Published in final edited form as:

Brain Res. 2006 October 20; 1116(1): 194–200.

Age- and transgene-related changes in regional cerebral metabolism in PSAPP mice

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Abstract

In parallel to imaging studies in humans with Alzheimer's disease (AD), we have mapped brain metabolic activity in transgenic mouse models of AD. Our aim in both is to provide new surrogate markers of progression to help clarify disease mechanisms and rapidly screen candidate therapeutics. Since previous findings of preferential reductions in posterior cingulate glucose metabolism may have been confounded by morphological abnormalities in previously studied "PDAPP" transgenic mice, we first assessed hippocampal and callosal anatomy in PSAPP (PS1 $\times$ APP) mice, another transgenic mouse model of AD, and found no major abnormalities. We then used fluorodeoxyglucose (FDG) autoradiography in older and younger PSAPP and wildtype mice to assess the functional state of 56 regions-of-interest across group, age and increasing amyloid load. Reductions in FDG uptake in aged transgenic mice, with significant interactions between group and age, were found in retrosplenial cingulate gyrus, found to be metabolically affected in persons affected by or at risk for AD, and in brain regions known to participate with retrosplenial cingulate in networks contributing to spatial learning deficits found in these animals. Like patients with AD, PSAPP mice have age-related metabolic reductions in posterior cingulate cortex, a finding that does not appear to be related to morphological abnormalities. If longitudinal studies support these progressive and preferential reductions in retrosplenial metabolism in PSAPP mice, these reductions could provide an indicator of disease progression, help bridge the gap between human and animal studies of AD, and aid in clarification of disease mechanisms and screening of promising treatments.

Keywords

Alzheimer's disease; Animal models; Functional brain imaging; Transgenic mice; Glucose uptake; Energy metabolism

1. Introduction

Fluorodeoxyglucose (FDG) positron emission tomography (PET) studies find that persons with Alzheimer's disease (AD) characteristically show preferential and progressive neocortical reductions in measurements of the cerebral metabolic rate for glucose or FDG uptake (Minoshima et al., 1994, 1995, 1997; Mielke et al., 1994; Reiman et al., 1996). The posterior cingulate cortex (PCC) appears to be particularly vulnerable (Minoshima et al., 1994, 1997;

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Reiman et al., 1996). We have previously reported that cognitively normal, late-middle-aged carriers of the apolipoprotein $\epsilon 4$ allele (APOE4), a common AD susceptibility gene, have functional brain abnormalities in the same neocortical regions as patients with probable AD, prior to the development of cognitive symptoms (Reiman et al., 2001), and furthermore, younger, 20- to 39-year-old carriers also show significant PET declines in these regions (Reiman et al., 2004), indicating a very early vulnerability in these cortices. In both studies, the most significant decline was in the PCC. Valla et al. (2001) examined the lamina of the human postmortem PCC using a marker of oxidative metabolic capacity, finding that each layer in AD patients was significantly decreased relative to controls and no similar decrements were found in the neighboring primary motor cortex. These studies all indicate a vulnerability of the PCC in AD and in those at risk for developing AD. It is hoped that, through in vivo imaging, one will be able to detect the earliest signs of AD, possibly as in the PCC, and intervene before significant damage has occurred.

In parallel to these studies of humans, we have launched a program examining the pattern of metabolic decline in emerging animal models of AD. Our goal is similar to that in our human program: if we can detect early and robust signs of dysfunction, in this case due to mutant amyloid overproduction, potential interventions can be tested prior to overt signs of cognitive dysfunction and perhaps even amyloid deposition. We have previously examined the "PDAPP" mouse, a human mutant amyloid-overexpressing line (Games et al., 1995), at several different ages (from 2 to 24 months of age) and at each gene dose (wildtype, heterozygotic, and homozygotic; Reiman et al., 2000 and unpublished observations). We sought to test, and our results showed, a preferential, highly significant, and possibly progressive reduction in FDG uptake in the PCC. However, findings from this analysis may have been confounded by the choice of slightly different cingulate regions due to the underlying truncated corpus callosum (CC) commissure and altered hippocampal anatomy observed in the PDAPP mice (Gonzalez-Lima et al., 2001; Valla et al., 2006). This measurement could be further confounded in functional imaging studies performed at a lower spatial resolution due to the combined effect of morphological differences and partial-volume averaging (Valla et al., 2002).

