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Research report

Cellular determinants of reduced adaptability of the aging brain: neurotransmitter utilization and cell signaling responses after MDMA lesions

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Abstract

Senescence is accompanied by the loss of neurons and synapses, and the maintenance of function depends on adaptive change at the levels of synaptic activity and cellular responsiveness. In the current study, we administered the neurotoxin MDMA, to young and aged mice and assessed the effects on indices of neuronal activity and cell signaling mediated through adenylyl cyclase. Young mice given MDMA showed 80% depletion of dopamine in the caudate and 30% depletion in the cerebral cortex; measurements of dopamine turnover indicated a compensatory upregulation of the activity of the remaining neurons in the caudate but downregulation in the cerebral cortex. Serotonin levels were comparatively less affected but serotonin turnover was decreased significantly in both regions. At the level of cell signaling, the young mice showed heterologous upregulation of adenylyl cyclase activity and a consequent enhancement of responses mediated through neurotransmitter receptors. In aged mice, MDMA treatment produced the same degree of lesioning but substantially different changes in neuronal activity and cell signaling. In the cerebral cortex, dopamine turnover was increased, and serotonin turnover decreased, effects opposite in direction to those seen in young mice. In the aged group, MDMA elicited heterologous loss of adenylyl cyclase responses instead of displaying the supersensitivity that had been seen in the young group. The aging brain thus displays maladaptation to the loss of monoaminergic input, effects that may augment the functional impairment associated with neurodegenerative disorders or stroke.

Theme: Development and regeneration

Topic: Aging process

Keywords: Adenylyl cyclase; Aging brain; Dopamine; Methylenedioxymethamphetamine (MDMA); Serotonin (5-HT)

1. Introduction

Aging is associated with increased susceptibility to neuronal loss and disruption of cerebral function, either as a component of senescence, or as a consequence of neurodegenerative diseases or stroke. The adaptability of the brain to neuronal or synaptic loss involves plasticity at the levels of cell signaling and a great deal of attention has been paid to the role of neurotrophic factors in the prevention of cell death or restoration of function. It is increasingly evident that neurotransmitters themselves play a key role in this process. Stimulation of nicotinic cholinergic receptors can prevent neuronal death caused by simulated hypoxic/ischemic injury or by deprivation of growth factors [13,40]. Similarly, stimulation of catecholaminergic receptors can offset aging-related deterioration of behavioral performance [5] and recovery after surgical lesions depends on the function of catecholamine systems [10]. It is thus important to note that lesions of catecholaminergic pathways in the aging brain can produce a different spectrum of behavioral effects from those seen after lesioning of the young brain [30].

Abbreviations: AC, adenylyl cyclase; ANOVA, analysis of variance; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine (serotonin); HVA, homovanillic acid; MDMA, methylenedioxymethamphetamine; PXT, paroxetine

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The issue of impaired adaptability of the aged brain to the loss of monoaminergic inputs is critical to our understanding of deterioration of function in Parkinson's disease, recovery from stroke and, as is now evident, geriatric depression. Elderly depressives are less responsive to monoamine reuptake inhibitors, the mainstay of antidepressant therapy [6,23,25], and animal models of geriatric depression that incorporate monoaminergic lesions show corresponding differences from effects in young animals [33]. Accordingly, the current study addresses the issue of whether there is a cellular basis for the aging-related impairment in the response to neuronal loss. We have concentrated on the effects of MDMA, a widely-abused neurotoxic amphetamine analog. Although MDMA was once thought to target 5-HT neurons selectively [14], evidence now suggests that its primary actions are directed toward DA systems [22,28]; the spectrum of actions depends on a variety of factors, including age [4]. We compared the degree of DA and 5-HT lesioning achieved in young and aged mice and then focused on two aspects of adaptation: neurotransmitter turnover and cellular signaling cascades. Transmitter turnover measures the utilization of the remaining stores of DA and 5-HT, which need to compensate for the partial loss of neuronal projections. Similarly, denervation typically evokes supersensitivity of cell signaling response elements, a major adaptation to the loss of input. We focused on the AC signaling cascade for several reasons. First, deterioration of AC signaling is an important correlate of aging [11,19,27]. In the peripheral sympathetic nervous system, the responsiveness of cardiovascular adrenergic target tissues to neurotransmitter stimulation declines markedly in the elderly, effects which reflect loss of specific receptor coupling mechanisms as well as of cellular intermediates in the signaling cascade, such as G-proteins and AC itself [3,11,19–21,27,29,38,39]. In the central nervous system, impaired DA and 5-HT responses have also been reported [15], although the underlying causes have not been fully characterized. In addition, we have demonstrated age-related disparities in the AC response of the aged brain to alterations of hormonal status or to lesions of sensory afferent inputs (olfactory bulbectomy) that replicate the endocrine and monoaminergic alterations thought to underlie human geriatric depression [7,31–36]. Accordingly, our underlying hypothesis is that damage to cell signaling capabilities may be particularly relevant in aging, where failure of compensatory upregulation of responsiveness would exacerbate, rather than offset, the effects of neuron loss. We have tested this hypothesis in mice in a relatively 'early' stage of senescence. Neurodegeneration, synaptic dysmorphology and neuronal loss are likely to be present when very old animals are used, obscuring primary effects of lesioning on synaptic or cellular function [16]. Accordingly, we have concentrated on 24-month-old mice, rather than examining animals at the very extreme of the life span.

2. Methods

Studies were carried out in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Male C57BL/6N mice were bred and housed four per cage with free access to food and water. Animals were selected at 3 and 24 months-of-age and given saline or MDMA (four injections of 20 mg/kg i.p., spaced 2 h apart; MDMA obtained from Sigma Chemical Co., St Louis, MO). One week later, animals were subjected to cervical dislocation and the brains were rapidly removed and dissected into different regions, placed on dry ice, and stored at −70°C. This dose regimen has been used previously to elicit neurobehavioral damage in rodents [17,24].

