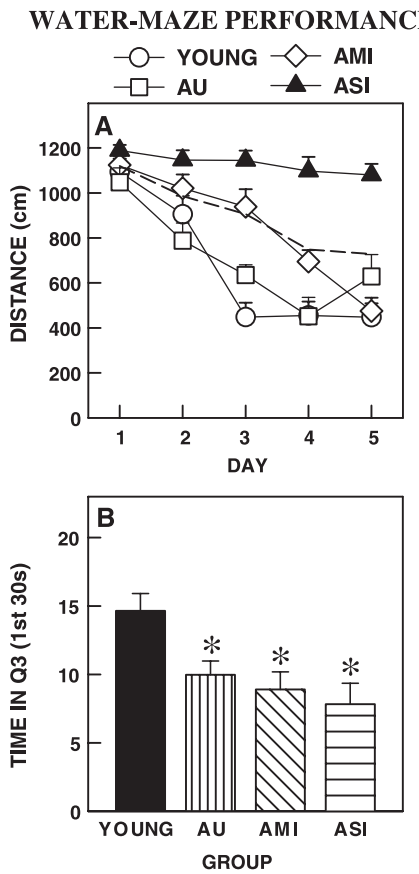

Fig. 1. (A) Average distance to the platform (mean + S.E.M.) in daily blocks of four-trial sessions recorded during the acquisition phase of the water maze in young adult (YOUNG), aged unimpaired (AU), aged moderately impaired (AMI), and aged severely impaired (ASI) rats. The interrupted line shows the average performances of all aged rats. (B) Average time (mean + S.E.M.) spent in the training quadrant (Q3) during the first 30 s of the probe trial. Statistics: * significantly different from YOUNG, $P<.05$ (only shown for the probe-trial performances).

When the analysis considered the young rats and the three subpopulations of aged rats separately, it showed significant Subpopulation [$F(3,28)=149.4$, $P<.001$] and Day [$F(4,112)=33.3$, $P<.001$] effects, as well as a significant Subpopulation × Day interaction [$F(12,112)=4.52$, $P<.001$] on the distances. The Subgroup effect was due to a significantly longer distance in AMI and ASI rats as compared to YOUNG or AU rats ($P<.001$ in each case), but also to a significantly longer distance in ASI rats as compared to AMI rats ($P<.001$). The difference between YOUNG and AU was not significant. The Day effect was due to overall distances, which decreased significantly from 1 day to the next day ($P<.05$, at least). Finally, the interaction can be interpreted as reflecting a between-day shortening of the path lengths, which was rapid in YOUNG and AU rats, slower in AMI rats, and almost absent in ASI rats. ANOVA of the latencies (not illustrated) yielded a comparable picture in which the same effects and the same differences were significant.

Finally, ANOVA of the probe trial performances showed a significant Age effect [$F(1,30)=15.4$, $P<.001$], due to the fact that aged rats spent less time in the probe quadrant than their younger counterparts. Consideration of the different subpopulations showed a significant Subpopulation effect [$F(3,28)=5.5$, $P<.01$], which can be explained as follows: performances of YOUNG rats were significantly better than in AU, AMI, and ASI rats ($P<.01$, at least), but the difference between AU, AMI, and ASI rats was not significant. It is, however, noteworthy that when the distance to reach the virtual platform location for the first time was considered, we found the following average values: YOUNG 281.6 ± 52.8, AU 568.1 ± 158.0, AMI 866.2 ± 135.5, and ASI 1070.0 ± 152.3 (all values in centimeters). Statistical analysis showed that the difference between YOUNG and AU rats was significant, that YOUNG rats performed significantly better than AMI and ASI rats, and that AU rats were significantly better than ASI rats ($P<.05$ in each case).

3.2. Accumulation of [^{3}H]choline, [^{3}H]noradrenaline, and [^{3}H]serotonin in cortical slices

In order to simplify the presentation of all analyses, the Age effect will be presented briefly, and the Subpopulation effect will be considered more extensively hereafter.

Data are shown in Fig. 2. ANOVA of the [^{3}H]choline accumulation (Fig. 2A) showed a significant Age effect [$F(1,30)=17.4$, $P<.001$], the accumulation being reduced by 25% in all aged rats as compared to their young counterparts. When analysis considered the four populations, there was a Subpopulation effect [$F(3,28)=6.2$, $P<.01$] that was due to a significant reduction of the values found in AU, AMI, and ASI rats as compared to their younger counterparts ($P<.001$). The difference amongst accumulation values found in AU, AMI, and ASI rats was not significant.

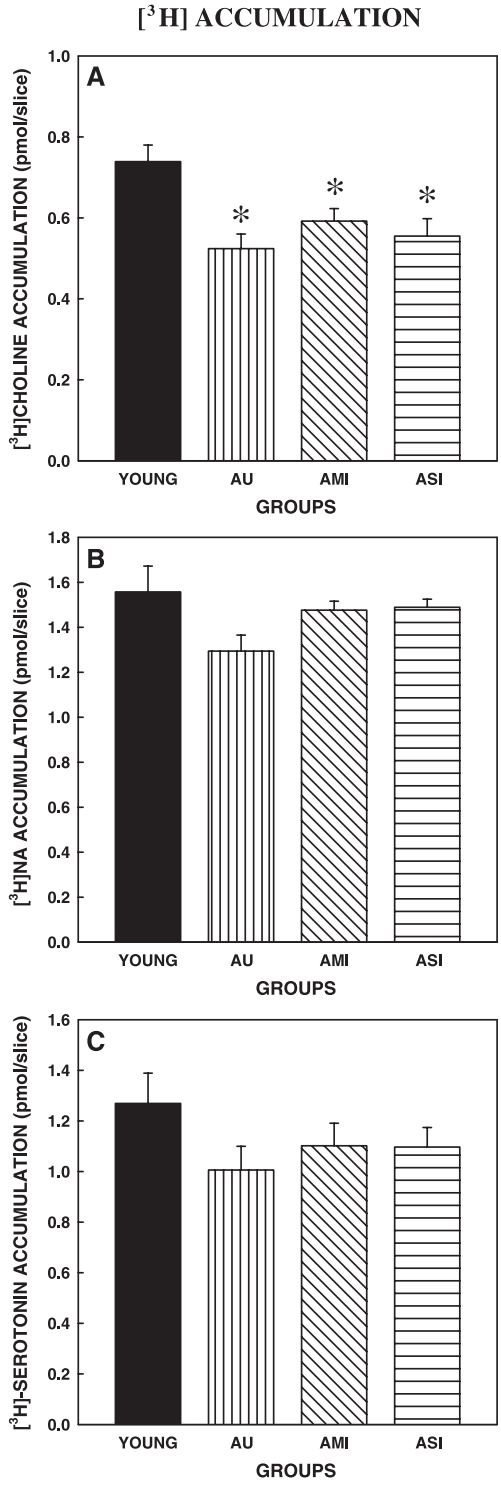

Fig. 2. Accumulation of (A) [^{3}H]choline, (B) [^{3}H]NA, and (C) [^{3}H]5-HT (average + S.E.M., in pmol/slice) in slices prepared from the frontoparietal cortex of young adult (YOUNG), aged unimpaired (AU), aged moderately impaired (AMI), and aged severely impaired (ASI) rats. Statistics: * significantly different from YOUNG, $P < .05$. There were eight rats per group and seven to eight slices per rat.

ANOVA of the [^{3}H]NA accumulation (Fig. 2B) showed no significant Age effect [$F(1,30) = 2.4$]. In addition, there was no significant Subpopulation effect [$F(3,28) = 2.4$].

ANOVA of the [^{3}H]5-HT accumulation (Fig. 2C) showed a tendency towards a significant Age effect [$F(1,30) = 3.3$, $P < .10$], the accumulation being reduced by 16% in the aged rats. Consideration of the subpopulations showed no significant Subpopulation effect [$F(3,28) = 1.3$].

3.3. Baseline outflow of tritium

Data are shown in Table 1. ANOVA of the baseline outflow of [^{3}H] in cortical slices preincubated with [^{3}H]choline showed a significant Age effect on only the absolute release [$F(1,30) = 6.4$, $P < .05$]. Consideration of the subpopulations failed to show a significant Subpopulation effect on both the relative (%) and absolute (nCi) release [$F(3,28) = 1.1$ and 2.1, respectively].

ANOVA of the baseline outflow in cortical slices preincubated with [^{3}H]NA showed no significant Age effect on the relative [$F(1,30) < 1.0$] or absolute release [$F(1,30) = 1.8$]. There was no significant Subpopulation effect on either the absolute [$F(3,28) < 1.0$] or the relative release [$F(3,28) = 1.1$].

ANOVA of the baseline outflow in cortical slices preincubated with [^{3}H]5-HT showed a significant Age effect on the relative release [$F(1,30) = 7.1$, $P < .05$], but not on the absolute release [$F(1,30) < 1.0$]; the relative release was increased by 15% in the aged rats. There was a significant Subpopulation effect on neither the relative nor the absolute release [$F(3,28) = 2.3$ and 0.6, respectively].

