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Effect of age on brain metabotropic glutamate receptor subtype 5 measured with [^{18}F]FPEB PET

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Abstract

Objective: Metabotropic glutamate receptor subtype 5 (mGluR5) is integral to the brain glutamatergic system and cognitive function. This study investigated whether aging is associated with decreased brain mGluR5 availability.

Methods: Cognitively normal participants (n = 45), aged 18 to 84 years, underwent [^{18}F]FPEB positron emission tomography scans to quantify brain mGluR5. Distribution volume (V_T) was computed using a venous or arterial input function and equilibrium modeling from 90 to 120 min. In the primary analysis, the association between age and V_T in the hippocampus and association

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Declaration of Competing Interest

The authors have no actual or potential conflicts of interest.

Availability of data

The data will be availability upon reasonable request from the principal investigators upon establishment of a data sharing agreement and when planned analyses are complete.

Supplementary materials

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cortex was evaluated using a linear mixed model. Exploratory analyses assessed the association between age and V_T in multiple brain regions. The contribution of gray matter tissue alterations and partial volume effects to associations with age was also examined.

Results: In the primary analysis, older age was associated with lower [^{18}F]FPEB binding to mGluR5 ($P = 0.026$), whereas this association was not significant after gray matter masking or partial volume correction to account for age-related tissue loss. *Post hoc* analyses revealed an age-related decline in mGluR5 availability in the hippocampus of 4.5% per decade ($P = 0.007$) and a non-significant trend in the association cortex ($P = 0.085$).An exploratory analysis of multiple brain regions revealed broader inverse associations of age with mGluR5 availability, but not after partial volume correction.

Conclusion: Reductions in mGluR5 availability with age appear to be largely mediated by tissue loss. Quantification of [^{18}F]FPEB binding to mGluR5 may expand our understanding of age-related molecular changes and the relationship with brain tissue loss.

Keywords

Aging; mGluR5; Glutamate receptor; [^{18}F]FPEB; PET

1. Introduction

Metabotropic glutamate receptor subtype 5 (mGluR5) is important in the functioning of the glutamatergic system (Sanacora et al., 2008) and cognitive performance (Homayoun and Moghaddam, 2010). These receptors are located primarily on neurons postsynaptically (Shigemoto et al., 1997; Shigemoto et al., 1993), but are also found presynaptically Gereau and Conn (1995) and on glia (Daggett et al., 1995). mGluR5 has highest expression in the hippocampus, intermediate expression in the striatum, cerebral cortex, deep cerebellar nuclei, and thalamus, and lowest expression in the cerebellum (Daggett et al., 1995). mGluR5, along with mGluR1, is a member of the group 1 mGluRs. Group 1 mGluRs are coupled to intracellular phospholipase C and stimulate cyclic AMP formation and arachidonic acid and calcium release to impact neuroplasticity, neuronal excitability, synaptic transmission, and gene expression (Hollmann and Heinemann, 1994). mGluR5 receptors have been extensively studied in mechanisms underlying hippocampus-dependent memory processes and shown to be involved in long-term potentiation (LTP) (Bikbaev et al., 2008; Bortolotto et al., 2005; Lu et al., 1997; Manahan-Vaughan and Braunewell, 2005; Neyman and Manahan-Vaughan, 2008) and long-term depression (LTD) (Lee et al., 2005; Neyman and Manahan-Vaughan, 2008), which have been associated with memory performance (Bikbaev et al., 2008; Lee et al., 2005; Lu et al., 1997; Manahan-Vaughan and Braunewell, 2005).

Animal models have suggested that changes in hippocampal mGluR5 expression play a role in both normal and pathologic cognitive aging (Lee et al., 2005). Until recently, the only means of studying the effects of age on mGluR5 in humans was by postmortem analyses, which have been scarce and limited in the brain regions studied (Tsamis et al., 2013). The advent of mGluR5 radioligands for positron emission tomography (PET) has enabled the study of aging effects on this receptor in living individuals. At least four

previous neuroimaging studies have utilized mGluR5 ligands to investigate mGluR5 and aging (Deschwanden et al., 2011; Akkus et al., 2013; DuBois et al., 2016; Leurquin-Sterk et al., 2016). These studies have used the mGluR5 ligands [^{11}C]ABP688 (Akkus et al., 2013; Deschwanden et al., 2011; DuBois et al., 2016) or [^{18}F]FPEB (Leurquin-Sterk et al., 2016) and have yielded variable results (see Discussion).

