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Regional cerebral glucose uptake in the 3xTG model of Alzheimer's disease highlights common regional vulnerability across AD mouse models

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Abstract

We have previously used fluorodeoxyglucose (FDG) autoradiography to detect the pattern of metabolic declines in two different transgenic mouse models of fibrillar beta-amyloid pathology in Alzheimer's disease (AD), including the PDAPP mouse, which overexpresses a mutant form of human APP, and the PSAPP mouse, which overexpresses mutant forms of the human APP and PS1 genes. In this study, we used the same approach to study a triple-transgenic (3xTG) model of AD, which overexpresses human APP, PS1 and tau mutations, and progressively develops amyloid plaques, neurofibrillary tangles, and synaptic dysfunction. Densitometric measurements from 55 brain regions were characterized and compared in 2, 12, and 18 month-old 3xTG and wildtype control mice (n=12/group). By 18 months of age, the 3xTG mice had significant reductions in FDG uptake in every measured brain region, including cortical and subcortical gray matter, cerebellar and brainstem regions. However, regional differences in normalized FDG

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uptake were apparent in the 2- and 12-month-old 3xTG mice, in a brain network pattern reminiscent of our previous analyses in the other mouse models. This prominently included the posterior cingulate/retrosplenial cortex, as in each previously-analyzed model. Overall, our analyses highlight consistencies in brain glucose uptake abnormalities across multiple mouse models of amyloid-associated pathophysiology. These mouse brain regional changes are homologous to alterations seen in PET scans from human AD patients and could thus be useful biomarkers for early testing of novel interventions.

Keywords

Alzheimer's disease; animal models; functional brain imaging; transgenic mice; glucose uptake; energy metabolism

1. Introduction

Human in vivo imaging, such as fluorodeoxyglucose positron emission tomography (FDG PET), has shown that persons with Alzheimer's disease (AD) demonstrate consistent glucose uptake reductions across large areas of association cortex (Minoshima et al., 1994, 1995, 1997; Mielke et al., 1994; Reiman et al., 1996). Such studies have also demonstrated that cognitively normal, late-middle-aged carriers of the apolipoprotein 4 allele (APOE4), a common AD susceptibility gene, have functional brain abnormalities in the same cortical regions as patients with probable AD, prior to the development of cognitive symptoms (Reiman et al., 2001), and further, younger, 20-39 year-old, carriers also show significant PET declines in these regions (Reiman et al., 2004), indicating a very early vulnerability in these cortices. In particular, the posterior cingulate cortex (PCC) demonstrated the most significant decline in these studies. We have previously (Valla et al., 2001) examined the lamina of the human postmortem PCC using a marker of oxidative metabolic capacity, and found 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 indicate a particular vulnerability of the PCC in AD and in those at risk for developing AD that may provide utility toward the early detection and tracking of the disease.

We have applied similar glucose uptake measures to emerging animal models of AD, with the same goal of early detection and tracking of progression and vulnerability. We have examined the "PDAPP" mouse, the first mutant APP-overexpressing line with plaque deposition (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; Valla et al., 2008). In addition, we have assessed the doubly-transgenic "PSAPP" mouse (PS1 [Duff et al., 1996] × TG2576 [Hsiao et al., 1996]; Holcomb et al., 1998) at approximately 4 and 16 months of age (Valla et al., 2006b). In both lines of mice, our results showed a preferential, highly significant, and age-related reduction in fluorodeoxyglucose (FDG) uptake in the posterior cingulate/retrosplenial cortex, an area with considerable homology to human PCC. In the PSAPP mice, 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. The former two areas, with sensory input through the laterodorsal thalamus, comprise a portion of a circuit essential for spatial learning tasks (Aggleton et al., 1996; Vann et al., 2000, 2003), and thus the glucose uptake declines could be directly related to such behavioral deficits in these mice (Arendash et al., 2001).

In the current study, we have applied FDG autoradiography to a triple-transgenic mouse model of AD which displays progressive amyloid plaque deposition as well as neurofibrillary tangles (Oddo et al., 2003ab). We hypothesized that the transgenic mice

would display an age-related vulnerability in posterior cingulate/retrosplenial cortex as compared to wildtype mice, and that our measures would show steeper functional decline with the addition of neurofibrillary pathology in this model.