Table 1
Relative (in percent of [^{3}H] accumulation) and absolute (in nCi) baseline outflows of tritium in frontoparietal cortical slices preincubated with [^{3}H]choline, [^{3}H]NA, or [^{3}H]5-HT

Variable	Subpopulations			
	YOUNG ($N=8$)	AU ($N=8$)	AMI ($N=8$)	ASI ($N=8$)
[^{3}H] outflow in slices incubated with [^{3}H]ACh				
In percent of accumulated [^{3}H]choline	1.40 ± 0.09	1.57 ± 0.09	1.47 ± 0.07	1.60 ± 0.09
In nCi	0.78 ± 0.03	0.63 ± 0.05	0.66 ± 0.02	0.65 ± 0.02
[^{3}H] outflow in slices incubated with [^{3}H]NA release				
In percent of accumulated [^{3}H]NA	0.94 ± 0.05	0.93 ± 0.04	0.90 ± 0.02	0.89 ± 0.03
In nCi	0.79 ± 0.07	0.67 ± 0.05	0.74 ± 0.03	0.73 ± 0.03
[^{3}H] outflow in slices incubated with [^{3}H]5-HT release				
In percent of accumulated [^{3}H]5-HT	1.77 ± 0.10	2.09 ± 0.15	2.03 ± 0.04	2.08 ± 0.06
In nCi	0.51 ± 0.04	0.47 ± 0.05	0.51 ± 0.04	0.52 ± 0.04

Data from slices of the frontoparietal cortex of young (YOUNG), aged unimpaired (AU), aged moderately impaired (AMI), and aged severely impaired (ASI) rats are shown.

3.4. Evoked overflow of tritium

Data are shown in Fig. 3. ANOVA of the electrically evoked overflow of [^{3}H]ACh (Fig. 3A) showed a significant Age effect [relative release: $F(1,30) = 11.4$, $P < .01$; absolute release: $F(1,30) = 85.1$, $P < .001$], the relative release being reduced by 22% and the absolute one by 44% in the aged rats. ANOVA of the evoked release of [^{3}H]ACh in the subpopulations also showed a significant Subpopulation effect on both the relative and absolute release [$F(3,28) = 5.3$ and 28.5, respectively, $P < .01$]. Concerning the relative release, the effect was due to the fact that values in AMI and ASI rats were significantly lower than in YOUNG rats. Relative values in AU rats tended to be significantly lower than in YOUNG rats ($P = .08$). The differences between AU, AMI, and ASI rats were not significant. On the absolute release, the effect was due to significantly lower values in AU, AMI, and ASI rats as compared to YOUNG rats ($P < .001$). The difference between AU, AMI, and ASI rats failed to be significant.

ANOVA of the electrically evoked overflow of [^{3}H]NA (Fig. 3B) showed a significant Age effect [relative release: $F(1,30) = 12.0$, $P < .01$; absolute release: $F(1,30) = 12.2$, $P < .01$], the relative release being reduced by 11% and the absolute one by 20% in the aged rats. ANOVA of the evoked release of [^{3}H]NA in the subpopulations also showed a significant Subpopulation effect on both the relative (%) and absolute (nCi) release [$F(3,28) = 3.8$ and 4.8, respectively, $P < .05$]. Concerning the relative release, this effect was due to significantly lower values in AU, AMI, and ASI rats as compared to YOUNG rats ($P < .05$). The difference between AU, AMI, and ASI rats was not significant. The effect on the absolute release was due to values that were significantly lower in AU, AMI, and ASI as compared to YOUNG rats.

ANOVA of the electrically evoked overflow of [^{3}H]5-HT (Fig. 3C) showed a significant Age effect [relative release: $F(1,30) = 32.4$, $P < .001$; absolute release: $F(1,30) = 14.2$, $P < .001$], the relative release being reduced by 21% and the absolute one by 34% in the aged rats. ANOVA of the evoked release of [^{3}H]5-HT in the subpopulations also showed a significant Subpopulation effect on both the relative (%) and absolute (nCi) release [$F(3,28) = 11.9$ and 4.6, respectively, $P < .01$]. Concerning the relative release, this effect was due to significantly lower values in AU, AMI, and ASI rats as compared to YOUNG rats ($P < .001$). The difference between AU, AMI, and ASI rats was not significant. The effect on the absolute release was due to values that were significantly lower in AU, AMI, and ASI as compared to YOUNG rats.

3.5. Effects of oxotremorine on electrically evoked release of [^{3}H]ACh

Data are shown in Fig. 4. ANOVA showed no significant Age effect, but there was a significant overall effect of the Drug [$F(1,60) = 592.6$, $P < .001$] and the Concentration

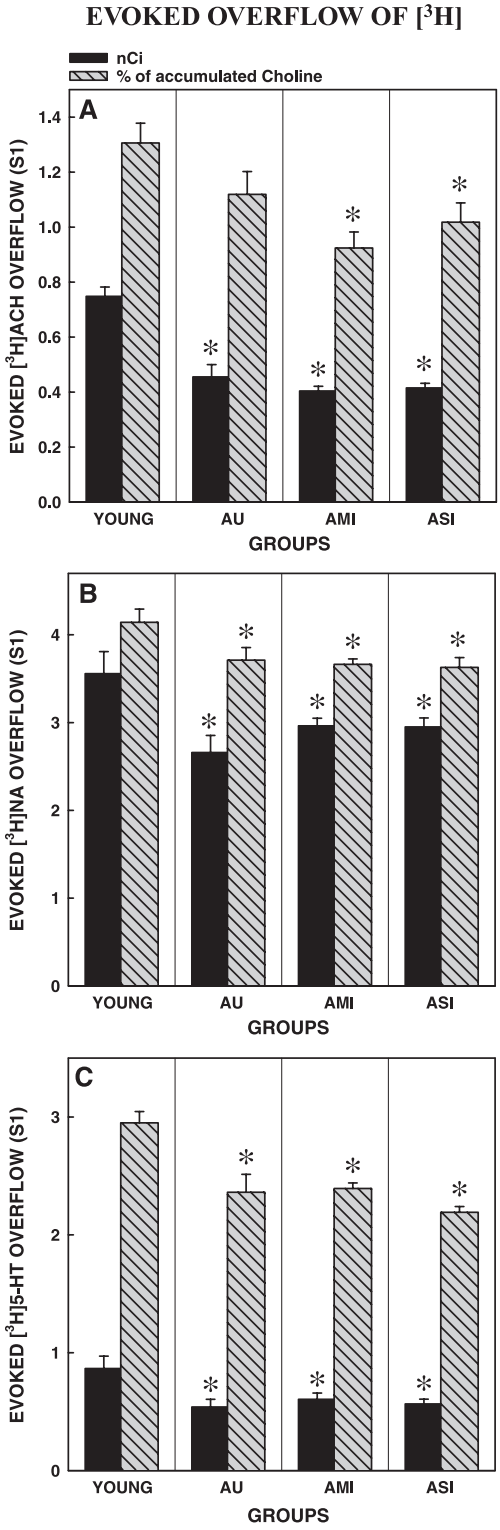

Fig. 3. [^{3}H]overflow evoked at S_1 by electrical field stimulation in slices prepared from the frontoparietal cortex and preincubated with (A) [^{3}H]choline, (B) [^{3}H]NA, and (C) [^{3}H]5-HT. Data are means (+S.E.M.) shown as either 'absolute' (in nCi; solid bars) or 'relative' (in percent of accumulation; hatched greyish bars) release. Group abbreviations are as in Fig. 2. Statistics: * significantly different from YOUNG, $P < .05$. Note that values are given in nCi (filled bars) and percent of [^{3}H] accumulation (hatched bar) for each group in each bar graph. There were eight rats per group and seven to eight slices per rat.

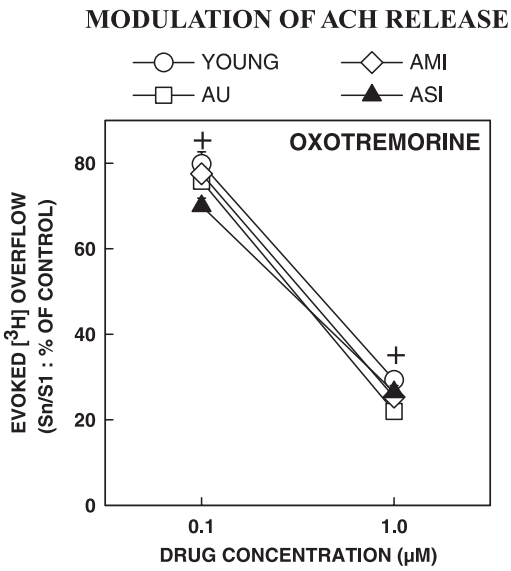

Fig. 4. Effects of oxotremorine on the evoked [^{3}H] overflow in slices prepared from the frontoparietal cortex and preincubated with [^{3}H]choline. The effects of two concentrations of the drug on the evoked overflow of [^{3}H] are expressed as a percentage of the respective control ratios (no drug). Group abbreviations are as in Fig. 2. Statistics: $^+$significantly different from control, $P<.05$ (no drug). There were eight rats per group and three to four slices per rat.