2.1. Neurotransmitters and metabolites

Concentrations of DA, 5-HT and their metabolites DOPAC, HVA and 5-HIAA were quantitated by high performance liquid chromatography with a C18 column and electrochemical detection [1]. Briefly, each region was weighed and deproteinized with 0.2 N perchloric acid containing 3,4-dihydroxybenzylamine (Sigma) as an internal standard. The tissue was then disrupted by ultrasonication, sedimented at 15,000×*g* and the supernatant solution was filtered through a 0.2 μm Nylon-66 microfilter (MF-1 centrifugal filter, Bioanalytical Systems, W. Lafayette, IN). Aliquots of 25 μl, representing 2.5 mg of original wet weight of brain tissue, were then injected directly onto the chromatographic system. Results were calculated using a standard curve constructed with varying concentrations of the authentic compounds (Sigma), with correction for sample recovery using the internal standard.

2.2. [^{3}H]PXT binding

The cell membrane fraction was prepared from brain regions by techniques described previously [18]; membrane protein was analyzed with Folin reagent. The suspension was then used immediately for determinations of AC activity (see below) and [^{3}H]PXT binding [18], using paroxetine[phenyl-6′-^{3}H] (specific activity 25.4 Ci/mmol, New England Nuclear Corp., Boston, MA) with or without addition of 100 μM serotonin (Sigma) to displace specific binding. Incubations lasted 120 min at 20°C, and were stopped by addition of 5 ml of ice-cold buffer, followed by vacuum filtration and washing onto Whatman GF/C filters, pre-soaked in 0.05% polyethyleneimine (Sigma). Non-specific binding was approximately 20% of the total in forebrain and brainstem, which highly express the 5-HT transporter, and 40% in the cerebellum, which has much lower transporter expression. Because of the large number of tissues involved in this study, 2 treatment groups×2 age groups×3 tissues×6 animals per group, it

was not practicable to run Scatchard analyses on each. Accordingly, we examined binding at 85 pM [^{3}H]PXT, a concentration above the K_d [18,33], but nevertheless below full saturation of the binding site. The strategy of using a single, subsaturating ligand concentration enables the detection of drug- or age-induced changes regardless of whether the changes are in affinity or capacity.

2.3. AC activity

AC activity was evaluated in the same membrane preparations according to established protocols [35,36]. Membrane aliquots were incubated for 10 min at 30°C with final concentrations of 100 mM Tris–HCl (pH 7.4), 10 mM theophylline, 1 mM adenosine 5′-triphosphate, 10 mM $MgCl_2$, 1 mg/ml bovine serum albumin, and a creatine phosphokinase-ATP regenerating system consisting of 10 mM sodium phosphocreatine, 8 I.U. phosphocreatine kinase, and 10 μM GTP (all reagents from Sigma). The enzymatic reaction was stopped by placing the samples in a 90–100°C water bath for 5 min, followed by sedimentation at 3000×*g* for 15 min, and the supernatant solution was assayed for cAMP using radioimmunoassay kits (Amersham Corp., Chicago, IL). Preliminary experiments showed that the enzymatic reaction was linear well beyond the 30-min time period and was linear with membrane protein concentration; concentrations of cofactors were optimal and, in particular, the addition of higher concentrations of GTP produced no further augmentation of activity.

Enzyme activity was evaluated under several different conditions. First, basal activity was evaluated. Second, the maximal G-protein-linked response was evaluated in samples containing 10 mM NaF. Third, maximal total activity of the AC catalytic unit, independent of receptors or G-proteins, was evaluated with 10 mM $MnCl_2$ [12,42]. Finally, neurotransmitter receptor-mediated effects were evaluated with either 100 μM 5-HT or DA [33,35]. The concentrations of all the agents used here have been found previously to be optimal for effects on AC and were confirmed in preliminary experiments.

PXT binding and AC determinations were evaluated in terms of tissue concentration (binding per g tissue), and as specific activity relative to other membrane proteins (binding per mg protein).

Because of the larger amounts of tissue required for determinations of [^{3}H]PXT binding and AC activity, brain dissections for these determinations differed somewhat from those used for the neurotransmitter and metabolite determinations. Given the similarity of age- and drug-related effects on neurotransmitters and metabolites in the cerebral cortex and caudate (see below), we did not separate these regions for PXT binding and AC determinations, and instead used the intact forebrain. In addition, we evaluated effects on the brainstem and cerebellum, two regions shown previously to show specific age-related differences in AC reactivity to lesions and drug treatments [7,32,33,35,36].

2.4. Data analysis

Data are presented as means and standard errors, with intergroup comparisons by multivariate ANOVA (data log-transformed whenever variance was heterogeneous). For an initial global test, the results were divided into five categorical groupings: DA and metabolites, 5-HT and metabolite, PXT binding, AC activity, and membrane protein concentration. Each grouping was then subjected to ANOVA using factors of age, MDMA treatment, brain region, and the multiple determinations within each category (a repeated measure, since more than one type of determination was made on each sample). In each case, whenever we found a significant effect of MDMA treatment or interaction of MDMA with the other variables, data were then subdivided into the individual regions, which were then re-evaluated using ANOVA. Finally, individual differences were established using Fisher's Protected Least Significant Difference. For all tests, significance was assumed at the level of $P<0.05$ for main treatment effects and at $P<0.1$ for interactions [37]. Some data are presented as the percentage change from age-matched control groups but the statistical analyses were conducted on the unmanipulated data.