2. Results

A summary of the groups analyzed is presented in Table 1. The transgenic mice showed higher weight than age-matched wildtype controls, significantly so at the youngest age, but otherwise were comparable. Both male and female mice were examined together. No gross abnormalities of the brain were noted in this transgenic strain, in contrast to what we have reported previously in PDAPP mice (Gonzalez-Lima et al., 2001; Valla et al., 2006a). Genotype was confirmed via PCR.

Regional densitometric analysis of the FDG autoradiographs demonstrated significant changes in FDG uptake with age in 3xTG mice, relative to wildtype. An initial 2×3 (genotype $\times$ age) ANOVA on raw (nonnormalized) FDG uptake data showed a significant effect of genotype ($p < 0.05$) in every measured region except the optic tract and *post hoc* t-tests between genotypes at each age showed that the every measured region, including PCC, was significantly lower in the 3xTG mice at 18 months (Table 2). This demonstrates a profound progression of the disease phenotype in 3xTG mice between 12 and 18 months of age but also confounds the normalization of the data to account for dosing variability and potential tracer-kinetic processes unrelated to brain metabolism. This was previously accomplished by a ratio utilizing the averaged whole brain uptake. Similar normalization could be accomplished utilizing a ratio to white matter, such as optic tract; however, 3xTG optic tract showed approximately 31% lower FDG uptake than wildtype and would thus similarly erase the obvious differences between groups.

3xTG mice at 18 months of age show profound and widespread glucose uptake declines. To test our hypothesis of early vulnerability in PCC in the transgenic mice, the 18 month-old cohorts were removed from the analysis, and the 2 and 12 month-old groups were assessed. Within this analysis, no significant effects of genotype were found in the whole brain average nor in the optic tract (Table 3); thus, the whole brain average could be used to normalize per our standard protocol. Following normalization, 2×2 (genotype $\times$ age) ANOVAs were performed for each region, followed by post hoc t-tests at a reduced alpha level of 0.01. Consistent with our previous findings in other transgenic strains (Reiman et al., 2000; Valla et al., 2006b), 3xTG mice showed a prominent, statistically significant effect of genotype in retrosplenial PCC; post hoc analyses indicated a significant difference between groups at 12 months of age. Several subcortical ROIs demonstrated main effects of genotype (Table 3), the majority of these in primary sensory, motor, and attention/arousal systems, again consistent with previous findings in other strains. In contrast to the global decreases in 3xTG mice at 18 months, these changes were a mix of increases and decreases in uptake, likely reflecting changes in the regional functional inter-relationships rather than direct effects of pathology. Within the basal forebrain, the nucleus accumbens and lateral and medial septal nuclei showed main effects of genotype, trending nonsignificantly higher in the 3xTG mice in the post hoc t-tests. This finding is consistent with our previous findings indicating that nuclei with direct connections to the hippocampal formation, particularly the subiculum, are preferentially impacted (Valla et al., 2006b). The primary auditory cortex and medial geniculate showed significant genotype and age effects, as in PSAPP mice; however, PSAPP mice demonstrated relatively heavy thioflavin-positive amyloid deposition in medial geniculate and inferior colliculus, whereas the other subcortical nuclei at those levels demonstrated no visible amyloid staining (Valla et al., 2006b). In 3xTG mice, the inferior colliculus appeared functionally intact and no thioflavin staining was apparent in

any of the auditory nuclei or cortices. Cerebellar involvement, as here in crus 1 and lobules 1-5, has also been a consistent and unexpected feature in all three analyzed mouse models.