To assess the association between age and brain mGluR5 availability, we utilized [^{18}F]FPEB, a PET radioligand that binds to mGluR5 with high specificity. [^{18}F]FPEB PET has been validated in several human studies (Park et al., 2015; Sullivan et al., 2013), and may provide superior image quality, greater specific binding, and greater test-retest reproducibility than [^{11}C]ABP688 (Lim et al., 2014; Wong et al., 2013). By measuring mGluR5 availability using [^{18}F]FPEBPET in healthy adults spanning a wider age range than previously studied, we aimed to understand the changes in brain mGluR5 expression that occur across the human lifespan. Given the evidence for mGluR5 involvement in hippocampal synaptic plasticity and memory performance (Bikbaev et al., 2008; Bortolotto et al., 2005; Lee et al., 2005; Lu et al., 1997; Manahan-Vaughan and Braunewell, 2005; Neyman and Manahan-Vaughan, 2008), as well as the specific association between hippocampal mGluR5 binding and memory performance in aged rats Menardand Quirion (2012), our primary analyses focused on hippocampus, but included a composite association cortical region of interest (ROI). However, we also conducted exploratory analyses in multiple brain regions. Finally, we assessed the contribution of gray matter (GM) tissue alterations and partial volume effects to the association between brain mGluR5 availability and age.

2. Materials and methods

2.1. Participants

Participants included cognitively normal individuals who were enrolled as healthy controls in studies performed at the Yale PET Center using the [^{18}F]FPEB ligand to quantify brain mGluR5 availability (Abdallah et al., 2017; Mecca et al., 2020; Park et al., 2015). Particpants were excluded for major medical or psychiatric illnesses or cognitive impairment. Since smoking status may confound the measurement of mGluR5 availability (Akkus et al., 2013), current tobacco users were also excluded. Additional exclusion criteria for participants over the age of 60 included a Clinical Dementia Rating score >0 (Morris,1993), a Mini-Mental Status Examination score (Folstei nan dFolstein,1975) <27, or an education-adjusted Logical Memory II score consistent with Mild Cognitive Impairment or Dementia (Chen et al., 2018). All study protocols were approved by the Yale University Human Investigation Committee and Radiation Safety Committee. All participants provided written informed consent prior to participation.

2.2. Magnetic resonance imaging

All participants underwent magnetic resonance imaging to exclude structural abnormalities and to co-register to the PET scan to allow for partial volume correction (PVC). Magnetic Resonance Imaging (MRI) was performed on a 3T Trio (Siemens Medical Systems, Erlangen, Germany) with a circularly polarized head coil. MR acquisition consisted of a

Sag 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence with 3.34 ms echo time, 2500 ms repetition time, 1100 ms in-version time, 7° flip angle, and 180 Hz/pixel bandwidth. Images are 256 × 256 × 176 with a pixel size of 0.98 × 0.98 × 1.0 mm.

2.3. Positron emission tomography

Participants underwent positron emission tomography (PET) scans after initiation of a bolus plus constant infusion protocol (K_{bol} = 190 min) (Park et al., 2015) and up to 185 MDq of [^{18}F]FPEB was administered intravenously. A dynamic PET scan was performed on the HRRT (207 slices, resolution $<$3 mm FWHM), the highest resolution human PET scanner (de Jong et al., 2007). Data from 90–120 min were used for analysis.

2.4. Reconstruction and Co-registration

PET images were reconstructed using the MOLAR algorithm (Carson et al., 2003) with correction for motion using an optical detector (Vicra; NDI Systems, Waterloo, Canada) (Jin et al., 2013) as well as the FSL-FLIRT mutual-information algorithm and a summed template image taken from either 60 to 70 min or 90 to 100 min of scanning. Each participant's MRI was registered to the summed motion-corrected template PET image.

2.5. Gray matter masking and partial volume correction

MR images were segmented into GM, white matter (WM), and cerebrospinal fluid (CSF) using Statistical Parametric Mapping version 12 (SPM12; Wellcome Trust Centre for Neuroimaging). PVC was performed in participant space according to previously described procedures (Mecca et al., 2017). Binary mask images of GM and WM were created in participant space and smoothed to the system resolution (~3 mm). For each dynamic PET frame, GM voxels were corrected for spill-in and spill-out of activity, assuming activity in CSF was zero and WM activity was uniform and was estimated from each image time frame.

2.6. Regions of interest

ROIs were obtained based on the Automated Anatomical Labeling for SPM2 (AAL) template (Tzourio-Mazoyer et al., 2002), with non-linear transformation of the MRI to a template MRI using BioImage Suite(version 2.5; www.bioimagesuite.com). for the primary analyses, using the published list of AAL regions, a hippocampal ROI was defined to include bilateral regions 42–43. The composite association cortical ROI comprised bilateral frontal regions 3–16, parietal regions 59–62, temporal regions 81–82, 85–86, 89–90, and occipital regions 49–54 (See Supplementary Methods).

2.7. Tracer kinetic modeling

Distribution volume (V_T) was computed using a venous or arterial input function and equilibrium modeling from 90 to 120 min. Use of either venous or arterial data was based on the initial study protocol, and in cases for which both were available, venous data was used since previous studies show excellent agreement between venous and arterial input functions (Park et al., 2015).The blood data input function was obtained from the time-activity curve of the metabolite-corrected concentration of [^{18}F]FPEB, with blood samples taken

immediately prior to the scan and at predetermined intervals during the 60 to 120 min period for each study. V_T was calculated as the average concentartion of tracer in tissue divided by the average concentration in plasma, for the 90 to 120 min interval post-injection, based on the assumption of equilibrium during this time frame. Parametric images of V_T were generated for non-masked data and after gray matter masking and PVC.