[$F(1,60)=551.8$, $P<.001$]. The only significant interaction was between the Drug and Concentration factors [$F(1,60)=539.7$, $P<.001$]; it reflected the concentration-dependent decrease of the release induced by oxotremorine as compared to the control conditions (-23% and -74% at 0.1 and 1 μM, respectively). ANOVA of the electrically evoked overflow of [^{3}H]ACh under the different drug conditions in the four subpopulations showed no significant Subpopulation effect [$F(3,56)<1.0$], but there were significant overall effects of the Drug [$F(1,56)=781.9$, $P<.001$] and of the Concentration [$F(1,56)=794.7$, $P<.001$] factors, as well as a significant Drug × Concentration [$F(3,56)=779.1$, $P<.001$] interaction. This interaction reflected the concentration-dependent decrease of the release induced by oxotremorine as compared to the control conditions.

3.6. Effects of UK 14,304 on electrically evoked release of [^{3}H]NA

Data are shown in Fig. 5. ANOVA showed no significant Age effect, but there was a significant effect of the Drug [$F(1,60)=1174.7$, $P<.001$] and the Concentration [$F(1,60)=636.3$, $P<.001$]. The only significant interaction was between the Drug and Concentration factors [$F(1,60)=638.3$, $P<.001$]; it reflected the concentration-dependent decrease of the release induced by UK 14,304 as compared to the control conditions (-30% and -59% at 0.01 and 0.1 μM, respectively). ANOVA of the electrically evoked overflow of [^{3}H]NA under the different drug conditions in the four subpopulations showed no significant Subpopulation effect [$F(3,56)<1.0$], but there were significant overall effects of the Drug [$F(1,56)=1526.3$, $P<.001$] and of the Concentration [$F(1,56)=858.3$, $P<.001$] factors, as well as a significant Drug × Concentration interaction [$F(1,56)=862.3$, $P<.001$]. The Drug × Concentration interaction can be interpreted as just mentioned for analysis of the Age effect.

3.7. Effects of CP 93,129 on electrically evoked release of [^{3}H]5-HT

Data are shown in Fig. 6. ANOVA showed no significant Age effect, but there was a significant effect of the Drug [$F(1,60)=312.1$, $P<.001$] and the Concentration [$F(1,60)=102.2$, $P<.001$]. The only significant interaction was between the Drug and Concentration factors [$F(1,60)=102.4$, $P<.001$]; as for both other drugs, it reflected the concentration-dependant decrease of the release induced by CP 93,129 in comparison with the control conditions (-28% and -55% at 0.0316 and 0.316 μM, respectively). ANOVA of the electrically evoked overflow of [^{3}H]5-HT under the different drug conditions in the four subpopulations showed no significant Subpopulation effect [$F(3,56)<1.0$], but there were significant overall effects of the Drug [$F(1,56)=140.1$, $P<.001$] and of the Concentration [$F(1,56)=140.4$, $P<.001$] factors, as well as a significant Drug × Concentration interaction [$F(1,224)=145.8$, $P<.001$], which can be interpreted as for the analysis of the Age effect.

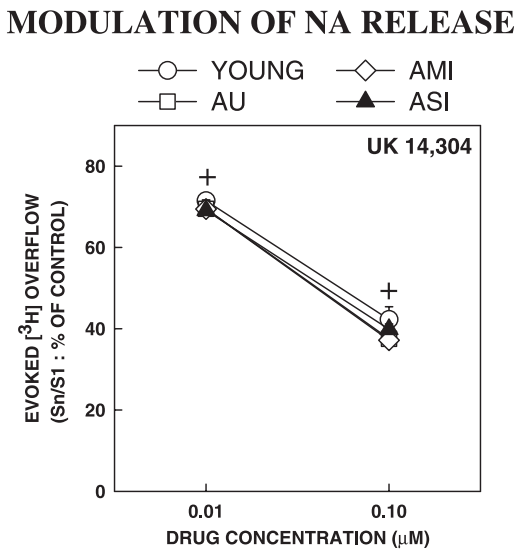

Fig. 5. Effects of UK 14,304 on the evoked [^{3}H] overflow in slices prepared from the frontoparietal cortex and preincubated with [^{3}H]NA. The effects of two concentrations of the drug on the evoked overflow of [^{3}H] are expressed as a percentage of the respective control ratios (no drug). Group abbreviations are as in Fig. 2. Statistics: $^+$significantly different from control, $P<.05$ (no drug). There were eight rats per group and three to four slices per rat.

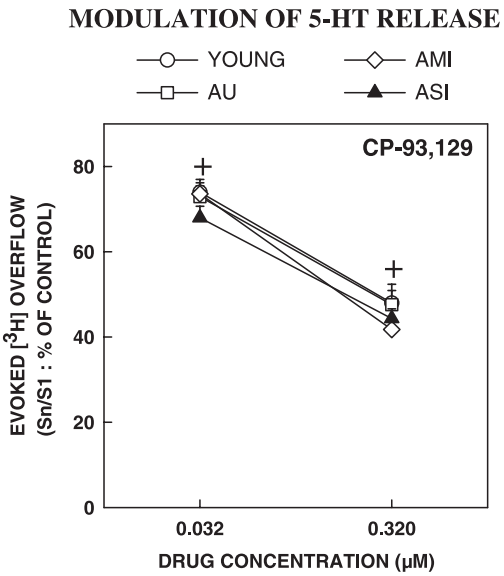

Fig. 6. Effects of CP 93,129 on the evoked [^{3}H] overflow in slices prepared from the frontoparietal cortex and preincubated with [^{3}H]5-HT. The effects of two concentrations of the drug on the evoked overflow of [^{3}H] are expressed as a percentage of the respective control ratios (no drug). Group abbreviations are as in Fig. 2. Statistics: $^+$significantly different from control, $P<.05$ (no drug). There were eight rats per group and three to four slices per rat.

3.8. Choline acetyltransferase and acetylcholinesterase activity

Data are shown in Table 2. ANOVA of the ChAT activity in the frontoparietal cortex or in the hippocampus failed to show a significant Age [−7%; $F(1,30)<1.0$] or Subpopulation [+2.5%; $F(3,28)<1.0$] effect. Conversely, ANOVA of the ChAT activity found in the striatum showed a significant Age effect [−23%; $F(1,30)=8.33$], while the Subpopulation effect only tended to be significant [$F(3,28)=2.7$, $P=.06$], due to values in AU, AMI, and ASI rats that tended to be significantly lower than in the YOUNG group ($P<.10$ for all three comparisons).

ANOVA of the AChE activity in the cortex homogenates, using an Age (Young, Aged) × Assay condition (control, ethopropazine, galanthamine, physostigmine) design, showed significant effects of Age [$F(1,30)=5.0$, $P<.05$] and Assay condition [$F(3,90)=801.3$, $P<.001$] factors, as well as a significant interaction between both [$F(3,90)=5.4$, $P<.01$]. The Age effect was due to a significantly lower overall AChE activity in aged as compared to the young rats (−14%). The Assay condition effect was due to a significant inhibition of AChE activity by ethopropazine (−9%, $P<.05$), galanthamine (−91%, $P<.001$), and physostigmine (−99%, $P<.001$), as compared to the control condition. The interaction can be interpreted as reflecting the fact that whereas YOUNG and aged rats differed significantly from each other in the absence of cholinesterase inhibitors ($P<.001$) or in the presence of ethopropazine ($P<.01$), they did no longer differ in the presence of galanthamine or physostigmine. When the Subpopulation factor was substituted for the Age factor (YOUNG, AU, AMI, ASI), ANOVA showed a marginal Group effect [$F(3,28)=2.8$, $P=.059$], a significant effect of the Assay condition [$F(3,84)=976.4$, $P<.001$], and an interaction between both factors [$F(9,84)=2.4$, $P<.05$]. While the Assay condition effect can be explained as above, the interaction was mainly due to the fact that AU, AMI, and ASI rats showed a significantly lower activity than YOUNG rats in the absence of cholinesterase inhibitors ($P<.05$) or in the presence of ethopropazine ($P<.05$), while none of these differences was significant in the presence of galanthamine or physostigmine.

In the hippocampus, ANOVA only showed a significant effect of the Assay condition, whether the Age or the Subpopulation factors were considered [$F(1,30)=1.1$ and $F(3,28)=0.6$]. This effect can be interpreted as for the cortex.

Finally, whether the Age or the Subpopulation factor was considered, ANOVA of the data from the striatum yielded a picture that, in terms of overall effects and corresponding 2 × 2 comparisons, was similar to that characterizing the cortex. The age-related reduction of AChE activity was of about 17% in the absence of cholinesterase inhibitors.