3. Results

Global statistical analyses (Table 1) indicated significant effects of age and MDMA treatment on all categories of the measured variables, with significant regionally-selective effects (main effects treatment, age and region, interactions among all three variables). Within each category, effects of aging and MDMA treatment were also different for the various measures (interactions of treatment×measure, age×measure, region×measure, treatment×age×measure, treatment×region×measure). For categories of neurotransmitters and their metabolites, this indicates selective effects on specific chemical entities, whether the native biogenic amine or its catabolic products. For PXT binding, this denotes selective effects directed toward absolute activity (activity per g tissue) or to the specific activity relative to other membrane proteins (activity per mg protein). For AC activity, the interaction of main variables with the specific measures also denotes differential effects on absolute vs. specific activity, as well as differences among the five different types of AC measurement (basal activity and effects of fluoride, manganese, dopamine and serotonin).

There were significant effects of both age and MDMA treatment on the membrane protein concentration, so that some of the differences in PXT binding and AC activity are potentially related to more generalized effects on cell

Table 1
Global statistical analyses[a]

	DA and metabolites	5-HT and metabolites	PXT binding	AC activity	Membrane protein
Treatment main effect	$P<0.0001$	NS	NS	NS	$P<0.04$
Age main effect	$P<0.03$	$P<0.0008$	$P<0.005$	$P<0.0001$	$P<0.0001$
Region main effect	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$
Treatment×age	NS	$P<0.003$	NS	$P<0.03$	NS
Treatment×region	$P<0.0001$	NS	NS	NS	NS
Age×region	NS	NS	NS	NS	NS
Treatment×age×region	NS	NS	NS	NS	NS
Treatment×measure	$P<0.0001$	NS	$P<0.04$	$P<0.06$	–
Age×measure	NS	NS	$P<0.0001$	$P<0.0001$	–
Region×measure	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$	–
Treatment×age×measure	$P<0.0002$	NS	NS	NS	–
Treatment×region×measure	$P<0.0001$	NS	NS	NS	–
Age×region×measure	NS	NS	NS	NS	–
Treatment×age×region×measure	NS	NS	NS	NS	–

[a] For each category, the global analysis combined all related measures in a repeated-measures design. For DA and 5-HT, analyses assessed each amine and its related metabolites. For PXT binding, analyses assessed both the concentration and specific activity of binding sites. For AC, analyses assessed all five measures, both as concentration and specific activity. Interactions with 'measure' thus denote selectivities toward specific types of measurements within each category.

membranes. In some situations, evaluation of the membrane protein concentration is necessary to correct for intersample variability in recovery of the cell membrane fraction. Here, samples from animals in the different treatment groups of a given age were all prepared simultaneously and processed in parallel, making it unlikely that there are systematic differences in recovery of membranes; in this case, systematic shifts in membrane protein concentration instead reflect changes in cell size and the attendant alteration of surface-to-volume ratio. Biologically-relevant effects on membrane protein concentrations are typical of aging and brain lesions, since changes in axon/synapse number, or replacement of damaged cells with glia, can all contribute to altered membrane protein concentrations. Monoamine transporters, neurotransmitter receptors, G-proteins and AC are all integral membrane proteins and are thus affected by alterations in cell size as well as by age- or drug-induced effects on specific expression of these proteins. Accordingly, we present PXT binding and AC measurements both as activity per g tissue and per mg protein to separate specific alterations in expression over-and-above effects on all membrane constituents. From the standpoint of cell function (signal produced per mass of tissue), activity per g of tissue is the relevant measure, whereas values adjusted for membrane protein establish drug effects on the expression of individual proteins.

In light of the significant interactions of aging and MDMA treatment with region and measures within each category, results are presented with subdivision into each region and measure. For clarity, comparisons are presented first between young and aged control mice, and then for the effects of MDMA on the two populations, shown as the percentage of the corresponding age-matched control values.

3.1. Neurotransmitters and metabolites

In control mice, there were only minor age-related differences in the levels of monoamines and their metabolites (Fig. 1, left panels). In the caudate nucleus, there were significant overall differences in the two age groups compared across all measurements, but for individual measurements only DOPAC showed a slightly higher level in the aged group, at the margin of statistical significance. Cerebral cortical levels of DA and its metabolites were indistinguishable in young versus aged mice, and levels of 5-HT and 5-HIAA were unchanged in all regions. In the hippocampus, concentrations of DA and its metabolites were too low for reliable quantitation.

Upon lesioning with MDMA, both young and aged mice showed equivalent, massive (80%) depletion of DA in the caudate nucleus (Fig. 1, upper right panel). DA metabolites were also reduced, although to a smaller extent than DA itself. Thus, the turnover of DA (ratio of total DA metabolites to DA) increased after lesioning, representing hyperactivity of the remaining nerve terminals (Table 2); the lack of a significant interaction of treatment×age indicated that the increase in aged mice was not distinguishable from that seen in young mice. Despite the equivalent loss of DA in the two age groups, aged mice showed a significantly smaller reduction in HVA (Fig. 1, upper right panel). The aged caudate also showed significantly different effects of MDMA on the 5-HT system: the young animals showed little or no 5-HT depletion (Fig. 1, upper right panel) and a decrease in 5-HT turnover (Table 2); aged animals showed increases in both 5-HT and 5-HIAA and no change in turnover.