2.8. Statistical analysis

SPSS version 24.0 (IBM Corp.) and Matlab R2018a (Mathworks, Inc.) were used to perform statistical analyses. The association of participant characteristics and injected dose with age were examined using unpaired *t*-tests or Pearson's correlation. To reduce the probability of Type 1 error due to multiple comparisons, our primary analyses focused on hippocampus and a composite association cortical ROI (Supplementary Methods). Because the analysis of multiple brain regions in the same participant is a repeated measure, we utilized linear mixed models to assess the association between age and mGluR5 distribution volume (V_T) or gray matter fraction in hippocampus and association cortex (within-participant factor). A participant-specific random intercept was included. Unstructured, compound symmetry, and heterogenous variance-covariance structures were used with the best-fit determined by the Bayesian information criterion. Secondary analyses utilized a similar model with exploratory ROIs listed in Table 3. Post-hoc analyses were performed for each region using univariate linear regression and Pearson's correlation with age as an independent variable and regional V_T as the outcome. To evaluate the contribution of GM tissue alterations and partial volume effects to mGluR5 changes with age, similar models were used to assess the association between age and mGluR5 V_T for gray-matter masked and PVC data, as well as for gray matter fraction. In all cases, tests were 2-tailed and an alpha of 0.05 was used to define statistical significance.

3. Results

3.1. Participant characteristics

The study sample included 45 cognitively normal, non-smoking individuals of whom 57.8% were female (Supplementary Table 1). Demographic characteristics stratified by age group are displayed in Table 1. The mean age of participants was 48.53 ± 21.72 years (range 18–84 years) and did not differ between males (49.79 ± 23.00 years) and females (47.62 ± 21.16 years, $P = 0.744$). Input functions were derived from venous blood for 71.1% of participants and arterial blood for the remaining 29.9%. Ages significantly differed between participants with arterial (36.92 ± 13.67 years) and venous (53.25 ± 22.77 years, $P = 0.021$) derived input functions, which was expected since the studies with older participants did not include arterial sampling. Importantly, input function source was not associated with significant differences in V_T for either the association cortex (venous 24.81 ± 5.43, arterial 26.50 ± 7.84, $P = 0.413$) or hippocampus (venous 21.13 ± 4.91, arterial 24.63 ± 6.45, $P = 0.055$). Overall, the sample had a mean BMI 26.88 ± 4.85 kg/m^2 and a mean injected dose of 156.9 ± 38.5 MBq and neither BMI (Pearson's r = 0.200, $P = 0.188$) or injected dose (Pearson's r = 0.179, $P = 0.239$) was associated with age.

3.2. Associations between age and mGluR5 availability in the hippocampus and association cortex

Linear mixed model analysis, including age, region (hippocampus, composite association cortex), and the age*region interaction as predictors, demonstrated a significant effect of age ($F(1,43) = 5.3$, $P = 0.026$), but not age*region ($F(1,43) = 2.0$, $P = 0.160$) on V_T. *Post hoc* analyses showed that age had a significant negative association with mGluR5 availability (V_T) in the hippocampus, but not the association cortex (Table 2, Fig. 1A and 1D). mGluR5 availability in the hippocampus decreased by 4.5% per decade. When corrected for atrophy with gray matter masking, the average V_T for the entire sample increased by 18.7% in association cortex and by 11.3% in hippocampus, and mGluR5 availability was no longer associated with age ($F(1,43) = 2.7$, $P = 0.106$) in either region (Table 2, Fig. 1B and 1E). PVC further increased the average V_T for the entire sample by 49.6% for association cortex and by 25.4% for hippocampus, compared to the gray matter masked data. After PVC, mGluR5 availability was no longer associated with age ($F(1,43) = 0.18$, $P = 0.670$) in association cortex or hippocampus after PVC (Table 2, Fig. 1C and 1F).

3.3. Association between age and mGluR5 availability in exploratory ROIs

Exploratory analyses were performed to investigate the association between age and mGluR5 availability in multiple ROIs. The linear mixed model analysis, including age, region, and the age*region interaction as predictors, demonstrated a significant effect of age ($F(1,45) = 6.9$, $P = 0.012$), region ($F(17,765) = 59.4$, $P < 0.0001$), and age*region ($F(17,765) = 9.6$, $P < 0.0001$) on V_T. Age had a significant negative association with mGluR5 availability (V_T) in the lateral temporal, medial temporal, precuneus, anterior cingulum, posterior cingulum, insula, precentral gyrus, postcentral gyrus, cuneus, cerebellum, thalamus, and caudate ROIs (Table 3). When better accounting for possible atrophy with gray matter masking, a significant effect on V_T persisted for age ($F(1,45) = 4.1$, $P = 0.049$), region ($F(17,765) = 60.5$, $P < 0.0001$), and age*region ($F(17,765) = 3.9$, $P < 0.0001$). The association of age with mGluR5 availability remained significant in the anterior cingulum, insula, precentral gyrus, cuneus, cerebellum, thalamus, and caudate ROIs (Table 3). After PVC was performed, no significant effects of age remained on V_T ($F(1,45) = 0.26$, $P = 0.616$); however, region ($F(17,765) = 56.9$, $P < 0.0001$) and age*region ($F(17,765) = 2.7$, $P < 0.0001$) remained significant predictors of V_T. Further analysis of all AAL regions yielded similar results (Fig. 2, Supplementary Table 2), but indicated subregions of the cerebellum may have age associated declines in mGluR5 in the cerebellar crus that persist after corrections for volume loss and partial volume effects. In *post hoc* analyses, no significant associations between mGluR5 availability and age remained in exploratory ROIs (Table 3). Because a previous human PET study using [^{18}F]FPEB in a younger sample of participants (aged 22–66 years) found a significant decline in mGluR5 with age in the precentral gyrus, postcentral gyrus, cuneus, and inferiorlateral temporal lobe (Leurquin-Sterk et al., 2016), we performed *post hoc* exploratory analyses on the subset of participants who were aged ≤ 66 years (Supplementary Table 3 and Supplementary Table 4). Increased age was significantly associated with lower mGluR5 availability (V_T) in all regions except the hippocampus and thalamus. Most of these associations remained significant after gray matter masking and PVC. In a similar analysis in the participants aged > 66 years, mGluR5 availability was not associated with age (data not shown).