To further this work, this FDG autoradiography study was specifically designed to test the hypothesis of significant and progressive reductions in PCC uptake in another transgenic mouse model of AD lacking the morphometric confound. We have now examined the doubly transgenic PSAPP mouse (PS1 (Duff et al., 1996) $\times$ TG2576 (Hsiao et al., 1996; Holcomb et al., 1998)). We first confirmed that this mouse model does not show the severe white matter alterations present in the PDAPP mouse, and thus we would be able to reliably identify the cingulate levels within each group. We then tested the hypothesis that aged (16-month-old) PSAPP mice have preferential reductions in FDG uptake in one of our three PCC regions. Subsequently, we investigated whether the implicated regions demonstrated this reduction in younger animals and whether age-related reductions in the PSAPP were greater than in wildtype mice.

2. Results

A summary of the groups analyzed is presented in Table 1. The transgenic mice showed significantly lower weight than age-matched controls but otherwise were comparable. Both male and female mice were examined together.

The results of the morphometric analyses of the 16-month-old mice are summarized in Table 2. The only anteroposterior (AP) distance demonstrating statistical significance was the ratio of CC over whole brain AP distance, primarily due to a nonsignificant increase in whole brain length concurrent with a slight shortening of the CC commissure. Estimates of volume of the hippocampus showed no significant differences: the PSAPP showed somewhat higher volume

estimates, likely due to their correspondent lengthening of the brain and hippocampal formation as the ratio of hippocampus to whole brain volume altered the directionality. Further analysis of the hippocampal formation as percent hemi-brain area at each level demonstrated reductions in the relative size of the hippocampal formation throughout its anterior aspect, with significant ($p < 0.01$, 2-tailed t tests) reductions appearing in 3 levels. The PSAPP hippocampus was relatively smaller until the far posterior levels, when slight lengthening of hippocampal AP distance in the PSAPP became apparent as an enlargement of the % area represented relative to wildtype.

Analysis of the imaging data demonstrated that, overall, FDG uptake in the PSAPP and wildtype mice was largely similar (at both ages). No significant differences were found in the whole brain average nor in the optic tract (Table 3), indicating that any differences would be localized to specific ROIs and would not likely result from a generalized potentiation or depression across the entire brain. The initial analysis of the 16-month-old mice, following normalization, demonstrated significant ($p < 0.05$, 2-tailed t tests) FDG decreases in several ROIs in the PSAPP relative to wildtype, including the retrosplenial cingulate, anteroventral thalamus, and medial mammillary nuclei. Several additional sensorimotor and arousal-related ROIs were also altered. These results provided the impetus to complete the analysis with additional younger animals. Subsequently, we analyzed both age groups with the omnibus 2×2 ANOVA. Main effects of age were numerous, but developmental changes may be confounded by potential subtle methodological differences (due to serial processing); these are not reported. A significant effect of group (transgenic vs. wildtype) without a significant interaction was deemed to represent an age-independent change. Significant main effects of group accompanied by significant group $\times$ age interactions, or significant interactions alone, likely indicated an age-related change effected by the transgene. ROIs showing significant group effects and/or interactions were followed up with post hoc t tests.