As in our previous analyses, staining for fibrillar pathology revealed little opportunity for direct effects of pathology on our functional measures; however, in the earlier strains, pathology failed to correlate with function because the pathology was much more widespread than the functional changes. In the current study, however, fibrillar pathology was not widespread and the functional changes more so. Thioflavin-S staining revealed a pattern of progressive, highly region-specific amyloid plaque deposition in the brains of the 3xTG mice. No positive staining was found in 2 month-old mice, while 40% of 12 month-old 3xTG mice sampled demonstrated thioflavin-positive plaque deposition in the anterior dorsal hippocampus, localized primarily in the subiculum and spreading into CA1. In half of these (20% overall), scattered plaques were also visualized in the ventral hippocampus and amygdala. At 18 months of age, all 3xTG mice demonstrated a high density of thioflavin-positive deposition in the anterior dorsal hippocampus, subiculum and CA1, and 45% showed positive staining in the ventral hippocampus and amygdala, often densely deposited throughout the CA fields, subiculum, and dentate. Further, approx 30% of 18 month-old 3xTG mice showed labeling in the medial and lateral septal nuclei of the basal forebrain. Thioflavin-positive deposition was not apparent elsewhere in these groups nor in any region in wildtype mice. AT8-positive tau immunohistochemistry targeted to the posterior hippocampal formation and midbrain/brainstem often revealed hyperphosphorylated tau in hippocampal formation of 3xTG at 18 months (66%), and none in this region in WT or younger 3xTG. In other posterior areas available for labeling, the red nucleus stood out, showing positive staining in a large number of cells in 3xTG mice. Aged WT mice showed some but fewer positively-labeled cells, as did both 3xTG and WT mice occasionally at the younger ages. Similarly, positive labeling in white matter tracts was apparent in some mice from all groups, with the oldest 3xTG showing a qualitative increase in this marker.

3. Discussion

This analysis confirmed our hypothesis that significant declines in PCC FDG uptake are a consistent feature of mouse models that over-express human mutant forms of APP. Glucose uptake declines in this cortex are similar to the PET-determined homologous regional decline seen in patients with AD and in those at-risk of developing AD, and thus may be a particularly relevant biomarker in the disease course.

One striking feature in this particular mouse model was the pronounced and widespread decline in FDG uptake in 3xTG mice at 18 months of age: all measured regions declined significantly in the 3xTG. These regional declines may or may not reflect a global reduction in the cerebral metabolic rate for glucose (CMR_{gl}); we cannot rule out other potential changes in glucose metabolism or distribution. However, quantitative PET studies find progressive reductions in whole brain CMR_{gl} , particularly in the latter stages of the disease (Alexander et al., 2002). This was not a feature in our previous analyses, including the PDAPP mice up to 24 months or the PSAPP mice to 16 months; neither demonstrated such a global decline. One may speculate that the addition of the mutant tau transgene in this model leads to an eventual global deterioration. In this limited tau series, however, no negative correlative trend was apparent between qualitative tau IHC and whole-brain FDG uptake.

The consistent PCC FDG uptake decrease in APP-overexpressing mouse models of AD is similar to the prominent decrease in this homologous region and other association cortices in AD patients (Minoshima et al., 1994, 1995, 1997; Mielke et al., 1994; Reiman et al., 1996). In fact, PCC FDG uptake is the *only* significant functional change that was found consistently in aged PDAPP (Reiman et al., 2000), PSAPP (Valla et al., 2006b), and 3xTG

mouse models. Glucose uptake was also shown to be significantly decreased in aged PDAPP PCC by another group (Dodart et al., 1999), using a slightly different data normalization procedure. Of note, the hippocampal subiculum, which shares prominent reciprocal connections with PCC, was significantly decreased in the PDAPP, and trended lower in both PSAPP and 3xTG. Other hippocampal structures—CA1, CA3, and dentate gyrus—showed no significant regional changes or consistent trends across studies. Three other regions showed consistent changes across at least 2 of our studies: mediodorsal thalamus uptake was significantly decreased in PDAPP and PSAPP mice, but not changed in 3xTG (at 12 months); the rostral caudoputamen/striatum uptake was significantly increased in PDAPP and 3xTG mice, but not changed in PSAPP; and the brainstem vestibular nuclei uptake was significantly increased in PSAPP and 3xTG, but not analyzed in PDAPP. Thus, the effect in the PCC is unique and uniquely reliable.

To speculate as to the cause of the reliable functional change in PCC, all the assessed transgenic models, with their varying assortment of human genes and mutations, share two principal commonalities: (mutant) APP overexpression, and subsequent increases in soluble amyloid leading to plaque deposition. Both of these intracellular species, holoprotein and cleavage product, have been demonstrated to interact directly and independently with mitochondria, changing mitochondrial function in a region-specific fashion as well (Valla et al., 2007). This dysfunction that could drive the bioenergetic changes shown here, either locally or by impacting efferent connections, and PCC may be a critical common node within the dysfunctional circuits.