Table 2
Activity of ChAT (nmoles/mg protein/min) and of AChE (nmoles/mg protein/min) under various assay conditions (without additional drugs or in presence of ethopropazine, galanthamine, or physostigmine) in homogenates prepared from the frontoparietal cortex, the hippocampus, or the striatum of young (YOUNG), aged unimpaired (AU), aged moderately impaired (AMI), and aged severely impaired (ASI) rats

Variable	Subpopulations			
	YOUNG ($N=8$)	AU ($N=8$)	AMI ($N=8$)	ASI ($N=8$)
ChAT activity				
Cortex	0.36 ± 0.02	0.32 ± 0.01	0.33 ± 0.02	0.35 ± 0.03
Hippocampus	0.59 ± 0.02	0.56 ± 0.03	0.61 ± 0.04	0.62 ± 0.05
Striatum	0.31 ± 0.03	0.25 ± 0.02	0.24 ± 0.02	0.24 ± 0.02
AChE activity (no additional drugs)				
Cortex	34.7 ± 1.8	28.2 ± 1.6	28.0 ± 1.6	31.8 ± 2.3
Hippocampus	46.1 ± 1.4	42.3 ± 4.4	45.0 ± 2.6	40.7 ± 5.03
Striatum	330.4 ± 24.5	288.1 ± 13.5	265.9 ± 13.0	264.4 ± 17.5
AChE activity (+ 10 μM ethopropazine)				
Cortex	31.5 ± 1.8	26.2 ± 1.6	26.5 ± 1.2	29.4 ± 2.3
Hippocampus	44.2 ± 1.1	39.9 ± 3.9	41.2 ± 2.4	37.0 ± 4.6
Striatum	314.7 ± 21.8	260.9 ± 17.0	249.1 ± 13.5	242.3 ± 16.1
AChE activity (+ 10 μM galanthamine)				
Cortex	2.9 ± 0.2$^\#$	2.3 ± 0.2$^\#$	2.1 ± 0.2$^\#$	3.3 ± 0.7$^\#$
Hippocampus	3.9 ± 0.1$^\#$	3.4 ± 0.4$^\#$	4.1 ± 0.3$^\#$	3.6 ± 0.6$^\#$
Striatum	17.6 ± 3.0$^\#$	8.7 ± 2.6$^\#$	11.7 ± 2.2$^\#$	11.0 ± 2.3$^\#$
AChE activity (+ 10 μM physostigmine)				
Cortex	0.2 ± 0.04$^\#$	0.4 ± 0.03$^\#$	0.5 ± 0.05$^\#$	0.5 ± 0.04$^\#$
Hippocampus	0.7 ± 0.03$^\#$	0.6 ± 0.1$^\#$	0.7 ± 0.04$^\#$	0.6 ± 0.1$^\#$
Striatum	1.1 ± 0.4$^\#$	0.5 ± 0.3$^\#$	0.4 ± 0.02$^\#$	0.8 ± 0.3$^\#$

Statistics: $^\#$significantly different from AChE activity found in the same structure without additional drugs, $P<.05$.

Regression analysis showed a significant positive correlation between ChAT activity and AChE activity measured in the absence of cholinesterase inhibitors and in the presence of ethopropazine, in the cortex (r=.84 and .8, respectively, $P<.001$), the hippocampus (r=.61 and .56, respectively, $P<.001$), and the striatum (r=.84 and .9, respectively, $P<.001$). None of the variables were significantly correlated under identical assay conditions across structures.

3.9. Concentrations of monoamines

Data are shown in Table 3. ANOVA of the concentrations of NA, DA, DOPAC, 5-HT, and 5-HIAA failed to show any significant Age or Subpopulation effect in the cortex, as well as in the hippocampus. There were, however, significant Age and Subpopulation effects on the concentration of NA [$F(3,30)=13.2$, $F(3,28)=5.87$, $P<.01$] and DA in the striatum [$F(3,30)=16.22$, $F(3,28)=5.42$, $P<.01$, $P<.01$]. In all three populations of aged rats, the striatal concentrations of NA and of DA were significantly lower than in the young rats ($P<.01$).

3.10. Correlations and multiple regression analyses

The computation of simple correlations (two variables) between behavioral and neuropharmacological (uptake, outflow, and overflow in slices) or neurochemical variables (concentration of neurotransmitters, metabolites, or enzymatic activity) did not show any other significant correlation than (i) between acquisition performance and AChE activity in the cortex, but only in the presence of galanthamine (r=.43, P=.036), (ii) probe trial performance and the relative basal NA outflow (r=.50, P=.014) or the concentration of NA in the hippocampus ($r=-.44$, P=.031). Multiple regression analysis did not yield any information suggesting that a combination of various neurobiological parameters (e.g., evoked overflow of [^{3}H]ACh, [^{3}H]NA, and [^{3}H]5-HT]) could account for the behavioral outcome among aged rats.

Table 3
Concentration of monoamines (mean ± S.E.M. in ng/mg protein) in homogenates prepared from the frontoparietal cortex, the hippocampus, or the striatum of young (YOUNG), aged unimpaired (AU), aged moderately impaired (AMI), and aged severely impaired (ASI) rats

Variable	Subpopulations			
	YOUNG (N=8)	AU (N=8)	AMI (N=8)	ASI (N=8)
NA				
Cortex	3.95 ± 0.22	3.95 ± 0.18	3.94 ± 0.21	4.49 ± 0.42
Hippocampus	3.34 ± 0.13	3.26 ± 0.22	3.56 ± 0.19	3.41 ± 0.24
Striatum	0.94 ± 0.09	0.67 ± 0.06 *	0.56 ± 0.05 *	0.74 ± 0.07 *
DOPAC				
Cortex	1.26 ± 0.33	1.11 ± 0.36	1.33 ± 0.08	1.33 ± 0.16
Hippocampus	0.51 ± 0.04	0.57 ± 0.07	0.49 ± 0.03	0.54 ± 0.06
Striatum	21.31 ± 1.95	16.73 ± 1.38	15.58 ± 1.04	18.07 ± 1.86
DA				
Cortex	1.53 ± 0.13	1.45 ± 0.19	1.70 ± 0.11	1.98 ± 0.27
Hippocampus	0.40 ± 0.08	0.49 ± 0.16	0.37 ± 0.03	0.46 ± 0.06
Striatum	153.9 ± 10.0	122.9 ± 8.3 *	113.7 ± 7.5 *	119.4 ± 4.0 *
5-HIAA				
Cortex	2.86 ± 0.18	2.78 ± 0.21	3.00 ± 0.32	3.46 ± 0.19
Hippocampus	4.25 ± 0.22	4.30 ± 0.21	5.12 ± 0.29	5.19 ± 0.41
Striatum	7.19 ± 0.51	7.04 ± 0.43	6.72 ± 0.34	7.74 ± 0.58
5-HT				
Cortex	5.25 ± 0.41	4.09 ± 0.31	4.51 ± 0.24	4.98 ± 0.41
Hippocampus	3.59 ± 0.09	3.20 ± 0.19	3.42 ± 0.17	3.57 ± 0.22
Striatum	5.67 ± 0.43	4.86 ± 0.25	4.69 ± 0.23	5.09 ± 0.43

Statistics: * significantly reduced as compared to YOUNG, $P<.05$.

4. Discussion

As in our previous study (Birthelmer et al., in press), our data confirm that a population of aged rats can be subdivided into different subpopulations characterized by different levels of performances in a Morris water-maze reference-memory task. The three subpopulations comprised normal (young and aged), moderately impaired, and severely impaired rats. In our previous experiment, clear-cut age-related neurochemical and neuropharmacological alterations could be identified in the hippocampus. However, we were unable to establish any relationship between the different levels of performances and cholinergic, noradrenergic, or serotonergic markers in the hippocampus, whether from a static (e.g., neurotransmitter concentrations in homogenates) or a dynamic (e.g., electrically evoked transmitter release in slices) point of view. These results urged us to focus our attention on other brain regions, in particular on another major target of the basal forebrain, namely, the frontoparietal cortex. As previously found in the hippocampus, age-related deficits were also evident in this part of the brain. Overall, the aged rats exhibited a reduced accumulation of [^{3}H]choline (−25%), [^{3}H]NA (−9%), and [^{3}H]5-HT (−16%). They also showed a weaker electrically evoked release (absolute values expressed in nCi) of [^{3}H]ACh (−44%), [^{3}H]NA (−20%), and [^{3}H]5-HT (−34%). The responses of young and aged rats to drugs acting at muscarinic, α_2, and 5-HT$_{1B}$ autoreceptors were roughly comparable: the muscarinic agonist oxotremorine weakened the release of [^{3}H]ACh, the α_2 agonist UK 14,304 decreased the release of [^{3}H]NA, and the 5-HT$_{1B}$ agonist CP 93,129 reduced that of [^{3}H]5-HT. The ChAT activity was not significantly reduced in the frontoparietal cortex (or the hippocampus), but was lower in the striatum. The activity of AChE was reduced (−14%; Table 2), but the concentrations of DA, DOPAC, NA, 5-HT, and 5-HIAA (Table 3) were not significantly altered in cortical (or hippocampal) homogenates of aged rats. In the striatum, the concentrations of DA and NA were reduced. None of all these age-related changes, was subpopulation-dependent.