In the final group $\times$ age ANOVA analyzing the normalized regional FDG data (Table 3), PSAPP mice show statistically significant *age-related* metabolic decreases in the retrosplenial gyrus, anteroventral thalamus, and laterodorsal thalamus. Each of these ROIs was marked by virtually no difference from wildtype at 4 months to significant decreases at 16 months. Medial mammillary nucleus showed only an effect of group, decreased at both ages. Several other ROIs also demonstrated group main effects alone, primarily decreases and significantly different post hoc only in the older PSAPP, if at all: monocular primary visual cortex, reticular thalamus, ventromedial and ventrolateral thalamus, ventroposterolateral and ventroposteromedial thalamus, subthalamus, and lateral hypothalamus. A few ROIs demonstrated group effects with significant post hoc *increases* in activity: lateral septal nucleus, periaqueductal gray, vestibular nuclei, and cerebellar lobules 1–5. Oddly, the primary auditory pathway showed significant group effects and interactions, except for a nonsignificant interaction in the cortex, and one of the largest changes, in the inferior colliculus. Inferior colliculus, medial geniculate, and primary auditory cortex all show significant post hoc increases in the older PSAPP mice. Interestingly, during the examination of thioflavin S-stained slides, it was noticed that the inferior colliculus and medial geniculate demonstrated relatively heavy thioflavin-positive amyloid deposition in the PSAPP, whereas the other subcortical nuclei at those levels typically demonstrated no visible amyloid staining (Fig. 1). Lateral entorhinal cortex, mediodorsal and parafascicular thalamus, rostral caudoputamen, and the gigantocellular reticular nucleus showed significant omnibus group $\times$ age interactions, in all cases due to an inversion of the activity change between old and young.

3. Discussion

Morphological measurements have indicated that the PSAPP mouse model does not show the severe anatomical alterations seen in the PDAPP mice previously analyzed (Gonzalez-Lima

et al., 2001; Valla et al., 2006), although the 16-month-old PSAPP mice do show modest size reductions in the hippocampal formation. We did not explicitly analyze the 4-month-old PSAPP mice, but visual examination during the densitometric imaging revealed no outstanding alterations, in contrast to the PDAPP mice. It is possible that these older PSAPP mice have recovered from a similar developmental agenesis, but in any case, they differ greatly from the PDAPP, whose anatomical defects were profound and present at all ages examined, from 2 to 24 months.

The FDG analysis confirmed our hypothesis that significant age-related declines in posterior cingulate FDG uptake (Fig. 2) can be found in a transgenic mouse model of AD that does not demonstrate significant truncation of the CC commissure, similar to the declines we see in patients with AD and in those at risk of developing AD. Decreases in FDG uptake were found in the aged PSAPP retrosplenial cingulate gyrus, anteroventral thalamus, and laterodorsal thalamus, but were not found in young mice. These areas, with sensory input through the laterodorsal thalamus, comprise a portion of a circuit essential for spatial learning tasks (Aggleton et al., 1996; Vann and Aggleton, 2003; Vann et al., 2000).

Behavioral studies confirm changes in both cognitive and behavioral domains in these mice. Arendash et al. (2001) completed a thorough analysis of the behavioral deficits in the PSAPP at 5–7 and 15–17 months of age. They found progressive increases in open field activity, a progressive decrement in an agility test, and cognitively, a progressive cognitive impairment in water maze acquisition. They also found a nonprogressive and profound impairment in balance beam performance and increased y-maze arm entries, indicative of generalized heightened activity. Gordon et al. (2001) also assessed PSAPP spatial performance in the radial arm water maze at approximately 15–16 months of age. A PSAPP deficit in this task was found and also seems to be progressive, measurable in older mice but not at 5–7 (Arendash et al., 2001) or 11–12 months of age (Morgan et al., 2000). The PSAPP analyzed in our study demonstrated aberrant behavior whenever (even mildly) stressed at both ages: frequent rapid circle-running, exaggerated startle, and increased aggression as compared to wildtype. Regrettably, we did not quantify this behavior, although it may be apparent in the behavioral measures taken by Arendash et al. (2001), epitomized by the progressively increasing open field activity and decreasing agility, and the stable impairment on other sensorimotor and anxiety-based assessments. It is also possible that these anxiety-based effects are impacting spatial memory performance (Diamond et al., 1999). Group-only effects in the lateral septum as well as in the cerebellar lobules could also play into the sensorimotor/arousal effects, but they also provide a link to the limbic system and involvement in retention of spatial learning (Hilber et al., 1998), respectively. Cerebellar involvement (as vermis lobules 1–6) was also shared by the PDAPP mice in our initial study (Reiman et al., 2000). Further investigation into this latter effect may be of interest since neither transgenic strain demonstrates amyloid deposition in the cerebellum.