In the cerebral cortex (Fig. 1, middle right panel), MDMA treatment elicited age-dependent differences in DA and metabolites resembling that seen in the caudate,

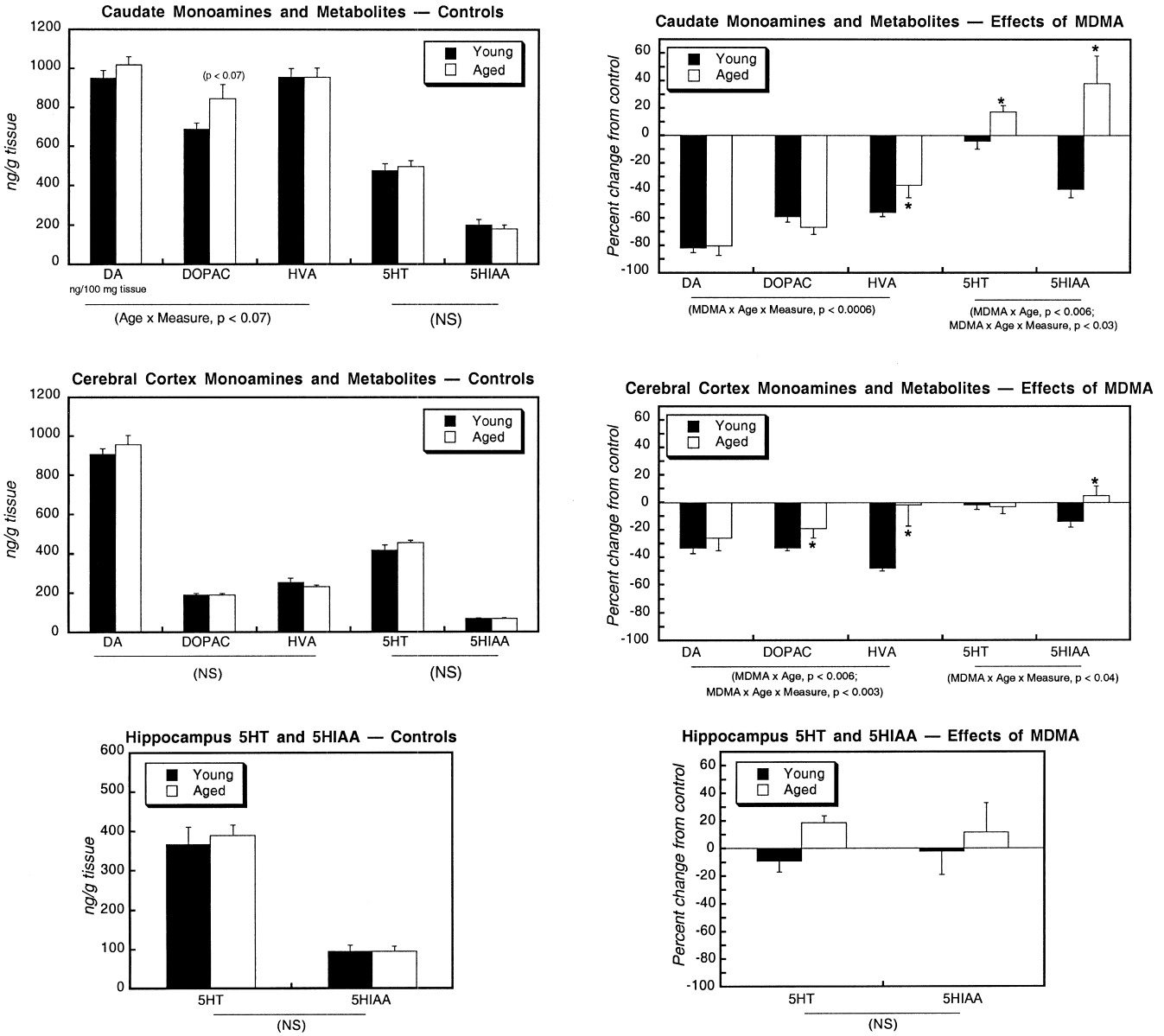

Fig. 1. Effects of aging and MDMA treatment on levels of DA and its metabolites, DOPAC and HVA, and on 5-HT and its metabolite, 5-HIAA. Note the different units for caudate DA (top left panel). Data represent means and standard errors obtained from five to eight animals in each group. The left-hand panels depict the differences between young and aged control mice, whereas the right-hand panels depict the effects of MDMA as the percent change from the corresponding control values. ANOVA across all measures for each monoamine appears at the bottom of each panel and asterisks denote individual values where the aged group differs from the young group. Across all three regions, ANOVA indicates significant, regionally-selective and/or measure-selective (DA vs. DOPAC vs. HVA) differences in the DA system between young and aged controls: main effect of region ($P<0.0001$) and interactions of age×measure ($P<0.06$) and region×measure ($P<0.0001$). No age-dependent differences were seen for the 5-HT system in the control group: main effect of region ($P<0.0001$), interaction of region×measure ($P<0.0001$), but no interaction of age with the other variables. For the effects of MDMA, ANOVA across all regions indicates significant age-dependent effects for both DA and 5-HT systems. For DA and its metabolites, there were significant interactions of MDMA×region ($P<0.0001$), MDMA×age ($P<0.008$), MDMA×age×measure ($P<0.0001$), MDMA×region×measure ($P<0.0001$), and MDMA×age×region×measure ($P<0.07$). For 5-HT and 5-HIAA, there were significant interactions of MDMA×age ($P<0.0003$) and MDMA×age×region ($P<0.05$).

but the effects of MDMA in general were of much smaller magnitude. In the cortex, both DOPAC and HVA showed smaller reductions in the aged group than in young animals. Most notably, DA turnover (Table 2) showed a completely different reaction to MDMA in the two age groups (treatment×age interaction, $P<0.006$, indicating that effects in aged mice were distinguishable from those in young mice). In young mice, the ratio decreased slightly, whereas in aged mice, the ratio increased after treatment. Neither age group showed any significant loss of cerebrocortical 5-HT after MDMA treatment, but nevertheless, differences in synaptic activity were found for