4.1. Aging and cognitive dysfunctions

Previous findings demonstrated that aging is not necessarily associated with alterations of learning and memory capabilities in rats (Gallagher and Burwell, 1989; Rapp and Amaral, 1992). Our present results indicate that part of the aged rats showed reference-memory performances in a water-maze task that were close to those found in young adult rats. However, at the same age, other rats from the same population, and thus with the same experience, had difficulties or even failed to learn the location of the platform. The Morris water maze is considered a task measuring spatial learning, but to reach the platform, the rats have to produce a motor response (e.g. swim around while searching for the platform). As aging may be accompanied by motor and sensorimotor perturbations (Ingram et al., 1994), there is a risk that cognitive measures are "contaminated" by sensorimotor biases. Although such possible contaminations have been a major issue in a review by Lindner (1997), there are arguments in the literature suggesting that motor, sensorimotor, and cognitive dysfunctions may be dissociated, at least in some tasks (e.g., Gage et al., 1984; Gallagher and Burwell, 1989; Markowska et al., 1989). Furthermore, we previously reported (Stemmelin et al., 1999) that these different aspects of behavioral performances were not correlated with each other in a population of rats that aged under conditions identical to the present ones. Finally, only the distance to the platform was considered in the present study, and this variable is generally regarded as poorly related to sensorimotor biases. For all these reasons, one may assume that the individual differences found in our population of aged rats most probably reflected different degrees of cognitive dysfunctions. In the recent study by Birthelmer et al. (in press), the subpopulations were derived from the average performances displayed by the rats over the five acquisition days. Interestingly, the differences between subpopulations were also found in the probe trial. In our present study, the three subpopulations of aged rats exhibited probe-trial performances, which did not differ significantly from each other. As the testing procedure was exactly the same in both studies, we do not know how to account for this difference. It is, nevertheless, noteworthy that when we considered the distance swum during the probe trial before the virtual location of the platform was reached for the first time, we again found that the performances of the YOUNG and AU rats did not differ significantly from each other, and that those displayed by ASI rats were the worst. These differences suggest that noncognitive factors (e.g., greater fatigability in aged rats?) may have interfered with probe-trial performances calculated over a duration of 30 s.

The water-maze task requires correct spatial learning capabilities (e.g., D'Hooge and De Deyn, 2001) and, as the hippocampus is generally considered to play a crucial role in processing spatial information (e.g., O'Keefe and Nadel, 1978; Hollup et al., 2001), the fact that we paid attention to neuropharmacological characteristics of the frontoparietal cortex may seem peculiar. However, it must be emphasized that the reasons of this choice were multiple. First, as stated above, the study on neuropharmacological functions in the hippocampus of aged rats (Birthelmer et al., in press) was completed when the present one was started. Second, the frontoparietal cortex is a target of nucleus basalis magnocellularis cholinergic neurons, and lesions of this nucleus not confining to only its cholinergic component (e.g., Galani et al., 2002), and thus damaging other types of neurons or fibers, were shown to impair rats in a water-maze task (e.g., Mandel et al., 1989; Mundy et al., 1990; Dekker et al., 1994). Interestingly, there is evidence that age-related behavioral deficits are not exclusively associated to cholinergic dysfunctions in the basal forebrain (e.g., Stemmelin et al., 2000). Third, for deriving the subpopulations, it seemed important to us to use the same behavioral criterion as the one used in two earlier experiments (Stemmelin et al., 1999, 2000) and our last experiment on neuropharmacological properties of the aged hippocampus (Birthelmer et al., in press). Finally, water-maze performances were previously found to correlate also with non-hippocampal neurochemical markers, in particular with NA concentration, as well as 5-HIAA/5-HT and homovanillic acid/DA ratios in the frontoparietal cortex (Stemmelin et al., 2000).

4.2. Aging and cholinergic/monoaminergic markers in the frontoparietal cortex

Age-related reductions of ChAT and AChE activity in cortical regions were reported in a few previous studies (ChAT: Ishimaru et al., 1991; AChE: Sirviö et al., 1988b, 1989). However, it seems that such alterations are not found consistently (e.g., Decker, 1987; Sirviö et al., 1988a). In two of these studies (Sirviö et al., 1988b, 1989), it is even suggested that ChAT activity may be unaltered, when AChE activity is reduced by aging. Our present results are in line with the latter studies. The fact that ChAT activity was not significantly altered, while AChE activity was weaker, may be related to the role of these enzymes in cholinergic function. ChAT is not the rate-limiting factor in acetylcholine synthesis, and there is more ChAT present in cholinergic neurons than required for normal function. Therefore, the relationship of ChAT activity with cholinergic functions may be looser than that of AChE, which is involved in the inactivation of the neurotransmitter and may therefore be proportional to the cholinergic activity. We also confirm that the level of cortical ChAT or AChE activity, and perhaps more generally that of cholinergic function, does not necessarily correlate with the performances of aged rats assessed in a water-maze reference-memory task (e.g., Lee et al., 1994; Stemmelin et al., 2000).

In Alzheimer patients, it has been argued that ACh can be hydrolyzed by butyrylcholinesterase (Mesulam et al., 2002), and it was described that the AChE activity may

decrease while that of butyrylcholinesterase (BChE) may increase (e.g., Greig et al., 2001). Therefore, we measured the hydrolysis of acetylthiocholine iodide in the absence and presence of drugs inhibiting AChE and/or BChE. Our data indicate that almost all acetylthiocholine iodide was hydrolyzed by AChE and that the activity of AChE was comparable in all subgroups of aged rats. However, it is noteworthy that the hydrolysis of AChE was slightly but significantly altered by ethopropazine, a selective inhibitor of BChE. It was also dramatically but not completely reduced by the more selective AChE inhibitor galanthamine and was almost completely blocked by physostigmine. In the hippocampus of aged rats, ChAT activity and AChE activity were not significantly changed, confirming our previous findings (Birthelmer et al., in press). Finally, in the striatum of aged rats, the ChAT and AChE activity was reduced significantly, confirming reduced cholinergic functions also in the striatum (e.g., Sirviö et al., 1989; Stemmelin et al., 2000). It is worth mentioning that AChE can also be found in non-cholinergic neurons (e.g., Satoh et al., 1983), particularly in dopaminergic neurons (e.g., Greenfield et al., 1983; Weston and Greenfield, 1986). Thus, part of the age-related changes in AChE activity might reflect non-cholinergic alterations.

An interesting observation concerned the regression analysis. The fact that ChAT and AChE activity were significantly correlated within each structure may sound trivial. However, that the activity of both enzymes was not correlated across structures suggests that, while altering central cholinergic functions, aging does not seem to do it equally in all three systems assessed in this study, i.e., in the NBM and hippocampal projection neurons and in the striatal interneurons.

Concerning the tissue concentrations of monoamines, we could not find any significant change in the cortex or the hippocampus of aged rats. The latter finding confirms the outcome of the recent study in which we focused on aging and hippocampal functions (Birthelmer et al., in press). For the cortex, controversial data can be found in the literature. For instance, on the concentration of serotonin, Gozlan et al. (1990) reported an age-related reduction, Valjakka et al. (1990) found no significant alteration, and van Luijtelaar et al. (1992) or Robson et al. (1993) observed an increase. Shores et al. (1999) found that an age-related loss of noradrenergic neurons occurred in the locus coeruleus. Petkov et al. (1988) and Luine et al. (1990) demonstrated a decrease of NA concentration in the cortex, but in other studies, this marker was unchanged (Ishida et al., 2001), slightly increased (Valjakka et al., 1990), or increased more markedly (Harik and McCracken, 1986), illustrating once again how heterogeneous neurochemical findings in aged rats may be. There is no exception for DA, which, in the cortex of aged rats, was also found to be decreased (Petkov et al., 1988; Luine et al., 1990) or increased (Godefroy et al., 1991). All these results might point towards the fact that in the present experiment, like in other ones, monoaminergic hippocampal innervations of the cortex do not seem to undergo dramatic alterations with aging, and that the corresponding neurotransmitter systems (dopaminergic, noradrenergic, and serotonergic) are not affected consistently in aged rats. Conversely, in the striatum of the aged rats of the present study, both NA and DA concentrations were found to be reduced, an observation in line with a more consensual literature about age-related catecholaminergic alterations in the striatum (Petkov et al., 1988; Gozlan et al., 1990; Marshall and Rosenstein, 1990; Dawson et al., 1999; Miguez et al., 1999).

It is also noteworthy that the uptake of [^{3}H]choline was altered by aging. Although the accumulation of [^{3}H]choline certainly reflected specific uptake (by axon terminals), as well as nonspecific binding (e.g., [^{3}H]choline to cell membranes), one may accept that, in a way or another, part of this accumulation was linked to the density of cholinergic terminals, to the functional status of the uptake sites, and/or to the storage capacity of the neurons.