Previous mouse models we have assessed, PDAPP and PSAPP mice, consistently demonstrated considerable, widespread thioflavin-positive plaque deposition with increasing age, throughout the hippocampal formation, in all cortices including primary sensory and motor, and in select subcortical nuclei (see, e.g., Valla et al., 2006b, Fig 1). In contrast, the 3xTG mice demonstrated a very limited distribution of positive-staining deposits, initially appearing in the anterior subiculum around 12 months of age, spreading into the CA fields, and then expanding into the basal forebrain, primarily within the midline septal nuclei, by 18 months. This is consistent with earlier reports (Oddo et al., 2003a), indicating that this is not due to limitations of thioflavin staining or a unique feature of our colony production. In our previous studies, amyloid deposition did not correlate with functional changes: deposition was so widespread as to encompass brain regions showing both significant decreases and increases, as well as no changes, in FDG uptake. Neurodevelopmental changes due to the transgene(s) could also contribute to these changes (Valla et al., 2008). In the current study, we found no thioflavin-positive staining in the PCC or adjacent areas, and no significant functional changes were found in the subiculum or the CA fields. However, the posterior cingulate cortex has direct reciprocal projections to the subiculum, where plaques become most prominent, as do the septal nuclei, which develop plaques as well. -amyloid-specific immunohistochemistry reveals copious amounts of intracellular, presumably soluble, amyloid in approx 1 year-old 3xTG mice, particularly in the CA fields (data not shown) and in much younger mice (Oddo et al., 2003a; Billings et al, 2005). As they can also explain the early functional alterations in young mice, soluble amyloid production or APP holoprotein overexpression itself (Anandatheerthavarada et al., 2003) are more likely drivers of the brain functional changes.

While our ability to monitor the regional PCC change in mouse brain using in vivo PET remains limited, as shown by our earlier work and others (Valla et al., 2002; Kuntner et al., 2009), it may be possible with the 3xTG model to assess whole brain CMR_{gl}, which would not be so confounded by limited spatial resolution. Thus, preclinical studies of interventional efficacy could use these mice as their own controls, following them longitudinally and assessing them periodically. Utilizing FDG uptake/metabolism as such a biomarker provides

an indicator of *functional*, rather than simply histopathological, progression, helping bridge the gap between ongoing in vivo human studies of AD and investigations of animal models toward the clarification of disease mechanisms and screening of promising treatments.

4. Experimental Procedure

The study was performed under protocols approved by the Institutional Animal Care and Use Committee at the Barrow Neurological Institute, St. Joseph's Hospital and Medical Center. 3xTG mice and controls (129/sv × C57BL6 background strain) were produced in our colony, which was derived from founders received directly from University of California, Irvine. Transgene status of mice in each cohort was blinded until the analysis was complete and was confirmed by PCR genotyping. Mice were group-housed in plexiglas shoebox-style cages with access to food and water ad libitum.

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 (FDG; American Radiolabeled Chemicals) in sterile saline and immediately placed in an individual clean cage in a dark, quiet cabinet 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; subsequently, these were used for thioflavin S staining. A second set was stained for mitochondrial cytochrome oxidase (C.O.) activity (Gonzalez-Lima & Garrosa, 1991; Valla et al., 2007) for morphometry, region identification, and future analyses of mitochondrial activity. The slides designated for autoradiography, along with ^{14}C autoradiographic standards (Amersham), were apposed to Kodak Ektaskan B/RA film in light-tight film cassettes and left undisturbed for 2-week exposure, after which all films were developed by hand using Kodak GBX developer and fixer (5 min each). During the blind administration, 9 mice were given tracer from a batch later found partially inactive. After unblinding, it was found that 1 mouse/group in the 12 and 18 month-old cohorts and 5 mice from the young WT cohort received this tracer and they were removed from the FDG analysis.