In a subset of 17 older adults aged 59–84 years who were recruited as CN controls for a study of Alzheimer's disease, the presence of neuropsychological testing afforded the opportunity for an exploratory investigation of the association between episodic memory and mGluR5 availability. These older participants were highly educated (17 ± 2 years, range 12–20) and had at least high average estimated premorbid intelligence (Wide Range Achievement Test 3 - reading subscale standard score 114 ± 7, range 92 to 120). In this sample, episodic memory was not associated with mGluR5 availability in the hippocampus ($r = 0.17$, $P = 0.52$ non-masked, $r = 0.24$, $P = 0.36$ GM masked, $r = 0.19$, $P = 0.46$ PVC) or the association cortex ($r = 0.11$, $P = 0.68$ non-masked, $r = 0.08$, $P = 0.77$ GM masked, $r = 0.10$, $P = 0.71$ PVC).

3.4. Associations between gray matter fraction and age

In a primary analysis of hippocampus and composite association cortex using a linear mixed model analysis, age ($F(1,45) = 47.4$, $P < 0.0001$), region ($F(1,45) = 357.4$, $P < 0.0001$), and age*region ($F(1,45) = 50.4$, $P < 0.0001$) were significant predictors of gray matter fraction. In an exploratory analysis of additional regions (Supplementary Table 2), age ($F(1,45) = 111.3$, $P < 0.0001$), region ($F(17,765) = 357.4$, $P < 0.0001$), and age*region ($F(17,765) = 15.7$, $P < 0.0001$) were significant predictors of gray matter fraction. Increasing age had a statistically significant association with gray matter fraction in all ROIs apart from the lateral parietal and lateral temporal regions (Supplementary Table 5). Gray matter fraction decline per decade ranged from 1.2% in composite association cortex to 8.1% in the caudate.

4. Discussion

This study investigated the relationship between age and [^{18}F]FPEB binding to brain mGluR5. In the primary analysis, increased age was significantly associated with lower mGluR5 availability in the hippocampus, which declined by 4.5% per decade, but not in the association cortex. When we evaluated the contribution of GM tissue loss and partial volume effects, we found that mGluR5 availability was no longer associated with age in either primary region. Exploratory analyses revealed multiple brain regions in which mGluR5 declined with advancing age. Many of these associations remained significant after GM masking, but none remained significant after PVC was performed. A reduced association of age with mGluR5 availability after atrophy correction suggests that this relationship is largely driven by tissue loss. Consistent with an atrophy mediated effect, older age was broadly associated with lower regional gray matter fraction.

4.1. Comparison with other human studies

PET has enabled the *in vivo* study of aging effects for a wide range of biomarkers, including glucose metabolism (Ishibashi et al., 2018), synaptic density (Michiels et al., 2021), and neuroreceptors (Wong et al., 1984). Previous human PET imaging studies that investigated the association between mGluR5 availability and age have yielded variable results. These studies have primarily used the mGluR5 ligand [^{11}C]ABP688 or [^{18}F]FPEB. The only dedicated aging study used [^{11}C]ABP688 in 31 healthy participants aged 20–77 years and found no association between age and mGluR5 in any brain region even prior to PVC of images (DuBois et al., 2016). In a study of brain mGluR5 availability with [^{11}C] ABP688