Taken together and compared to the literature, our present data suggest that there is considerable heterogeneity in the neurochemical changes related to aging, and that no serious prediction on the status of cortical cholinergic, noradrenergic, and serotonergic transmitter systems can be made in aged animals on the basis of the type of behavioral performances assessed herein. Factors such as strain, sex, rearing conditions, and even age (experiments in the literature being carried out on rats aged of 20 to more than 30 months) might account for both this variability and this poor reproducibility of neurochemical findings from one laboratory to another.

4.3. Aging and neurotransmitter release in the frontoparietal cortex

With the electrical stimulation conditions used herein, the evoked overflow of tritium by slices preloaded with [^{3}H]choline, [^{3}H]NA or [^{3}H]5-HT was repeatedly shown to be Ca^{2+}-dependent and sensitive to tetrodotoxin. Therefore, this overflow can be considered as representing action potential-induced exocytotic release of a neurotransmitter (e.g., Taube et al., 1977; Göthert and Schlicker, 1991; Goldbach et al., 1998).

The slices from aged rats preloaded with [^{3}H]choline, [^{3}H]NA, or [^{3}H]5-HT released less tritium in response to electrical field stimulation than slices from the young rats. The reductions were of −44%, −20%, and −34% for ACh, NA, and 5-HT, respectively, when the absolute overflow was considered. This reduced overflow might not be a direct consequence of a lower uptake of [^{3}H]NA or [^{3}H]5-HT, as this uptake was not altered by aging. Concerning [^{3}H]choline, this possibility cannot be completely excluded (but see below) as the accumulation of this precursor was reduced in aged rats, which might result from age-related alterations of the uptake carrier or of the density of cholinergic terminals. To our knowledge, there is

little information in the literature on the status of this carrier in the brain of aged rats. Forloni and Angeretti (1992) reported a decreased binding of hemicholinium to high-affinity uptake sites in the cortex of aged rats, confirming earlier data by Sirviö et al. (1988b), but it seems that the fundamental mechanism of these sites may be preserved (Wheeler, 1985). Concerning other sites, Druse et al. (1997) found that in the cortex of aged rats, the 5-HT reuptake sites were increased. The number of reuptake sites of NA seemed also increased by age as shown by Harik and McCracken (1986), but was decreased according to others (Shores et al., 1999), or even unchanged (McIntosh and Westfall, 1987). Our data might be indicative for an alteration of the uptake mechanisms and/or for a reduced number of cholinergic terminals, and a relative preservation of the noradrenergic and serotonergic ones.

The reduced overflow of [^{3}H]ACh cannot be ascribed to a lower uptake of [^{3}H]choline in the slices from aged rats. Indeed, when the relative values were taken into account (percent of [^{3}H] accumulation in the slices), a significant age-related decrease of the release was also observed: − 22%. This was also true for [^{3}H]NA and [^{3}H]5-HT: − 21% and − 11%, respectively. Such observations indicate that mechanisms involved in the exocytosis of neurotransmitters might also be altered by aging (e.g., Meyer et al., 1986; Giovannelli et al., 1988).

Concerning the evoked release of ACh and 5-HT, the decreases reported herein are not only quite comparable to the changes recently found in the hippocampus by Birthelmer et al. (in press). Despite some differences in the stimulation conditions, they are also in line with previous reports on the evoked release of ACh or 5-HT by cortical slices (e.g., Pedata et al., 1983; Meyer et al., 1984; Meyer and Judkins, 1993; Giovannelli et al., 1988; Schlicker et al., 1989; Araujo et al., 1990; Rylett et al., 1993; Tanaka et al., 1996). As was the case for the reduced uptake, part of these results might also reflect a reduced innervation of the cortex as, for instance, reviewed by Decker (1987) or reported by Turrini et al. (2001) for cholinergic fibers, by Nishimura et al. (1998) or van Luijtelaar et al. (1992) for serotonergic fibers, or by Ishida et al. (2000) for noradrenergic ones. However, there is at least one report suggesting that in the cortex, the evoked release of ACh may be unaltered by aging (Buyukuysal et al., 1998).

Concerning the overflow of NA, our present findings in the frontoparietal cortex of aged rats are at some variance with the age-related changes found by Birthelmer et al. (in press) on the evoked overflow of NA in hippocampal slices. Indeed, while decreased in the cortex, this overflow was not significantly altered in the hippocampus, suggesting that the noradrenergic changes related to aging do not occur uniformly through the entire brain or, if considered at the level of the source, within the locus coeruleus. An alternative explanation might be that, beside evenly distributed degeneration processes, homotypic or heterotypic compensatory mechanisms (e.g., collateral sprouting from intact fibers) are more powerful in the hippocampus than in the cortex. For instance, the sympathetic (and thus, noradrenergic) sprouting, which is elicited by a cholinergic denervation, might have been more potent in the hippocampus than in the cortex (Crutcher, 1987), thereby contributing to counterbalance the effects of noradrenergic dysfunctions in the former structure. Such issues would certainly deserve further investigations, all the more because some reports suggested that the presynaptic activity of noradrenergic terminals in the cortex may be unchanged (release: McIntosh and Westfall, 1987) or even increased (uptake: Harik and McCracken, 1986).

It is well established that, at the level of axon terminals in the rodent cortex, the release of ACh is inhibited by, e.g., the activation of muscarinic autoreceptors, which are presumably of the M_2 type (e.g., Douglas et al., 2001), while that of NA is inhibited by, e.g., the activation of α_2 autoreceptors (e.g., Starke, 2001). Finally, the release of 5-HT is inhibited by, e.g., the activation of 5-HT_{1B} autoreceptors (e.g., Hjorth et al., 2000). Our data are in line with these considerations, although the muscarinic agonist used (oxotremorine) was not a selective compound for M_2 receptors (both other ones, namely, UK 14,304 and CP 93,129, are considered selective for their respective receptor subtypes, e.g., Cambridge, 1981 and Macor et al., 1990, respectively). A few previous studies have shown that aging might, under some circumstances, alter the functionality of autoinhibitory mechanisms in the cortex. As concerns the cholinergic system, Araujo et al. (1990) reported that the sensitivity of M_2 receptors can be reduced by aging. Earlier reports showed that atropine facilitated the electrically evoked release of ACh (Pedata et al., 1983) or had no effect in case of K^+-evoked release (Meyer et al., 1984) and that oxotremorine decreased the K^+-evoked release of ACh (Meyer et al., 1984). However, when observed, these changes were not different between young and aged rats. Concerning the release of NA, Schlicker et al. (1989) reported that the electrically evoked release of NA was inhibited by application of NA and facilitated by that of phentolamine. Again, there was no difference between young and aged rats. Using other stimulation conditions, Gonzales et al. (1991) reported that the NMDA-evoked release of NA was altered by aging, a conclusion also supported by findings of Qi and Nomura (1988), who used potassium to stimulate the release of NA. Finally, as to 5-HT, Schlicker et al. (1989) showed that the electrically evoked release of 5-HT was facilitated by application of methiothepine and inhibited by that of 5-HT, but young and aged rats presented comparable responses to the drugs. From the comparison of our present results with the few experiments that used electrical stimulation to evoke a neurotransmitter release in slices, it seems that the presynaptic modulation of ACh, NA, and 5-HT release is not altered by aging in the cortex, an observation suggesting that the cortical autoreceptor functions associated with these transmitter systems remain relatively preserved over time. For the cholinergic, noradrenergic, and

serotonergic cortical innervations, this conclusion is valuable in the three subpopulations of aged rats.

5. Conclusions

Altogether, our present results suggest that in the frontoparietal cortex of rats, aging may affect cholinergic, noradrenergic, and serotonergic reuptake and/or release functions. In addition, the presynaptic autoinhibitory mechanisms involving muscarinic, α_2, and 5-HT_{1B} autoreceptors seemed preserved. All these observations do not exclude that also other modulation mechanisms involving, e.g., terminal heteroreceptors of the transmitter systems considered herein, or autoreceptors of other transmitter systems, might be affected by the aging process. In addition, it might be assumed that differential alterations in the functionality of the reuptake carrier, in the mechanisms underlying the release, or even in the presynaptic autoreceptors investigated herein do not seem to account for the marked interindividual differences found on performances among aged rats in the water-maze reference-memory task. The lack of differential alterations between the subgroups of aged rats might also be explained by the fact that the differences in the performances of these three groups were not sharp enough. Indeed, whereas aged rats were impaired more (ASI) or less (AU) and one subgroup showed intermediate performances (AMI) during the acquisition phase of the water-maze task, all showed comparable deficits in the probe trial. However, noncognitive factors may have contributed to this leveling of performances in the probe trial. After having explored age-related presynaptic neuropharmacological changes in the hippocampus (Birthelmer et al., submitted for publication) and the frontoparietal cortex (present study) without being able to relate them to differences in cognitive performances, it seems that other transmitter systems or other brain structures must be investigated in future studies. Systems using amino acids as neurotransmitters (GABA, glutamate), or a structure such as the striatum, might be potentially interesting topics.

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References

Amenta F, Liu A, Giannella M, Pigini M, Tayebati SK, Zaccheo D. Age-related changes in the density of muscarinic cholinergic M1 and M2 receptor subtypes in pyramidal neurons of the rat hippocampus. Eur J Histochem 1995;39:107–16.