Table 2
Effects of MDMA on neurotransmitter turnover[a]

Region	Treatment	(DOPAC+HVA)/DA		5-HIAA/5-HT	
		Young	Aged	Young	Aged
Caudate	Control	0.17±0.01	0.18±0.01	0.42±0.06	0.38±0.06
	MDMA	0.46±0.05*	0.59±0.12*	0.26±0.03*	0.41±0.07
Cerebral cortex	Control	0.49±0.03	0.44±0.02	0.17±0.01	0.15±0.01
	MDMA	0.44±0.02	0.55±0.04*	0.14±0.01	0.16±0.01
Hippocampus	Control	–	–	0.32±0.09	0.27±0.06
	MDMA	–	–	0.31±0.07	0.23±0.04

[a] Data represent means and standard errors obtained from five to eight animals in each group.
Asterisks denote significant differences between MDMA-treated animals and their corresponding control group. In the caudate, two-factor ANOVA indicates no age-dependent difference for the increase of dopamine turnover after MDMA (no interaction of treatment×age) but the decrease in 5-HT turnover seen in young animals was not observed in aged animals ($P<0.05$ for interaction of treatment×age). In the cerebral cortex, two-factor ANOVA indicates age-dependent differences for the effects of MDMA on both DA turnover and 5-HT turnover (respectively, $P<0.005$ and $P<0.05$ for interaction of treatment×age). There were no significant age-dependent effects on 5-HT turnover in the hippocampus.

serotonergic systems (Fig. 1, middle right panel). 5-HIAA tended to decrease in the young animals treated with MDMA, but to increase in the aged group, producing a statistically significant interaction of treatment×age ($P<0.03$). When these data were used to calculate the metabolite ratio (5-HIAA to 5-HT) the differences between young and old animals became even more apparent (Table 2). The ratio decreased in the young mice given MDMA but increased in the identically-treated aged animals (treatment×age interaction, $P<0.05$).

The hippocampus showed little or no MDMA-related changes in 5-HT or 5-HIAA levels (Fig. 1, bottom right panel) or in the turnover ratio (Table 2).

3.2. [^{3}H]PXT binding

In control mice, there were minor differences in PXT binding between the young and aged cohorts (Fig. 2). The concentration of sites (binding per g tissue) showed slightly higher values in the forebrain of aged mice (upper left panel) and lower values in the brainstem (middle left panel). Because membrane protein concentrations were higher in the aged group, the specific activity of PXT binding sites was actually lower in both regions when compared to other membrane proteins (upper and middle right panels). As expected from the distribution of 5-HT projections, binding in the cerebellum (lower panels) was much lower than in the forebrain or brainstem.

Lesioning with MDMA had only small (<10%) effects on PXT binding in the forebrain of either young or aged mice. The concentration of sites (Fig. 3, upper left panel) was slightly elevated in the young group and reduced in the aged group; however, these differences were not specific for the 5-HT transporter, since adjustment for membrane protein (upper right panel) eliminated the difference between the two age groups. In the brainstem (middle panels), neither young nor aged mice showed any differences in PXT binding on either the basis of concentration of specific activity. In the cerebellum (lower panels), binding was elevated (measured either as concentration or specific activity) after MDMA treatment of young mice but no effect was seen in aged mice; although changes elicited by MDMA were proportionally larger in the cerebellum, it should be kept in mind that the overall concentration of these sites was very low.

3.3. AC activity

In control forebrain, there were small differences in AC between young and aged mice (Fig. 2). On a concentration basis (activity per g tissue), activities for all measures tended to be slightly higher in the aged group (upper left panel); after adjustment for the concentration of other membrane proteins (upper right panel), values were higher for young animals, in keeping with earlier studies with aged rats [35]. Similar, slight reductions in AC specific activity, were seen in brainstem (middle panels) and cerebellum (lower panels). AC stimulation by DA was highly statistically significant ($P<0.0001$) and showed preferential effects in the forebrain ($P<0.0001$ for interaction of region×DA-induced stimulation), in keeping with the localization of 90% of DA projections in that region; nevertheless, DA-induced stimulation was statistically significant in all three regions ($P<0.0001$ for each). DA caused a 35% increase in AC over basal values in the forebrain and a 7–15% increase in the other two regions. DA stimulation over basal values did not differ with age (35±3% increase in young control forebrain, 32±1% in aged control forebrain). Although 5-HT also stimulated AC significantly across all three regions ($P<0.0001$), the response was limited to the cerebellum ($P<0.0001$), whereas the forebrain and brainstem showed no significant stimulation. This is a likely consequence of the fact that 5-HT receptors both stimulate and inhibit AC, so that the net effect depends on the relative balance of positive and negative inputs [33,35]. Alterations in AC signaling can thus be elicited by changes in either stimulatory or inhibitory responses.