The film 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). 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 & Franklin (2001) except that we subdivided their retrosplenial gyrus into three defined anteroposterior ROIs to localize hypothesized reductions in the PCC: posterior cingulate (approx 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. After the initial analysis of raw uptake values in all 3 cohorts, regional FDG data from each 2 and 12 month-old mouse was normalized to a “whole brain” value of 1000 nCi/g using the mean activity of all gray matter ROIs sampled in each respective mouse, in order to investigate regions preferentially affected or spared in the transgenic mice independent of the variation in absolute measurements. The two remaining age groups were analyzed with a 2×2 ANOVA encompassing transgene and age, with each omnibus F assessed at $\alpha = 0.05$. Significant effects were followed by post hoc Student's t-tests at a reduced $\alpha = 0.01$. 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 they are due to random fluctuations. Individual ROI scores with a Studentized residual > 3.0 in the initial 2 × 2 ANOVA were deemed outliers and excluded from the final analysis.

Thioflavin S staining utilized the dried brain sections used for FDG, representing a coronal series through the entire brain from approx the anterior callosal commissure to the tectum. Slides were fixed with 4% buffered PFA, washed with tap water, rinsed in purified H₂O, immersed in 4% thioflavin S in purified H₂O for 5 min, differentiated in 70% ethanol, rinsed twice with purified H₂O, and coverslipped with aqueous media. Fluorescing deposits were visualized on an Olympus BX61 microscope. Immunohistochemistry for phosphorylated tau (clone AT8) on remaining frozen coronal sections was restricted to the posterior hippocampal formation and midbrain. Frozen sections were fixed with 4% PFA, blocked with peroxide (3% for 5 min) and 3% BSA and 2% goat serum (1 hr), probed on-slide (tau AT8; Pierce), and visualized with DAB (Dako LSAB+ System) on the Olympus BX61 microscope.

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Abbreviations

FDG	fluorodeoxyglucose
PCC	posterior cingulate/retrosplenial cortex

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Table 1

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

	Wildtype	3xTG	P *
2 Month-old	N = 12	N = 12	
age (weeks)	8.6 $\pm$ 0.6	8.3 $\pm$ 2.2	0.643
weight (g)	21.9 $\pm$ 2.6	26.8 $\pm$ 4.8	0.006 *
12 Month-old	N = 12	N = 12	
age (weeks)	52.2 $\pm$ 1.5	52.0 $\pm$ 2.3	0.780
weight (g)	38.2 $\pm$ 5.3	42.7 $\pm$ 6.6	0.080
18 Month-old	N = 12	N = 12	
age (weeks)	76.6 $\pm$ 3.2	76.4 $\pm$ 1.8	0.860
weight (g)	40.8 $\pm$ 6.1	43.0 $\pm$ 7.8	0.459

*
2-tailed t-tests, uncorrected for multiple comparisons

Table 2

Raw (nonnormalized) FDG uptake in the 18-month-old 3xTG and wildtype mice, mean $\pm$ standard deviation.

	Wildtype	3xTG *
Whole brain average	914 $\pm$ 131	669 $\pm$ 81
Optic tract	370 $\pm$ 111	256 $\pm$ 65
Cingulate gyrus, retrosplenial	1058 $\pm$ 152	755 $\pm$ 74
Cingulate gyrus, posterior 2	1047 $\pm$ 114	786 $\pm$ 94
Cingulate gyrus, posterior	1054 $\pm$ 125	809 $\pm$ 95
Cingulate gyrus, middle	1064 $\pm$ 145	805 $\pm$ 101
Cingulate gyrus, anterior	929 $\pm$ 145	687 $\pm$ 80
Posterior parietal cortex	1003 $\pm$ 132	740 $\pm$ 106
Lateral entorhinal cortex	726 $\pm$ 128	518 $\pm$ 57
1° somatosensory cortex	926 $\pm$ 141	671 $\pm$ 84
1° somatosensory, barrel fields	1015 $\pm$ 121	771 $\pm$ 90
2° somatosensory cortex	927 $\pm$ 128	704 $\pm$ 84
1° auditory cortex	885 $\pm$ 118	625 $\pm$ 82
1° visual cortex, monocular	1045 $\pm$ 145	781 $\pm$ 97
Piriform cortex	766 $\pm$ 126	535 $\pm$ 78
CA1	759 $\pm$ 144	532 $\pm$ 76
CA3	772 $\pm$ 143	519 $\pm$ 70
Dentate gyrus	807 $\pm$ 138	547 $\pm$ 79
Subiculum	906 $\pm$ 142	650 $\pm$ 83
Medial mammillary nucleus	1127 $\pm$ 97	911 $\pm$ 96
Substantia nigra	727 $\pm$ 132	490 $\pm$ 73
Basolateral amygdala	785 $\pm$ 137	542 $\pm$ 82
Anteroventral thalamus	1163 $\pm$ 148	900 $\pm$ 114
Reticular thalamus	1070 $\pm$ 163	813 $\pm$ 94
Reuniens nucleus	818 $\pm$ 159	624 $\pm$ 120
Ventromedial thalamus	1100 $\pm$ 137	869 $\pm$ 105
Ventrolateral thalamus	1080 $\pm$ 137	840 $\pm$ 107
Mediodorsal thalamus	1076 $\pm$ 140	814 $\pm$ 100
Laterodorsal thalamus	1144 $\pm$ 130	913 $\pm$ 100
Lateral posterior thalamus	1098 $\pm$ 152	837 $\pm$ 109
Lateral habenula	863 $\pm$ 151	632 $\pm$ 120
Parafascicular thalamus	1013 $\pm$ 139	740 $\pm$ 90
Ventroposterolateral thalamus	1023 $\pm$ 152	747 $\pm$ 108
Ventroposteromedial thalamus	1064 $\pm$ 155	775 $\pm$ 110
Posterior thalamus	1083 $\pm$ 139	802 $\pm$ 117
Rostral caudoputamen	920 $\pm$ 142	727 $\pm$ 78
Caudal caudoputamen	744 $\pm$ 143	430 $\pm$ 61
Lateral globus pallidus	747 $\pm$ 138	484 $\pm$ 75
Subthalamus	898 $\pm$ 116	722 $\pm$ 98