in depression, the control group (n = 11, age range 23–62 years), reported no significant regional associations between age and mGluR5 (Deschwanden et al., 2011). However, the analyses were restricted to brain regions with significant findings in depression and may have missed associations between age and mGluR5 availability in other regions (e.g. hippocampus). Another study used [^{11}C]ABP688 with the primary goal of assessing the relationship between brain mGluR5 availability and smoking in a group of young (average age ~ 37 years old) smokers, non-smokers, and ex-smokers (Akkus et al., 2013). In the entire sample, older age was significantly associated with lower mGluR5 availability in the putamen and left occipital lobe, but when groups were analyzed separately, only the smokers and ex-smokers showed a significant association between age and mGluR5 availability. This study did not correct for partial volume effects, so gray matter atrophy may have accounted for the association of mGluR5 with age. Finally, a study of novelty-seeking temperament using the mGluR5 ligand [^{18}F]FPEB in 44 healthy participants aged between 22 and 66 years found a significant negative correlation between age and mGluR5 availability in the precentral gyrus, postcentral gyrus, cuneus, and inferiorlateral temporal lobe (Leurquin-Sterk et al., 2016). Interestingly, our *post hoc* analysis for comparison to this study found similar results in participants ≤ 66 years, in whom age was significantly associated with lower mGluR5 availability (V_T) in many ROIs, but not the hippocampus. These associations remained significant after gray matter masking and PVC. This exploratory analysis raises the possibility of a nonlinear relationship of mGluR5 availability and age across the lifespan, in which younger adults may have a higher rate of mGluR5 decline per unit tissue volume that plateaus in older adults. However, our results may also be influenced by sampling bias, with the loss of an aging effect when supranormal older participants of high education and intelligence are included in the analysis (See Limitations).

Only one previous human *post mortem* study has examined the association between age and mGluR5 expression. Tsamis et al. used immunohistochemistry to quantify mGluR5, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the caudate nucleus (Tsamis et al., 2013) in individuals aged 17–86 years. mGluR5, but not NMDA or AMPA receptors, were expressed on more neurons in older (80%) compared to younger (40%) healthy individuals. This was also true for older individuals with AD in whom mGluR5 was expressed in 92% of caudate neurons. The authors hypothesized that increased mGluR5 expression with age may reflect a compensatory response to synaptic loss to preserve excitability and function. In our study, mGluR5 in the caudate nucleus declined with age in the uncorrected, but not after correction for gray matter atrophy. The analysis by Tsamis *et al.* focused on the caudate nucleus and used a measure of relative expression (% positive neurons) that cannot differentiate between an increase in mGluR5 expression and a decrease in non-mGluR5 expressing neurons. Moreover, the immunohistochemical staining of mGluR5, which is largely cytoplasmic, may not reflect the same pool of receptors labeled by [^{18}F]FPEB. Therefore, differences between our findings and those of Tsamis et al. may related to measurement of different receptor pools.

4.3. Comparison with rodent studies

Several studies have explored associations between brain mGluR5 protein levels and age in rodents. Fang et al. investigated mGluR5 levels in wild type mice up to 16 months using [^{11}C]ABP688 PET imaging and ex vivo immunoblotting (Fang et al., 2017). Binding of [^{11}C]ABP688 to mGluR5 in the whole brain, striatum, hippocampus, or thalamus was not significantly different in older adult mice, compared to young adult mice. Conversely, immunoblotting suggested a decrease in mGluR5 levels with age. Hernandez *et al.* observed that older adult rats (22 months) compared to younger rats(aged 4 months) have lower mGluR5, mGluR3, and mGluR4 in the medial prefrontal cortex (Hernandez et al., 2018). Menard and Quirion provided evidence for a more complex relationship between age, memory, and mGluR5 expression in the hippocampus Menard and Quirion (2012). When older rats were stratified according or the presence or absence of memory impairment, the rats with memory impairment had significantly lower mGluR5 expression in CA1 compared to younger and unimpaired older rats. After training with the Morris Water Maze, mGluR5 expression in the impaired older rats increased to the levels of younger and unimpaired older rats. Our results of no significant association between age and hippocampal mGluR5 availability may be consistent with these findings considering that our group of healthy older adults are cognitively normal. In fact, our related work indicates that hippocampal mGluR5 availability is lower in cognitively impaired participants with AD. Although the human AD process is certainly different than that of impaired older rats without AD, there could be similar alterations in mGluR5 expression among processes that affect memory performance. Furthermore, assessing differential alterations in mGluR5 expression in humans after memory task training may be insightful. Additional studies, which do not phenotype by baseline memory performance, have shown that mGluR5 protein levels decrease with age. Using immunofluorescence, mGluR5 in CA3 of the hippocampus was significantly lower, but only with the specific comparison to 16-month-old mice that did not have lower mGluR5 than younger mice. Discrepancies between rodent studies may be due to heterogeneity in cognitive aging such that animals with preserved cognition have no significant changes in mGluR5. This may be similar to our finding in the entire cohort where age was not a significant predictor of brain mGluR5 availability.