Araujo DM, Lapchak PA, Meaney MJ, Collier B, Quirion R. Effects of aging on nicotinic and muscarinic autoreceptor function in the rat brain: relationship to presynaptic cholinergic markers and binding sites. J Neurosci 1990;10:3069–78.

Ashour MB, Gee SJ, Hammock BD. Use of a 96-well microplate reader for measuring routine enzyme activities. Anal Biochem 1987;166:353–60.

Aubert I, Rowe W, Meaney MJ, Gauthier S, Quirion R. Cholinergic markers in aged cognitively impaired Long–Evans rats. Neuroscience 1995;67:277–92.

Barnes CA, Nadel L, Honig WK. Spatial memory deficit in senescent rats. Can J Psychol 1980;34:29–39.

Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp Neurol 2000;163:495–529.

Bartus RT, Dean III, RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982;217:408–14.

Birthelmer A, Stemmelin J, Jackisch R, Cassel J-C. Presynaptic modulation of acetylcholine, noradrenaline and serotonin release in the hippocampus of aged rats with various levels of memory impairments. Brain Res Bull 2003 [in press].

Burnett DM, Bowyer JF, Masserano JM, Zahniser NR. Effect of aging on alpha-1 adrenergic stimulation of phosphoinositide hydrolysis in various regions of rat brain. J Pharmacol Exp Ther 1990;255:1265–70.

Buyukuysal RL, Ulus IH, Kiran BK. Age-related alterations in pre-synaptic and receptor-mediated cholinergic functions in rat brain. Neurochem Res 1998;23:719–26.

Cambridge D. UK-14,304, a potent and selective alpha2-agonist for the characterisation of alpha-adrenoceptor subtypes. Eur J Pharmacol 1981; 72:413–5.

Consolo S, Wang JX, Fiorentini F, Vezzani A, Ladinsky H. In vivo and in vitro studies on the regulation of cholinergic neurotransmission in striatum, hippocampus and cortex of aged rats. Brain Res 1986;374:212–8.

Crutcher KA. Sympathetic sprouting in the central nervous system: a model for studies of axonal growth in the mature mammalian brain. Brain Res 1987;434:203–33.

Dawson Jr, R, Pelleymounter MA, Cullen MJ, Gollub M, Liu S. An age-related decline in striatal taurine is correlated with a loss of dopaminergic markers. Brain Res Bull 1999;48:319–24.

Decker MW. The effects of aging on hippocampal and cortical projections of the forebrain cholinergic system. Brain Res 1987;434:423–38.

Dekker AJ, Winkler J, Ray J, Thal LJ, Gage FH. Grafting of nerve growth factor-producing fibroblasts reduces behavioral deficits in rats with lesions of the nucleus basalis magnocellularis. Neuroscience 1994;60: 299–309.

D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 2001;36:60–90.

Douglas CL, Baghdoyan HA, Lydic R. M2 muscarinic autoreceptors modulate acetylcholine release in prefrontal cortex of C57BL/6J mouse. J Pharmacol Exp Ther 2001;299:960–6.

Druse MJ, Tajuddin NF, Ricken JD. Effects of chronic ethanol consumption and aging on 5-HT2A receptors and 5-HT reuptake sites. Alcohol Clin Exp Res 1997;21:1157–64.

Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.

Fonnum F. A rapid radiochemical method for the determination of choline acetyltransferase. J Neurochem 1975;24:407–9.

Forloni G, Angeretti N. Decreased [^{3}H]hemicholinium binding to high-affinity choline uptake sites in aged rat brain. Brain Res 1992;570:354–7.

Gage FH, Bjorklund A, Stenevi U, Dunnett SB, Kelly PA. Intrahippocampal septal grafts ameliorate learning impairments in aged rats. Science 1984;225:533–6.

Galani R, Lehmann O, Bolmont T, Aloy E, Bertrand F, Lazarus C, et al. Selective immunolesions of CH4 cholinergic neurons do not disrupt spatial memory in rats. Physiol Behav 2002;76:75–90.

Gallagher M, Burwell RD. Relationship of age-related decline across several behavioral domains. Neurobiol Aging 1989;10:691–708.
Gallagher M, Pelleymounter MA. Spatial learning deficits in old rats: a model for memory decline in the aged. Neurobiol Aging 1988;9:549–56.
Ghirardi O, Giuliani A, Caprioli A, Ramacci MT, Angelucci L. Spatial memory in aged rats: population heterogeneity and effect of levocarnitine acetyl. J Neurosci Res 1992;31:375–9.
Giovannelli L, Giovannini MG, Pedata F, Pepeu G. Purinergic modulation of cortical acetylcholine release is decreased in aging rats. Exp Gerontol 1988;23:175–81.
Godefroy F, Bassant MH, Lamour Y, Weil-Fugazza J. Effect of aging on dopamine metabolism in the rat cerebral cortex: a regional analysis. J Neural Transm Gen Sect 1991;83:13–24.
Goldbach R, Allgaier C, Heimrich B, Jackisch R. Postnatal development of muscarinic autoreceptors modulating acetylcholine release in the septohippocampal cholinergic system: I. Axon terminal region: hippocampus. Brain Res Dev Brain Res 1998;108:23–30.
Gonzales RA, Brown LM, Jones TW, Trent RD, Westbrook SL, Leslie SW. *N*-methyl-D-aspartate mediated responses decrease with age in Fischer 344 rat brain. Neurobiol Aging 1991;12:219–25.
Göthert M, Schlicker E. Regulation of serotonin release in the central nervous system by presynaptic heteroreceptors. In: Feigenbaum F, Hanani M, editors. Presynaptic regulation of neurotransmitter release: a handbook. Tel Aviv: Freund Publishing; 1991. p. 845–76.
Gozlan H, Daval G, Verge D, Spampinato U, Fattaccini CM, Gallissot MC, et al. Aging associated changes in serotoninergic and dopaminergic pre- and postsynaptic neurochemical markers in the rat brain. Neurobiol Aging 1990;11:437–49.
Grady CL, Craik FI. Changes in memory processing with age. Curr Opin Neurobiol 2000;10:224–31.
Greenfield SA, Grunewald RA, Foley P, Shaw SG. Origin of various enzymes released from the substantia nigra and caudate nucleus: effects of 6-hydroxydopamine lesions of the nigro-striatal pathway. J Comp Neurol 1983;214:87–92.
Greig NH, Utsuki T, Yu Q, Zhu X, Holloway HW, Perry T, et al. A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase. Curr Med Res Opin 2001;17:159–65.
Harik SI, McCracken KA. Age-related increase in presynaptic noradrenergic markers of the rat cerebral cortex. Brain Res 1986;381:125–30.
Hersi AI, Rowe W, Gaudreau P, Quirion R. Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acetylcholine release in memory-impaired aged rats. Neuroscience 1995;69:1067–74.
Hjorth S, Bengtsson HJ, Kullberg A, Carlzon D, Peilot H, Auerbach SB. Serotonin autoreceptor function and antidepressant drug action. J Psychopharmacol (Oxford) 2000;14:177–85.
Hollup SA, Kjelstrup KG, Hoff J, Moser MB, Moser EI. Impaired recognition of the goal location during spatial navigation in rats with hippocampal lesions. J Neurosci 2001;21:4505–13.
Huguet F, Tarrade T. Alpha 2-adrenoceptor changes during cerebral ageing. The effect of *Ginkgo biloba* extract. J Pharm Pharmacol 1992;44:24–7.
Ingram DK, London ED, Goodrick CL. Age and neurochemical correlates of radial maze performance in rats. Neurobiol. Aging 1981;2:41–7.
Ingram DK, Spangler EL, Iijima S, Ikari H, Kuo H, Greig NH, et al. Rodent models of memory dysfunction in Alzheimer's disease and normal aging: moving beyond the cholinergic hypothesis. Life Sci 1994;55:2037–49.
Ishida Y, Shirokawa T, Miyaishi O, Komatsu Y, Isobe K. Age-dependent changes in projections from locus coeruleus to hippocampus dentate gyrus and frontal cortex. Eur J Neurosci 2000;12:1263–70.
Ishida Y, Shirokawa T, Komatsu Y, Isobe K. Changes in cortical noradrenergic axon terminals of locus coeruleus neurons in aged F344 rats. Neurosci Lett 2001;307:197–9.
Ishimaru H, Ogawa S, Fuji K, Fukuta T, Kameyama T, Nabeshima T. Aged-related changes in learning and memory, choline acetyltransferase activity and number of neuronal cells in rats. J Pharmacobio-Dyn 1991;14:321–5.
Kalaria RN, Andorn AC, Tabaton M, Whitehouse PJ, Harik SI, Unnerstall JR. Adrenergic receptors in aging and Alzheimer's disease: increased beta 2-receptors in prefrontal cortex and hippocampus. J Neuroche 1989;53:1772–81.
Krzywkowski P, Lagny-Pourmir I, Jazat F, Lamour Y, Epelbaum J. The age-related increase in galanin binding sites in the rat brain correlates with behavioral impairment. Neuroscience 1994;59:599–607.
Lee JM, Ross ER, Gower A, Paris JM, Martensson R, Lorens SA. Spatial learning deficits in the aged rat: neuroanatomical and neurochemical correlates. Brain Res Bull 1994;33:489–500.
Lindner MD. Reliability, distribution, and validity of age-related cognitive deficits in the Morris water maze. Neurobiol Learn Mem 1997;68:203–20.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
Luine V, Bowling D, Hearns M. Spatial memory deficits in aged rats: contributions of monoaminergic systems. Brain Res 1990;537:271–8.
Macor JE, Burkhart CA, Heym JH, Ives JL, Lebel LA, Newman ME, et al. 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one: a potent and selective serotonin (5-HT1B) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. J Med Chem 1990;33:2087–93.
Mandel RJ, Gage FH, Thal LJ. Enhanced detection of nucleus basalis magnocellularis lesion-induced spatial learning deficit in rats by modification of training regimen. Behav Brain Res 1989;31:221–9.
Markowska AL, Stone WS, Ingram DK, Reynolds J, Gold PE, Conti LH, et al. Individual differences in aging: behavioral and neurobiological correlates. Neurobiol Aging 1989;10:31–43.
Marshall JF, Rosenstein AJ. Age-related decline in rat striatal dopamine metabolism is regionally homogeneous. Neurobiol Aging 1990;11:131–7.
McIntosh HH, Westfall TC. Influence of aging on catecholamine levels, accumulation, and release in F-344 rats. Neurobiol Aging 1987;8:233–9.
Mesulam M, Guillozet A, Shaw P, Quinn B. Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and Alzheimer brain. Neurobiol Dis 2002;9:88–93.
Meyer EM, Judkins JH. The development of age-related deficits in several presynaptic processes associated with brain [^{3}H]acetylcholine release. Mech Ageing Dev 1993;72:119–28.
Meyer EM, St. Onge E, Crews FT. Effects of aging on rat cortical presynaptic cholinergic processes. Neurobiol Aging 1984;5:315–7.
Meyer EM, Crews FT, Otero DH, Larsen K. Aging decreases the sensitivity of rat cortical synaptosomes to calcium ionophore-induced acetylcholine release. J Neurochem 1986;47:1244–6.
Miguez JM, Aldegunde M, Paz-Valinas L, Recio J, Sanchez-Barcelo E. Selective changes in the contents of noradrenaline, dopamine and serotonin in rat brain areas during aging. J Neural Transm 1999;106:1089–98.
Millan MJ, Lejeune F, Gobert A. Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. J Psychopharmacol (Oxford) 2000;14:114–38.
Mundy WR, Barone S, Tilson HA. Neurotoxic lesions of the nucleus basalis induced by colchicine: effects on spatial navigation in the water maze. Brain Res 1990;512:221–8.
Narang N. In situ determination of M1 and M2 muscarinic receptor binding sites and mRNAs in young and old rat brains. Mech Ageing Dev 1995;78:221–39.
Nishimura A, Ueda S, Takeuchi Y, Matsushita H, Sawada T, Kawata M. Vulnerability to aging in the rat serotonergic system. Acta Neuropathol (Berlin) 1998;96:581–95.
Nyakas C, Oosterink BJ, Keijser J, Felszeghy K, de Jong GI, Korf J, et al. Selective decline of 5-HT1A receptor binding sites in rat cortex, hippocampus and cholinergic basal forebrain nuclei during aging. J Chem Neuroanat 1997;13:53–61.
O'Keefe J, Nadel L. The hippocampus as a cognitive map. Oxford: Oxford Univ. Press; 1978.