	Wildtype	3xTG *
Nucleus accumbens	727 ± 133	531 ± 77
Nuc. of vertical diagonal band	834 ± 140	638 ± 84
Medial septal nucleus	872 ± 140	657 ± 82
Lateral septal nucleus	817 ± 154	582 ± 83
Nucleus basalis	746 ± 139	497 ± 71
Anterior hypothalamus	662 ± 136	452 ± 86
Lateral hypothalamus	763 ± 140	543 ± 75
Dorsolateral geniculate	1029 ± 128	784 ± 109
Medial geniculate	961 ± 171	691 ± 87
Superior colliculus	999 ± 158	719 ± 96
Pontine nuclei	800 ± 149	561 ± 85
Inferior colliculus, central	887 ± 150	683 ± 99
Periaqueductal gray	634 ± 138	408 ± 74
Vestibular nuclei	1059 ± 140	841 ± 98
Reticular nucleus, giganto	1070 ± 163	813 ± 94
Cerebellar lobules 1-5	985 ± 142	650 ± 73
Simple lobule	734 ± 139	504 ± 71
Crus 1 lobule	728 ± 130	511 ± 69

* 3xTG mice demonstrated significantly lower FDG uptake than same-age wildtype in every region measured (2-tailed t-test, $p < 0.01$, uncorrected for multiple comparisons).

Table 3

Normalized regional FDG uptake (mean $\pm$ standard deviation) in 2 and 12 month-old 3xTG mice and wildtype controls.