4.4. Importance of accounting for atrophy and partial volume effects

A strength of our investigation is the comprehensive use of non-masked, gray matter masked, and partial volume corrected analyses - to account for changes in mGluR5 availability that may be due to gray matter volume loss. The importance of gray matter masking and partial volume correction is highlighted by our findings of a strong inverse association between age and gray matter fraction across many brain regions. It is also underscored by a wealth of previous studies that have reported a relationship between age and gray matter volume both cross-sectionally (Good et al., 2001; Jernigan et al., 1991; Salat et al., 2004; Sowell et al., 2003) and longitudinally (Resnick et al., 2003; Scahill et al., 2003; Storsve et al., 2014). The analyses that include gray matter masking and PVC likely provide better comparability to postmortem results and suggest that the decrease in mGluR5 may simply be mediated by age-related loss of gray matter. However, PVC introduces additional sources of variability due to measurement error associated with the correction procedure (Bettinardi et al., 2014). Non-masked analyses reflect the effects of aging on

absolute regional receptor numbers, which may better reflect excitatory glutamatergic activity in specific brain regions. Evidence for compensatory up-regulation of mGluR5 has been observed with age and neurodegeneration, perhaps associated with reorganization of neuronal connections (Tsamis et al., 2013). Overall, the presentation of all three forms of data optimally represents the effects of aging on mGluR5.

In aging analyses, the choice of PVC method is also an important consideration. Each PVC method makes different assumptions about tracer distribution, imaging physics, and reconstruction algorithms, and each has advantages and drawbacks (Erlandsson et al., 2012). One advantage of the Miiller-Gartner (MG) and other image-based methods over ROI-based methods is the ability to perform PVC for each voxel in the target regions. With the MG method (Muller-Gartner et al., 1992), all GM voxels are targeted, and the WM and CSF are treated as background regions. A limitation of the MG method is that it requires an accurate estimate of the WM regional activity and assumes uniform tracer uptake in WM. In addition, MG does not account for spill-in and spill-out between GM regions. Other image-based methods such as, 'iterative Yang' (IY) (Erlandsson et al., 2012) or region-based voxel-wise (RBV) (Thomas et al., 2011), provide a voxel-based PVC image, correct for PVE among GM regions, and correct for potential spill-in from CSF to GM. Of relevance to this analysis, PVC methods that exclude CSF may cause a loss in the power to detect age-related changes (Greve et al., 2016). Thus, approaches incorporating and comparing multiple methods of PVC may provide added insight into the robustness of correlation analyses between age and mGluR5.

4.5. Limitations

Our study has a number of important limitations. Because participants were enrolled from the normal control groups of several clinical protocols, potential sources of sampling bias must be considered. Among these is the possibility of supranormal aging in the oldest group who were highly educated with above average intelligence. The inclusion of these participants may tend to minimize brain aging effects and may account for the emergence of more aging effects when these participants are excluded. Moreover, the design is cross-sectional and although associations of receptor availability or gray matter volume with age are plausible age-related changes, longitudinal studies would be needed to confirm the age-related decline in mGluR5. Finally, because bias in V_T can result if equilibrium assumptions are not met, we investigated these possible effects by applying a tissue clearance correction to V_T estimates as previously validated Hillmer and Carson (2020) and again assessed the association between age and V_T in all primary and exploratory regions. This analysis revealed no evidence for associations between age and radioligand clearance rate, and V_T corrected for individual differences in tissue clearance correction did not substantively affect the primary results (data not shown).

4.5. Conclusion

Increased age was significantly associated with lower mGluR5 availability in the hippocampus, but not the association cortex. When we evaluated the contribution of GM tissue loss and partial volume effects, we found that mGluR5 availability was no longer associated with age in either region. Exploratory analyses revealed multiple brain regions

in which mGluR5 availability declined with advancing age, but none remained significant after PVC. These results suggest that age-related decline in mGluR5 availability is largely mediated by tissue loss. Further study is needed to define the regional pattern and temporal course of mGluR5 alterations with aging. Quantification of [^{18}F]FPEB binding to mGluR5 may expand our understanding of age-related molecular changes and the relationship with brain tissue loss.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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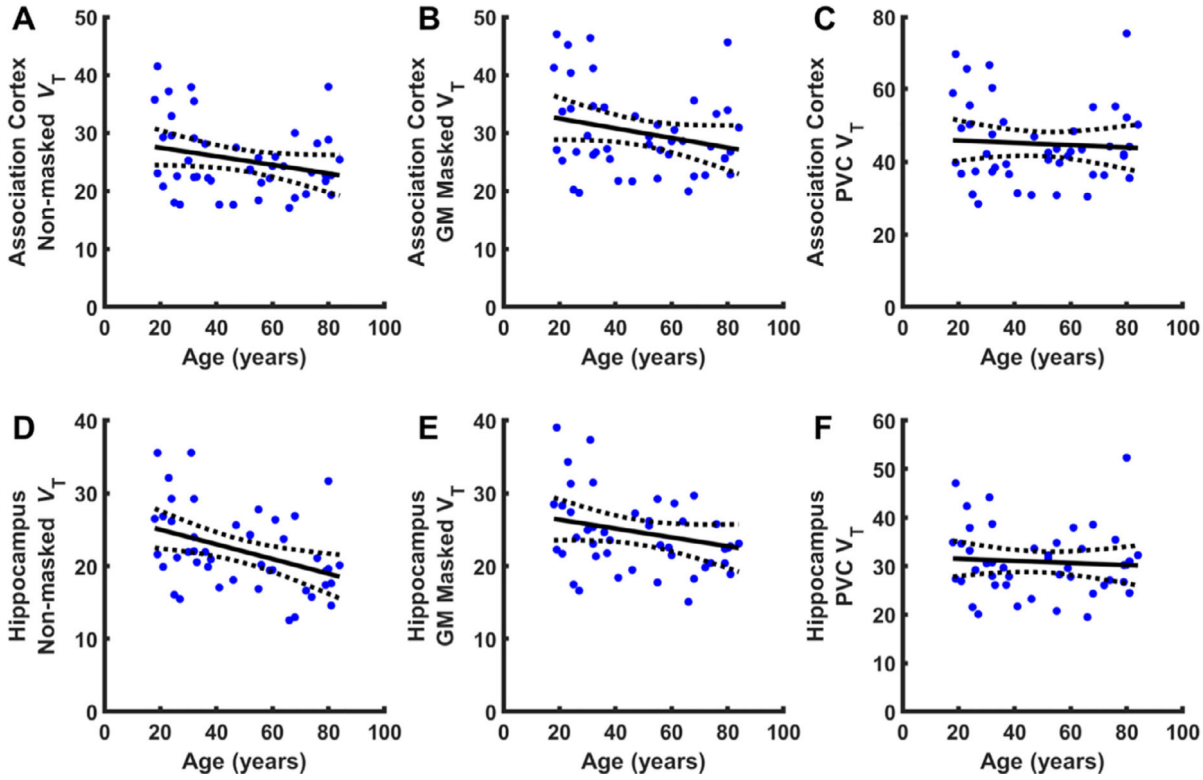