Unexpectedly, the subcortical primary auditory pathway showed a consistent age-related increase in FDG uptake in the PSAPP, with the auditory cortex showing an omnibus group effect. Thioflavin S staining also demonstrated that the inferior colliculus and medial geniculate shared an atypical amyloid burden (Fig. 1). The increased FDG uptake may indicate a mechanism to compensate for the visuospatial challenges the mice apparently develop, related to the retrosplenial cingulate dysfunction. It is also possible that the progressive amyloid burden is interacting with a genetic propensity of C57B6 mice (a component of the PSAPP background) to develop primary progressive auditory degeneration (Willott et al., 1993), but sensorimotor testing does not bear this out (Gordon et al., 2001).

The age-related results correspond chronologically to increasing amyloid load, but while amyloid deposition is pronounced in all areas of the cortex (as in Fig. 1), the glucose uptake

changes are not as widespread. We also see significant changes in the cerebellum, which shows no thioflavin-positive amyloid deposition. While plaque-associated metabolic decline in one ROI could be expected to drive changes throughout an interconnected network, there is also increasing support for the role of soluble and/or intracellular APP products in the disruption of cellular processes in AD (Gouras et al., 2005 for a review) as well as in transgenic animal models (Galvan et al., 2006).

In conclusion, we have verified that the PSAPP mouse model does not share the profound morphological changes that made the PDAPP mouse unsuitable for in vivo assessment. Furthermore, we have confirmed our hypothesis that significant, age-related, and therefore potentially progressive declines in a region of the posterior cingulate can be found in a transgenic mouse model of AD, independent of any significant morphological abnormalities, and extended the findings of significant declines to other regions known to underlie spatial learning and memory. Thus, we suggest that this finding in PSAPP mice could be used to provide a surrogate indicator of progression, help bridge the gap between human studies of AD and investigations of animal models, and be used productively to aid the clarification of disease mechanisms and screening of promising treatments.

4. Experimental procedures

The study was performed under protocols approved by Institutional Animal Care and Use Committees. PSAPP mice and wildtype littermate controls were purchased from Mind-genix Inc. (Rensselaer, NY). Transgene status of mice in each cohort was blinded until the analysis was complete.

To generate the autoradiographic images, each animal was given an i.p. injection of 18 μCi /100 g body weight [^{14}C]-fluoro-deoxy-D-glucose in sterile saline and immediately placed in an individual box in a dark, quiet room for the 45-min uptake period. Subsequently, the mouse was decapitated and its brain rapidly removed and frozen. Brains were stored at -20°C until sectioned in a precision cryostat at 40 μm and thaw-mounted on clean glass slides. Sections were evenly divided between three series, with every 3rd section assigned to its respective series, creating three matched sets of slides for each brain. The first of these was rapidly dried on a hotplate ($\sim 60^\circ\text{C}$) and stored for autoradiography. A second set was stained for mitochondrial cytochrome oxidase (C.O.) activity (Gonzalez-Lima and Garrosa, 1991) for the morphometric analysis and to aid in region identification, as well as for future analyses of mitochondrial activity. The final set was stained with thioflavin S to verify group membership by illuminating amyloid plaques. The slides from the 16-month-old mice designated for autoradiography, along with ^{14}C autoradiography standards (Amersham), were apposed to Kodak Biomax MR film in light-tight film cassettes and left undisturbed for 6-week exposure. This film, while having some advantages over the Kodak Ektascan we have typically used (nominally, sensitivity), yielded some background artifact upon development which slightly degraded the images. Thus, we returned to Ektascan film for the subsequent 4-month-old study and utilized an 8-week exposure. The outcomes were similar, but nonetheless, our routine within-subject data normalization, below, ensured comparability. All films were developed by hand using Kodak GBX developer and fixer.