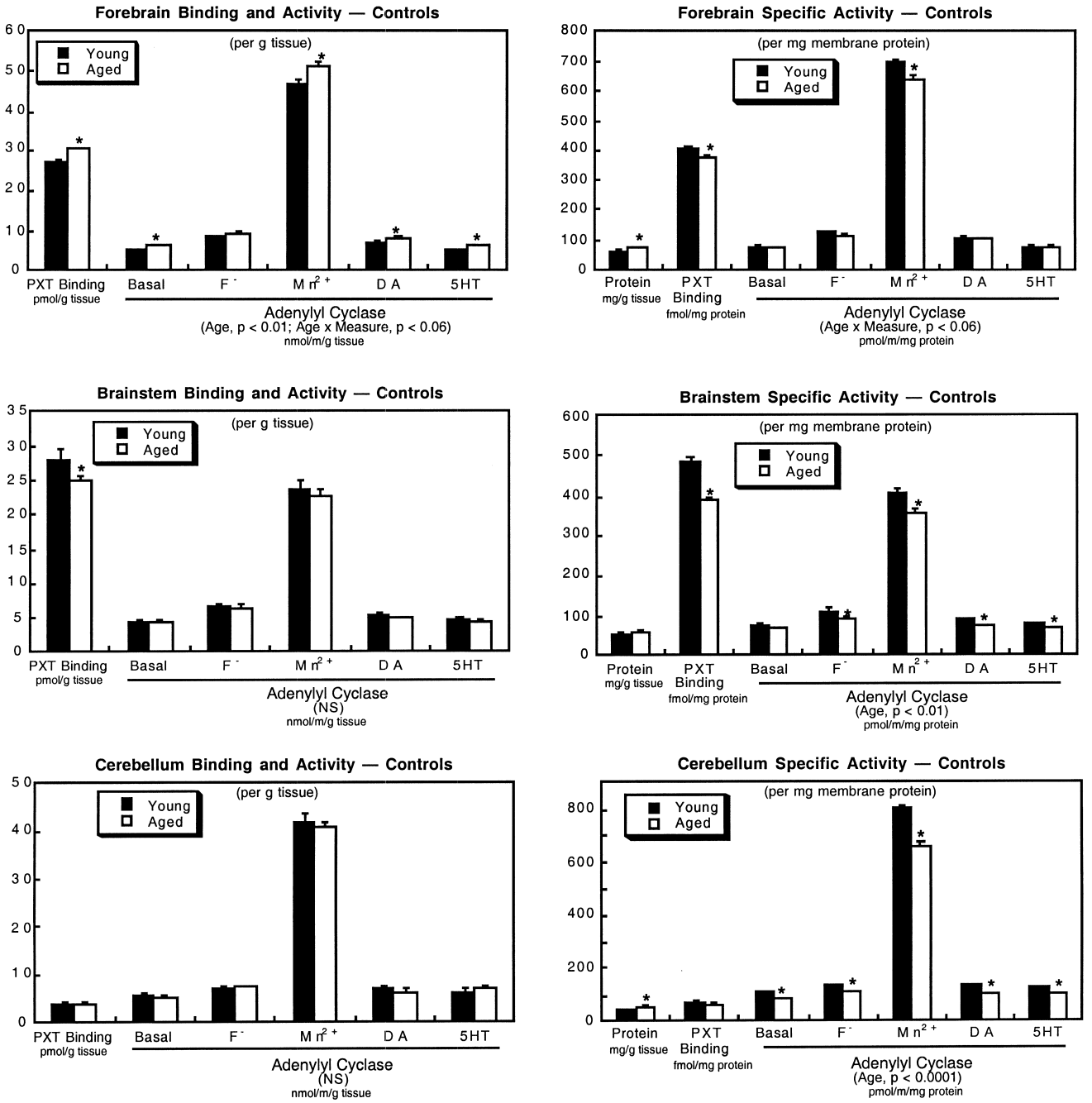

Fig. 2. Effects of aging on [^{3}H]PXT binding and AC activity assessed either as absolute concentration (left panels) or as specific activity relative to membrane protein (right panels). Data represent means and standard errors obtained from six to seven animals in each group. ANOVA across all AC measures appears at the bottom of each panel and asterisks denote individual values where the aged group differs from the young group. Across all three regions, ANOVA indicates significant age-related differences for tissue weight (main effect, $P<0.0002$; age×region interaction, $P<0.0001$), membrane protein (main effect of age, $P<0.0001$), [^{3}H]PXT binding (main effect of age for binding per mg protein, $P<0.006$), and AC (main effect of age for activity per mg protein, $P<0.0001$; age×region interaction, $P<0.06$).

Despite the fact that young and aged mice showed similar degrees of DA lesioning after MDMA treatment, there were profound differences in the effects of MDMA on AC activity in the two age groups (Fig. 3). On a concentration basis (activity per g tissue), young mice showed upregulation across all AC measures in the forebrain, whereas the aged group showed overall loss of activity and reactivity to DA or 5-HT (upper left panel). In this case, age-dependent differences were still evident after adjustment for membrane protein (upper right panel); after MDMA treatment, AC specific activity was relatively unchanged in young mice, but there was a global decrease in aged mice. Effects were much less notable in the brainstem, although the same direction of change was seen