Fig. 1. Association between age and mGluR5 availability.

In the top row, scatter plots depict the association between age and volume of distribution (V_T) in association cortex for (**A**) non--masked (V_T = −0.074*age + 28.89, Pearson's r = −0.26, P = 0.085), (**B**) gray matter-masked (V_T = −0.083*age + 34.08, Pearson's r = −0.246, P = 0.103), and (**C**) partial volume corrected (V_T = −0.032*age + 46.49, Pearson's r = −0.063, P = 0.682) data. In the bottom row, scatter plots depict the association between age and V_T in the hippocampus for (**D**) non-masked (V_T = −0.10* age + 27.03, Pearson's r = −0.394, P = 0.007), (**E**) gray matter-masked (V_T = −0.061*age + 27.59, Pearson's r = −0.231, P = 0.126), and (**F**) partial volume corrected (V_T = −0.021*age + 31.95, Pearson's r = −0.067, P = 0.663) data. Linear regression lines are plotted with their 95% confidence intervals. GM = gray matter, PVC = partial volume corrected

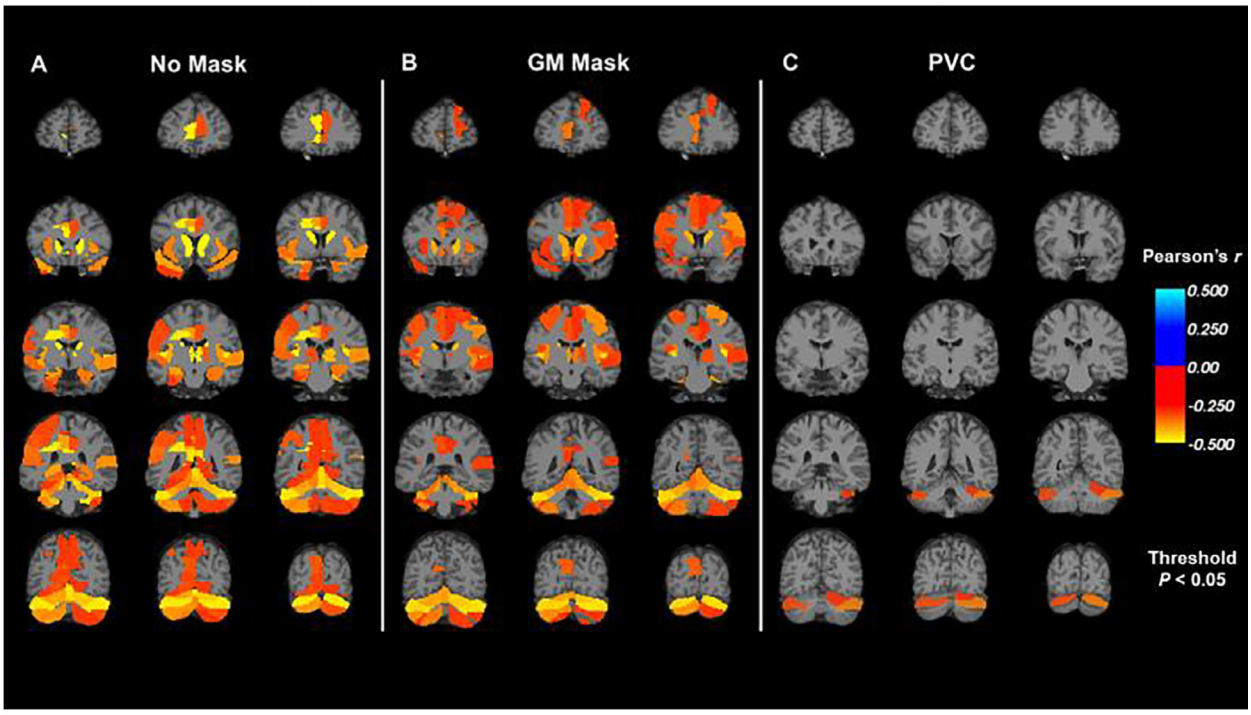

Fig. 2. Correlation maps of age and mGluR5 in all regions.