	2 month			12 month			Main Effects [†] :		Genotype $\times$ Age
	Wildtype	3xTG		Wildtype	3xTG		Genotype	Age	
Whole brain average	692 $\pm$ 106	613 $\pm$ 131		759 $\pm$ 140	721 $\pm$ 130		0.167	0.041 *	0.625
Optic tract	277 $\pm$ 49	265 $\pm$ 70		282 $\pm$ 91	326 $\pm$ 86		0.526	0.190	0.270
Cingulate gyrus, retrosplenial	1135 $\pm$ 56	1051 $\pm$ 75		1168 $\pm$ 43	1092 $\pm$ 61 **		<0.001 *	0.065	0.836
Cingulate gyrus, posterior 2	1210 $\pm$ 51	1130 $\pm$ 83		1175 $\pm$ 61	1158 $\pm$ 79		0.054	0.880	0.206
Cingulate gyrus, posterior	1227 $\pm$ 55	1194 $\pm$ 49		1184 $\pm$ 58	1201 $\pm$ 59		0.671	0.346	0.203
Cingulate gyrus, middle	1184 $\pm$ 42	1178 $\pm$ 64		1150 $\pm$ 56	1143 $\pm$ 55		0.724	0.065	0.985
Cingulate gyrus, anterior	1033 $\pm$ 40	1070 $\pm$ 47		1018 $\pm$ 45	986 $\pm$ 65		0.887	0.005 *	0.045 *
Posterior parietal cortex	1077 $\pm$ 73	1083 $\pm$ 54		1116 $\pm$ 50	1104 $\pm$ 66		0.884	0.166	0.654
Lateral entorhinal cortex	745 $\pm$ 49	806 $\pm$ 80		789 $\pm$ 54	792 $\pm$ 66		0.132	0.475	0.165
1° somatosensory cortex	999 $\pm$ 36	1017 $\pm$ 40		1041 $\pm$ 37	1027 $\pm$ 45		0.867	0.062	0.248
1° somatosensory, barrel fields	1176 $\pm$ 79	1157 $\pm$ 81		1136 $\pm$ 61	1137 $\pm$ 48		0.686	0.196	0.664
2° somatosensory cortex	1047 $\pm$ 45	1037 $\pm$ 46		1024 $\pm$ 34	1028 $\pm$ 48		0.837	0.269	0.626
1° auditory cortex	1029 $\pm$ 35	1016 $\pm$ 55		994 $\pm$ 38	945 $\pm$ 36		0.034 *	0.001 *	0.222
1° visual cortex, monocular	1159 $\pm$ 67	1197 $\pm$ 81		1177 $\pm$ 58	1156 $\pm$ 80		0.719	0.616	0.223
Piriform cortex	787 $\pm$ 39	774 $\pm$ 61		809 $\pm$ 59	790 $\pm$ 62		0.395	0.330	0.868
CA1	708 $\pm$ 55	724 $\pm$ 75		769 $\pm$ 41	810 $\pm$ 50		0.133	<0.001 *	0.514
CA3	718 $\pm$ 44	766 $\pm$ 61		778 $\pm$ 47	796 $\pm$ 48		0.052	0.010 *	0.366
Dentate gyrus	757 $\pm$ 81	739 $\pm$ 74		839 $\pm$ 49	836 $\pm$ 72		0.635	<0.001 *	0.746
Subiculum	958 $\pm$ 64	931 $\pm$ 67		978 $\pm$ 43	941 $\pm$ 39		0.075	0.414	0.761
Medial mammillary nucleus	1372 $\pm$ 119	1323 $\pm$ 116		1349 $\pm$ 118	1323 $\pm$ 104		0.311	0.756	0.754
Substantia nigra	764 $\pm$ 43	736 $\pm$ 38		760 $\pm$ 55	766 $\pm$ 26		0.426	0.344	0.228
Basolateral amygdala	785 $\pm$ 33	781 $\pm$ 41		832 $\pm$ 38	847 $\pm$ 37		0.637	<0.001 *	0.432
Anteroventral thalamus	1243 $\pm$ 122	1262 $\pm$ 123		1238 $\pm$ 58	1294 $\pm$ 69		0.230	0.663	0.553
Reticular thalamus	1225 $\pm$ 74	1199 $\pm$ 80		1173 $\pm$ 57	1163 $\pm$ 47		0.398	0.046 *	0.713
Reuniens nucleus	862 $\pm$ 99	929 $\pm$ 98		880 $\pm$ 60	900 $\pm$ 97		0.136	0.854	0.413
Ventromedial thalamus	1316 $\pm$ 54	1263 $\pm$ 64		1282 $\pm$ 75	1299 $\pm$ 61		0.414	0.961	0.101