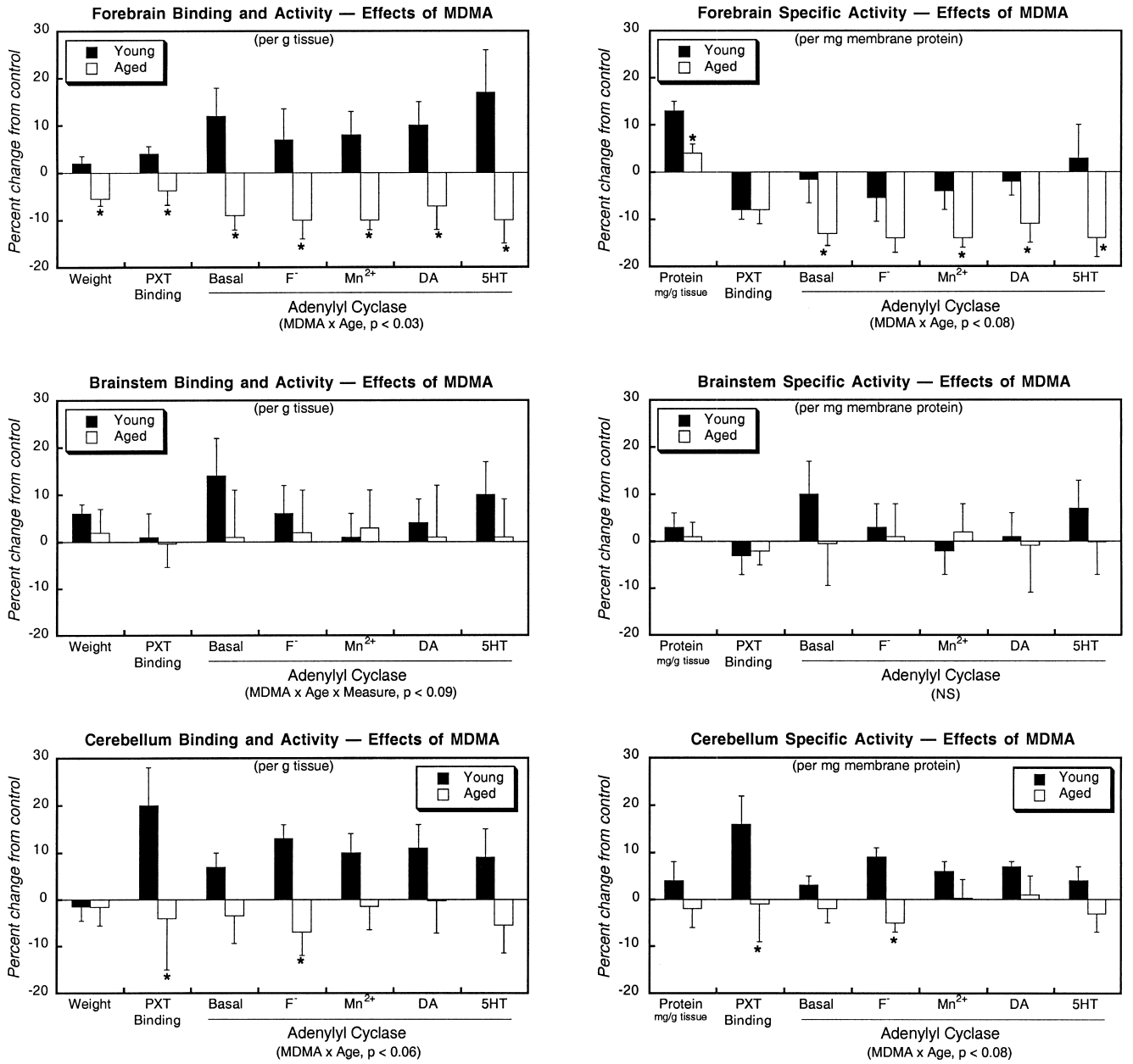

Fig. 3. Effects of MDMA on [^{3}H]PXT binding and AC activity assessed either as absolute concentration (left panels) or as specific activity relative to membrane protein (right panels). Data represent means and standard errors obtained from five to six animals in each group. ANOVA across all AC measures appears at the bottom of each panel and asterisks denote individual values where the aged group differs from the young group. Across all three regions, ANOVA indicates significant age-related differences for membrane protein (MDMA×age, $P<0.04$; MDMA×region, $P<0.05$), [^{3}H]PXT binding (MDMA×age, $P<0.05$ for binding per g tissue, $P<0.07$ for binding mg protein), and AC (MDMA×age, $P<0.01$ for activity per g, $P<0.05$ for activity per mg protein; $P<0.1$ for MDMA×age×measure for activity per g).

as for the forebrain: a tendency toward increases in activity per g tissue in young mice only. In the cerebellum (lower panels), there were global increases in all measures of AC after lesioning of young mice, and no change or a decrease in aged mice given the same treatment.

MDMA treatment caused an increase in the membrane protein concentration in the region (forebrain) possessing the highest concentration of DA (Fig. 3, upper right panel). Although a robust increase was seen in young mice, a significantly smaller effect was obtained in aged mice, despite the equivalent degree of lesioning as evidenced by DA depletion.

4. Discussion

The current results indicate that lesioning of dopaminergic pathways by MDMA produces dissimilar effects

in the aged brain as compared to young brain. These differences were apparent at two levels of organization: first, patterns of neural activity as indicated by measurements of monoamine transmitter turnover, and second, cell signaling responses mediated through AC. The age-related disparities were not apparent under basal conditions, as the non-lesioned groups displayed similar transmitter utilization rates and signaling profiles.

Administration of MDMA produced equivalent lesions in both age groups, evidenced by approximately 80% depletion of DA in the caudate. In young animals, the loss of dopaminergic inputs showed two adaptive responses at the level of neurotransmitter utilization. DA turnover increased, indicating a rise in neuronal activity in the remaining dopaminergic projections, a compensatory adaptation to the loss of DA inputs. Similarly, 5-HT turnover decreased in the young animals given MDMA; since 5-HT receptors provide inhibitory control over AC [2,33,35], this effect also may compensate for the loss of stimulatory DA inputs. The response to lesioning on neuronal activity in the caudate can thus be viewed as a coordinated effort to augment stimulatory responses while suppressing inhibitory inputs. In contrast, the aged animals specifically were deficient in the adaptive suppression of 5-HT activity and instead, showed upregulation of 5-HT utilization. Although the aged group exhibited overall increases in DA turnover equivalent to those in younger animals, the result was accomplished through somewhat different metabolic processes, involving a greater relative contribution of HVA. Since HVA is produced by deamination of DA, this result is in keeping with earlier observations of greater monoamine oxidase activity in senescence [34]. Higher monoamine oxidase may also account for the slightly greater DOPAC levels in aged controls.