The subsequent images of the mouse brain were captured on a backlit fiber optic lightbox with a Photometrics Sensys high-resolution CCD camera, digitized, and transmitted to Optimas Image Analysis software (Media Cybernetics). The C.O.-stained sections from the 16-month-old mice were first used to assess the presence and degree of callosal white matter and hippocampal morphology changes in the mice, as in Gonzalez-Lima et al. (2001). Section counts used to generate anteroposterior (AP) distances and area and volume measurements were performed in Optimas, as follows. Whole brain AP distance was calculated from distance

between coronal sections in one hemisphere containing the most rostral portion of the nucleus accumbens and the section immediately posterior to the superior colliculus. Sections including and between the two points were counted (including unstained sections) and multiplied by 40 μm , the section thickness. CC commissure AP distance was calculated by counting the number of coronal sections in which commissural fibers were visible within the CC. It should be emphasized that this measurement only refers to the commissural length of the CC, and not lateral portions. Hippocampal AP distance was calculated from distance between coronal sections in one hemisphere containing the point of first appearance of the dorsal hippocampus proper, specifically CA3 and dentate gyrus, and the caudal disappearance of the subiculum. Digital imaging was used to determine hemi-brain and hemi-hippocampal area through the extent of the hippocampal formation. In each sample, the initial section was randomly selected from the 1st, 2nd, or 3rd stained section containing the rostral-most hippocampus. Subsequently, every stained section (every 3rd actual section) as far as the caudal extent of the hippocampus was sampled. The percent of hemi-brain area occupied by hippocampus was calculated at each level. Using this systematic-random sample, we also applied the Cavalieri principle by multiplying each area (mm^2) by 0.120 mm to provide estimates of volume.

For the densitometric regional analysis, the light level was set and held constant across each session. Optical distortions were corrected by subtracting the background film. Regions-of-interest (ROIs) correspond to the delineations of Paxinos and Franklin (2001) except that we subdivided their retrosplenial gyrus into three defined anteroposterior ROIs to localize any hypothesized reductions in the PCC: posterior cingulate (approximately bregma -1.4), posterior cingulate level 2 (bregma -2.1), and retrosplenial (bregma -2.6). Optical densities were sampled bilaterally from each ROI using sampling windows of varying size and averaged to provide the regional measures. Subsequently, the density values from each film were independently converted to nCi/g using the ^{14}C standards apposed to each. To investigate regions preferentially effected or spared in the transgenic mice independent of the variation in absolute measurements, regional FDG data from each mouse were normalized to a "whole brain" value of 1000 nCi/g using the mean activity of all gray matter ROIs sampled in each respective mouse. The 16-month-old mice were first assessed with 2-tailed group t tests on each ROI. Ultimately, the two age groups were analyzed with a 2×2 ANOVA encompassing age and group (transgene), with each omnibus F assessed at $\alpha = 0.05$. Significant effects were followed by post hoc Student's t tests. We did not statistically correct for multiple comparisons; rather, we report the calculated significance. While the possibility remains that a portion of our results may be due to Type I error, the results do correspond to our previous studies, reducing the probability that they are due to random fluctuations. Individual ROI scores with a Studentized residual >3.0 in an initial 2×2 ANOVA were considered outliers and excluded from the final analysis.

Acknowledgements

This study was supported by the Harrington Research Center, the Barrow Neurological Foundation, the Arizona Alzheimer's Disease Consortium, and the Arizona Alzheimer's Disease Clinical Core (P30 AG019610).

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Fig. 1. Thioflavin S-stained fluorescent images of a PSAPP mouse illustrating the amyloid load in the subcortical primary auditory pathway, medial geniculate (arrow), and inferior colliculus (inset, outlined). Deposition in the auditory nuclei was coincident with highly significant progressive increases in FDG uptake. Other subcortical nuclei at these levels present very little or no apparent amyloid load, while the cortex shows a very high level, as in the primary and secondary visual and auditory cortices seen at top left.

Fig. 2.

Retrosplenial cingulate activity (i.e., normalized FDG uptake in nCi/g) in aged PSAPP and wildtype mice. Retrosplenial FDG activity showed both group main effects and a significant interaction between group and age ($p < 0.001$, 2×2 ANOVA) and was significantly lower in the aged PSAPP mice than in aged wildtype ($p < 0.001$, 2-tailed t test). No retrosplenial difference was seen between the two young groups ($p > 0.95$, 2-tailed t test). The abnormal, age-dependent reduction in retrosplenial cingulate activity could be a surrogate marker of AD-like progression in these mice.