	2 month			12 month			Main Effects [†] :		
	Wildtype	3xTG		Wildtype	3xTG		Genotype	Age	Genotype × Age
Ventrolateral thalamus	1292 ± 69	1232 ± 76		1258 ± 95	1281 ± 55		0.446	0.768	0.092
Mediodorsal thalamus	1239 ± 68	1183 ± 46		1233 ± 71	1235 ± 46		0.163	0.222	0.125
Laterodorsal thalamus	1331 ± 63	1274 ± 59		1330 ± 95	1311 ± 56		0.103	0.433	0.397
Lateral posterior thalamus	1213 ± 41	1197 ± 44		1231 ± 53	1211 ± 51		0.251	0.316	0.895
Lateral habenula	886 ± 74	951 ± 97		936 ± 82	976 ± 57		0.049 [*]	0.153	0.637
Parafascicular thalamus	1077 ± 46	1094 ± 40		1094 ± 31	1110 ± 52		0.227	0.248	0.978
Ventroposterolateral thalamus	1143 ± 46	1094 ± 60		1130 ± 45	1131 ± 41		0.131	0.461	0.123
Ventroposteromedial thalamus	1187 ± 30	1130 ± 65		1170 ± 48	1169 ± 39		0.069	0.485	0.083
Posterior thalamus	1169 ± 19	1119 ± 61		1198 ± 41	1163 ± 32		0.004 [*]	0.013 [*]	0.581
Rostral caudoputamen	1109 ± 68	1188 ± 61		1030 ± 36	1066 ± 39		0.001 [*]	<0.001 [*]	0.213
Caudal caudoputamen	805 ± 46	733 ± 62		766 ± 82	699 ± 63		0.002 [*]	0.096	0.910
Lateral globus pallidus	801 ± 45	764 ± 34		782 ± 35	773 ± 42		0.077	0.696	0.266
Subthalamus	1039 ± 68	1085 ± 51		999 ± 28	1058 ± 56 ^{**}		0.003 [*]	0.055	0.713
Nucleus accumbens	741 ± 38	809 ± 37 ^{**}		745 ± 91	827 ± 60		0.001 [*]	0.588	0.747
Nuc. of vertical diagonal band	921 ± 55	953 ± 56		904 ± 60	925 ± 50		0.148	0.212	0.762
Medial septal nucleus	937 ± 52	978 ± 26		936 ± 37	964 ± 29		0.006 [*]	0.502	0.554
Lateral septal nucleus	815 ± 33	858 ± 43		838 ± 39	857 ± 53		0.037 [*]	0.446	0.397
Nucleus basalis	785 ± 49	790 ± 31		793 ± 29	791 ± 52		0.898	0.723	0.785
Anterior hypothalamus	610 ± 37	703 ± 46 ^{**}		665 ± 68	727 ± 71		<0.001 [*]	0.046 [*]	0.418
Lateral hypothalamus	792 ± 52	811 ± 43		856 ± 45	856 ± 32		0.506	<0.001 [*]	0.484
Dorsolateral geniculate	1177 ± 29	1179 ± 55		1151 ± 48	1123 ± 47		0.398	0.011 [*]	0.329
Medial geniculate	1109 ± 42	1091 ± 49		1064 ± 40	992 ± 36 ^{**}		0.003 [*]	<0.001 [*]	0.057
Superior colliculus	1116 ± 39	1069 ± 35		1126 ± 32	1101 ± 57		0.016 [*]	0.143	0.435
Pontine nuclei	848 ± 40	870 ± 45		817 ± 53	876 ± 37		0.010 [*]	0.417	0.228
Inferior colliculus, central	1206 ± 168	1191 ± 70		974 ± 102	990 ± 81		0.986	<0.001 [*]	0.658
Periaqueductal gray	586 ± 79	655 ± 58		662 ± 59	674 ± 50		0.054	0.027 [*]	0.168
Vestibular nuclei	1215 ± 87	1288 ± 53		1190 ± 61	1235 ± 61		0.012 [*]	0.091	0.543

	2 month			12 month			Main Effects [†] :			Genotype × Age
	Wildtype		3xTG	Wildtype		3xTG	Genotype		Age	
Reticular nucleus, gigantot.	827 ± 44	829 ± 54		819 ± 44	903 ± 70	**	0.030	*	0.091	0.040
Cerebellar lobules 1-5	1055 ± 81	961 ± 105		1078 ± 91	999 ± 87		0.009	*	0.337	0.804
Simple lobule	758 ± 37	754 ± 35		775 ± 60	778 ± 72		0.966		0.291	0.848
Crus 1 lobule	788 ± 49	716 ± 57		777 ± 51	767 ± 71		0.048	*	0.321	0.125

* significant effects followed up by post hoc t-tests.

** significantly different from same-age wildtype, post hoc t-test, $P < 0.01$.

[†] Main effects of genotype, age and their interaction indicate the calculated P value associated with the omnibus 2×2 ANOVA.