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

Pedata F, Slavikova J, Kotas A, Pepeu G. Acetylcholine release from rat cortical slices during postnatal development and aging. Neurobiol Aging 1983;4:31–5.

Petkov VD, Petkov VV, Stancheva SL. Age-related changes in brain neurotransmission. Gerontology 1988;34:14–21.

Planas B, Kolb PE, Raskind MA, Miller MA. Galanin receptors in the hippocampus and entorhinal cortex of aged Fischer 344 male rats. Neurobiol Aging 1998;19:427–35.

Qi SB, Nomura Y. Age related changes in the inhibitory effect of clonidine on high K^+-evoked noradrenaline release from brain slices of rats. J Pharmacobio-Dyn 1988;11:483–5.

Rapp PR, Amaral DG. Individual differences in the cognitive and neurobiological consequences of normal aging. Trends Neurosci 1992;15: 340–5.

Rapp PR, Rosenberg RA, Gallagher M. An evaluation of spatial information processing in aged rats. Behav. Neurosci. 1987;101:3–12.

Robson L, Gower AJ, Kendall DA, Marsden CA. Age-related behavioural, neurochemical and radioligand binding changes in the central 5-HT system of Sprague–Dawley rats. Psychopharmacology 1993;113:274–81.

Rylett RJ, Goddard S, Schmidt BM, Williams LR. Acetylcholine synthesis and release following continuous intracerebral administration of NGF in adult and aged Fischer-344 rats. J Neurosci 1993;13:3956–63.

Sastry BV, Janson VE, Jaiswal N, Tayeb OS. Changes in enzymes of the cholinergic system and acetylcholine release in the cerebra of aging male Fischer rats. Pharmacology 1983;26:61–72.

Satoh K, Armstrong DM, Fibiger HC. A comparison of the distribution of central cholinergic neurons as demonstrated by acetylcholinesterase pharmacohistochemistry and choline acetyltransferase immunohistochemistry. Brain Res Bull 1983;11:693–720.

Schlicker E, Betz R, Göthert M. Investigation into the age-dependence of release of serotonin and noradrenaline in the rat brain cortex and of autoreceptor-mediated modulation of release. Neuropharmacology 1989;28:811–5.

Shores MM, White SS, Veith RC, Szot P. Tyrosine hydroxylase mRNA is increased in old age and norepinephrine uptake transporter mRNA is decreased in middle age in locus coeruleus of Brown–Norway rats. Brain Res 1999;826:143–7.

Sirviö J, Hervonen A, Valjakka A, Riekkinen PJ. Pre- and postsynaptic markers of cholinergic neurons in the cerebral cortex of rats of different ages. Exp Gerontol 1988a;23:473–9.

Sirviö J, Valjakka A, Jolkkonen J, Hervonen A, Riekkinen PJ. Cholinergic enzyme activities and muscarinic binding in the cerebral cortex of rats of different age and sex. Comp Biochem Physiol C 1988b;90:245–8.

Sirviö J, Pitkanen A, Paakkonen A, Partanen J, Riekkinen PJ. Brain cholinergic enzymes and cortical EEG activity in young and old rats. Comp Biochem Physiol C 1989;94:277–83.

Starke K. Presynaptic autoreceptors in the third decade: focus on alpha2-adrenoceptors. J Neurochem 2001;78:685–93.

Stemmelin J, Cassel JC, Will B, Kelche C. Sensitivity to cholinergic drug treatments of aged rats with variable degrees of spatial memory impairment. Behav Brain Res 1999;98:53–66.

Stemmelin J, Lazarus C, Cassel S, Kelche C, Cassel JC. Immunohistochemical and neurochemical correlates of learning deficits in aged rats. Neuroscience 2000;96:275–89.

Tanaka Y, Hasegawa A, Ando S. Impaired synaptic functions with aging as characterized by decreased calcium influx and acetylcholine release. J Neurosci Res 1996;43:63–76.

Taube HD, Starke K, Borowski E. Presynaptic receptor systems on the noradrenergic neurones of rat brain. Naunyn-Schmiedeberg's Arch Pharmacol 1977;299:123–41.

Turrini P, Casu MA, Wong TP, De Koninck Y, Ribeiro-da-Silva A, Cuello AC. Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy. Neuroscience 2001;105:277–85.

Valjakka A, Sirviö J, Pitkanen A, Riekkinen PJ. Brain amines and neocortical EEG in young and aged rats. Comp Biochem Physiol C 1990;96:299–304.

van Luijtelaar MG, Tonnaer JA, Steinbusch HW. Aging of the serotonergic system in the rat forebrain: an immunocytochemical and neurochemical study. Neurobiol Aging 1992;13:201–15.

Vizi ES, Kiss JP. Neurochemistry and pharmacology of the major hippocampal transmitter systems: synaptic and nonsynaptic interactions. Hippocampus 1998;8:566–607.

Weston J, Greenfield SA. Release of acetylcholinesterase in the rat nigrostriatal pathway: relation to receptor activation and firing rate. Neuroscience 1986;17:1069–88.

Wheeler DD. Aging of membrane transport mechanisms in the central nervous system—high affinity choline transport in rat cortical synaptosomes. Exp Gerontol 1985;20:73–80.

Zsilla G, Zelles T, Mike A, Kekes-Szabo A, Milusheva E, Vizi ES. Differential changes in presynaptic modulation of transmitter release during aging. Int J Dev Neurosci 1994;12:107–15.