Table 1A summary of the group statistics, mean $\pm$ standard deviation

	Wildtype	PSAPP
4-month-old	N=14	N=15
Age (weeks)	All 17 weeks of age	
Weight (g)	29.2 $\pm$ 3.6	23.9 $\pm$ 3.7 [*]
16-month-old	N=13	N=12
Age (weeks)	69.6 $\pm$ 0.4	69.5 $\pm$ 0.4
Weight (g)	42.6 $\pm$ 5.8	30.2 $\pm$ 2.6 [*]

* Significantly different from wildtype, $p < 0.001$, 2-tailed t test uncorrected for multiple comparisons.

Table 2

Results of the morphology analyses in the 16-month-old mice, mean±standard deviation

	Wildtype	PSAPP	<i>p</i>
Whole brain AP (mm)	6.8±0.3	7.1±0.4	0.10
Callosal AP (mm)	3.8±0.3	3.7±0.3	0.28
Callosal/Brain AP ratio	0.56±0.04	0.52±0.03	0.01*
HF AP (mm)	3.5±0.3	3.7±0.2	0.15
HF/Brain AP ratio	0.52±0.05	0.53±0.05	0.74
Hemi-brain volume (mm ³)	88.2±10.8	94.0±8.2	0.14
Hemi-HF volume (mm ³)	13.1±1.3	13.4±1.1	0.44
HF/Brain volume ratio	0.15±0.01	0.14±0.01	0.31
HF % of hemi-brain area:			
0 mm (from anterior pole)	1.6±1.3%	1.2±1.3%	0.48
0.36 mm	6.4±1.2%	5.4±1.4%	0.06
0.72 mm	9.6±1.0%	8.5±0.9%	0.008*
1.08 mm	11.9±1.3%	10.6±0.8%	0.004*
1.44 mm	18.1±5.7%	12.9±3.2%	0.009*
1.80 mm	22.5±6.3%	18.0±6.2%	0.08
2.16 mm	24.8±3.4%	24.0±5.0%	0.61
2.52 mm	22.4±3.5%	24.9±3.5%	0.06
2.88 mm	15.1±7.3%	19.7±5.6%	0.10
3.24 mm	10.6±6.2%	11.6±7.9%	0.78

HF=hippocampal formation. *p* indicates result of Student's 2-tailed *t* test.

Table 3

Regional FDG uptake values, mean±standard deviation, and the *p* values for main effect of group (wildtype, PSAPP) and group×age interaction