One major conclusion then, is that the adaptations to the loss of DA inputs after MDMA lesioning involves adjustments in neuronal activity extending to transmitter systems, like 5-HT, that are apparently spared (as evidenced by a lack of transmitter depletion). If this is true, then age-related differences in the response to MDMA may encompass regions displaying much smaller outright loss of DA projections. In the cerebral cortex, we found a much smaller degree of DA depletion (about 30%) and again, there were no differences in the efficacy of lesioning in the two age groups. However, just as found in the caudate, the aged brain responded differently to the insult. Young mice showed little or no change in cerebrocortical DA turnover but aged mice evinced hyperactivity. One implication is that the aged animals may suffer a more severe loss of DA function at equivalent degrees of DA depletion, necessitating a greater compensatory activation of the remaining nerve terminals. A potential explanation emerges when we consider effects on cell signaling (below), where the aged animals, but not young animals, display a loss of signaling efficiency. Just as in the caudate, the aged animals showed activation of cerebrocortical 5-HT systems despite no apparent transmitter depletion.

Because we found age-related differences in 5-HT turnover after MDMA lesioning, we also explored the possibility that changes in 5-HT transporter expression might account for the apparent alterations in presynaptic function. Measurement of the concentration of transporter sites, assessed with PXT binding per g tissue, indicated a significantly greater decrease in the aged group, an effect which would indeed increase 5-HT turnover by reducing the presynaptic recapture of 5-HT. However, it is unclear whether this represents a specific change in transporter expression, since, with correction for MDMA-induced effects in other membrane proteins (binding per mg protein), the age-related differences in PXT binding were no longer evident. In fact, on this basis, MDMA produced small (~10%) reductions in 5-HT transporter binding in the forebrain without distinction between young and aged mice, an effect which would enhance 5-HT utilization in both age groups. Furthermore, the largest proportional changes in transporter binding sites were seen in the cerebellum, a region relatively sparse in 5-HT projections. It is thus possible that alterations in transporter function contribute somewhat to the changes in net 5-HT utilization rates but is not clear whether measurements simply of transporter binding sites can distinguish the degree to which they influence this outcome.

Differences between aged and young mice were not limited to effects on neurotransmitter utilization or synaptic activity, but also were prominent at the level of cellular signaling as monitored by AC. Indeed, MDMA-induced lesions altered forebrain AC activity and reactivity to stimulation in opposite directions at the two ages. In young animals, the predominant response (activity per g tissue) was a global increase in all measures of AC activity, an adjustment that would help compensate for the loss of DA input by increasing the efficiency of transmitter-generated responses. In contrast, the aged mice showed a decrease, which would worsen the functional effects of the lesion. Both the sensitization in young animals and the desensitization in aged animals was heterologous, that is, the adjustments were made at a post-receptor locus: the effects are seen for basal, fluoride-stimulated and Mn^{2+}-stimulated AC activity, all of which do not require the participation of neurotransmitter receptors. This is in keeping with recent studies in developing tissues and with congestive heart failure, that indicate that the major cellular adjustments to altered rates of transmitter input are at the level of AC expression [8,9,26,41,42]. In the present case, at least some of the increased AC in young, lesioned animals, represents effects on a wider variety of membrane proteins, since the upregulation of AC was no greater than that of overall membrane protein. The increase in membrane protein in young forebrain after MDMA treatment may reflect either induction of multiple proteins, or a change in cell size consequent to the lesioning treatment; membrane protein increases as the surface-to-volume ratio increases, indicating cell shrinkage, cell damage, reactive axonal sprouting, or replacement of neurons with glia which are

smaller in size. Since both aged and young mice had the same degree of lesion, assessed by DA depletion, this implies that the young brain may exhibit greater axonal sprouting or gliosis after lesioning. Future studies can address this issue through evaluation of specific axonal and glial markers. In either case, though, the fact that the aged brain uniquely shows a deficit in AC signaling after lesioning is a likely contributor to the effects on transmitter utilization. Given a smaller cell signaling response, aged mice would have to increase impulse activity even more because of the maladaptive changes in AC. As already discussed, cerebrocortical DA and 5-HT utilization rates were higher after lesioning in aged animals than in young animals.

If global changes at the level of cell signaling provide the driving force for age-related differences in the effects of MDMA on neuronal activity, then what is the mechanism underlying such widespread change? It is highly unlikely that synaptic adjustments are simply a localized response to focal damage. Indeed, in the cerebellum, which is quite sparse in DA projections, we obtained the same type of increases in AC function in young animals given MDMA, whereas the aged group again showed either no adaptive increase, or even a decrease in AC signaling. Accordingly, it is likely that multiple circuits, activated or repressed by the lesion, converge on the AC signaling pathway to regulate its function in a heterologous manner. In earlier work, we obtained similar results after olfactory bulb lesions, which compromise the function of cortical monoaminergic circuits: aged rats showed a loss of AC reactivity whereas young rats showed upregulation [33]. It is entirely possible that such heterologous changes are triggered by hormonal differences. Both DA and 5-HT exert major control over the functioning of the HPA axis and glucocorticoids evoke differential effects on AC-based signaling in young vs. aged brain [35,36]. Again, future studies will need to address this issue.

Regardless of the mechanism underlying the maladaptive signaling responses in aged brain, the global loss of responsiveness is likely to contribute to the net effect on synaptic and behavioral function, augmenting the disruptive consequences of neuronal loss. Although we studied animals at 24 months-of-age so as to avoid the neurodegeneration associated with extreme aging [16], it is quite likely that study of even older animals will reveal greater differences in the response to lesioning. Age-related differences in cell signaling, and their repercussions for neurotransmitter disposition and synaptic activation, are likely to play a key role in the outcome of stroke and neurodegenerative disorders that are prominent in senescence.

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