	Aged		Young		Main effect: Group	Interaction: Group×Age
	Wildtype	PSAPP	Wildtype	PSAPP		
Optic tract	24±8	23±6	36±7	35±10	0.465	0.951
Whole brain	50±16	45±10	58±9	54±16	0.249	0.982
Whole brain/optic tract	2.0±0.3	2.1±0.3	1.6±0.2	1.6±0.1		
Cingulate gyrus, retrosplenial	1355±107	1187±91**	1141±62	1142±42	0.001*	0.001*
Cingulate gyrus, posterior 2	1265±76	1225±100	1193±55	1188±29	0.237	0.333
Cingulate gyrus, posterior	1245±98	1220±88	1208±58	1180±81	0.240	0.952
Cingulate gyrus, middle	1229±96	1184±78	1180±58	1147±44	0.050	0.760
Cingulate gyrus, anterior	1035±66	1037±87	1044±51	1026±51	0.657	0.559
Posterior parietal cortex	1061±50	1081±36	1122±43	1119±50	0.514	0.335
Lateral entorhinal cortex	863±72	879±86	896±65	832±57**	0.217	0.045*
Primary somatosensory cortex	1017±64	1047±54	1109±49	1107±54	0.372	0.310
Primary somatosensory, barrel fields	1183±78	1140±57	1080±41	1081±54	0.205	0.178
Secondary somatosensory cortex	1045±52	1068±44	953±29	973±43	0.076	0.906
Primary auditory cortex	989±81	1058±76**	900±63	927±59	0.016*	0.280
Primary visual, monocular	1152±111	1069±75	1146±48	1111±68	0.009*	0.275
Primary visual, binocular	1072±49	1044±71	1118±51	1095±82	0.166	0.891
Secondary visual cortex	1021±31	1018±73	1081±45	1054±79	0.465	0.465
Piriform cortex	874±52	909±72	787±41	793±38	0.149	0.306
CA1	672±38	680±88	860±62	865±24	0.688	0.931
CA3	731±18	742±90	794±41	813±74	0.408	0.825
Dentate gyrus	964±52	966±63	963±41	959±20	0.958	0.803
Subiculum	947±50	937±41	972±60	984±42	0.922	0.409
Medial mammillary nucleus	1364±124	1224±180	1112±159	1075±147	0.042*	0.229
Basolateral amygdala	764±36	791±47	806±37	815±29	0.093	0.366*
Anteroventral thalamus	1276±73	1187±48**	1082±72	1080±56	0.012*	0.016*
Reticular thalamus	1043±58	969±48**	966±53	949±68	0.006*	0.079
Reuniens nucleus	932±66	922±85	824±65	841±79	0.875	0.523
Ventromedial thalamus	1145±55	1057±35**	1105±62	1067±49	0.001*	0.081
Ventrolateral thalamus	1133±76	1070±60**	1119±50	1088±61	0.007*	0.337
Mediodorsal thalamus	1107±54	1039±28**	1057±50	1092±67	0.264*	0.001*
Laterodorsal thalamus	1196±58	1115±43**	1060±46	1060±43	0.003*	0.003*
Lateral posterior thalamus	1085±41	1056±35	1018±66	1026±46	0.465	0.171
Parafascicular thalamus	1038±38	1007±41	1037±46	1059±31	0.667	0.016*
Ventroposterolateral thalamus	1033±61	994±76	1022±56	995±46	0.048*	0.738
Ventroposteromedial thalamus	1115±62	1043±72**	1064±59	1045±42	0.008*	0.110
Posterior thalamus	1045±62	1028±61	1083±52	1049±50	0.100	0.602
Lateral habenula	1007±51	1015±49	953±66	970±68	0.434	0.794
Rostral caudoputamen	905±32	877±40	923±49	950±49	0.980	0.025*
Caudal caudoputamen	713±81	719±88	788±41	819±38	0.307	0.482
Lateral globus pallidus	680±43	672±67	826±36	848±45	0.567	0.269
Subthalamus	938±29	897±19**	914±49	907±46	0.027*	0.112
Nucleus accumbens	755±49	745±52	944±39	944±39	0.283	0.070
Nucleus of vertical diagonal band	1038±50	1043±53	865±81	918±62	0.184	0.184
Lateral septal nucleus	809±37	824±46	775±53	843±59**	0.004*	0.068
Anterior hypothalamus	798±64	762±86	830±71	832±70	0.395	0.356
Lateral hypothalamus	753±48	715±61	854±35	841±30	0.041*	0.310
Anterior prepectal area	1076±76	1077±88	1076±62	1023±38	0.939	0.904
Dorsolateral geniculate	879±55	887±53**	964±51	948±29	0.788	0.375*
Medial geniculate	928±103	1029±84	979±51	960±34	0.042*	0.004*

	Aged		Young		Main effect: Group	Interaction: Group×Age
	Wildtype	PSAPP	Wildtype	PSAPP		
Superior colliculus	989±21	986±40	1000±57	983±51	0.408	0.563
Pontine nuclei	762±28	724±34**	826±54	825±38	0.072	0.097*
Inferior colliculus, central	964±202	1310±242**	1043±105	1078±67	0.001*	0.001
Periaqueductal gray	708±62	786±70**	762±60	788±47	0.003*	0.121
Vestibular nuclei	1289±101	1359±114	1069±83	1139±83**	0.012*	0.997
Reticular nucleus, gigantocellularis	746±29	720±27**	872±51	908±56	0.685	0.015*
Cerebellar lobules 1–5	1057±60	1177±150**	1220±102	1238±55	0.014*	0.066
Simple lobule	956±84	1030±89	1191±63	1183±75	0.139	0.063
Crus 1 lobule	1207±125	1215±151	1370±73	1280±92	0.197	0.118

* =effects followed up by post hoc *t* tests.

** =significantly different from wildtype, post hoc *t* test, $p<0.05$.