Pearson's *r* was calculated for the correlation between age and mGluR5 availability in all AAL atlas regions for (**A**) non-masked, (**B**) gray matter masked, and (**C**) partial volume corrected [^{18}F]FPEB binding to mGluR5 (V_T). For these analyses, Pearson's r (effect size) maps were created by producing images with the voxels in each region set uniformly to the calculated effect size for that region. The color scale represents Pearson's *r*, which is displayed only for regions that had an uncorrected $P < 0.05$.

Table 1

Participant characteristics.

	Age (years)								
	<20	**21–30**	**31–40**	**41–50**	**51–60**	**61–70**	**71–80**	**81–90**	***P***
Count	3	9	8	3	7	5	7	3	
Sex (Male:Female)[a]	1:2	4:5	5:3	0:3	2:5	3:2	3:4	2:1	0.744
Blood Data Source (Venous:Arterial)[a]	1:2	7:2	5:3	0:3	4:3	5:0	7:0	3:0	0.021[c]
BMI (kg/m2)[b]	26.19± 3.40	25.29 ± 3.39	28.03 ± 2.95	25.25 ± 4.72	26.30 ± 3.37	27.13 ± 6.61	29.32 ± 8.24	26.21 ± 6.13	0.188
Injected Dose (mBq)[b]	139 ± 31	171 ± 24	130 ± 21	160 ± 26	144 ± 30	178 ± 2	161 ± 34	180 ± 2	0.239

BMI = body mass index

[a]Categorical variables are reported as frequencies by age group. *P* values are for an unpaired t-test for mean age in male and female groups or venous and arterial input function groups.

[b]Continuous variables are reported as mean ± standard deviation by age group. *P* value is for Pearson's correlations with age.

[c]$P < 0.05$

Table 2

Associations between age and mGluR5 availability in primary regions of interest.

	Non-maskedv			Gray Matter Masked			Partial Volume Corrected		
ROI	**V_T (mL/cm^3)**	**Pearson's r**	***P***	**V_T (mL/cm^3)**	**Pearson's r**	***P***	**V_T (mL/cm^3)**	**Pearson's r**	***P***
Association Cortex	25.30 ± 6.18	−0.26	0.085	30.04 ± 7.35	−0.246	0.103	44.94 ± 11.02	−0.063	0.682
Hippocampus	22.14 ± 5.56	−0.40 [a]	0.007	24.65 ± 5.70	−0.231	0.126	30.90 ± 7.03	−0.067	0.663

Data are represented as mean ± standard deviation. ROI = region of interest, V_T = volume of distribution

[a] $P < 0.05$, *P* values from Pearson's correlations uncorrected for multiple comparisons in different regions.

Table 3

Associations between age and mGluR5 availability in exploratory ROIs

	Non-masked			Gray Matter Masked			Partial Volume Corrected		
ROI	**V_T (mL/cm^3)**	**Pearson's r**	***P***	**V_T (mL/cm^3)**	**Pearson's r**	***P***	**V_T (mL/cm^3)**	**Pearson's r**	***P***
Amygdala	26.20 ± 6.23	−0.261	0.083	27.45 ± 6.45	−0.194	0.202	30.55 ± 7.03	−0.098	0.521
Anterior Cingulum	27.61 ± 7.27	−0.432 [a]	0.003	31.71 ± 7.91	−0.319 [a]	0.032	44.14 ± 10.83	−0.082	0.591
Caudate	24.14 ± 7.73	−0.642 [a]	<0.0005	29.48 ± 7.47	−0.429 [a]	0.003	37.79 ± 9.03	−0.166	0.276
Cerebellum	9.64 ± 2.66	−0.406 [a]	0.006	10.80 ± 3.01	−0.380 [a]	0.010	13.37 ± 3.57	−0.230	0.128
Cuneus	23.64 ± 5.94	−0.299 [a]	0.046	26.26 ± 6.54	−0.295 [a]	0.049	41.69 ± 10.15	−0.069	0.653
Frontal	25.65 ± 6.33	−0.288	0.055	30.62 ± 7.61	−0.279	0.063	45.50 ± 11.10	−0.065	0.671
Insula	28.90 ± 7.38	−0.402 [a]	0.006	30.87 ± 7.62	−0.354 [a]	0.017	42.14 ± 10.00	−0.153	0.315
Lateral Parietal	24.99 ± 6.32	−0.281	0.061	29.56 ± 7.36	−0.259	0.086	46.21 ± 11.44	−0.121	0.428
Lateral Temporal	26.73 ± 6.72	−0.318 [a]	0.033	30.52 ± 7.52	−0.231	0.126	43.75 ± 10.83	−0.063	0.683
Medial Temporal	23.19 ± 5.75	−0.375 [a]	0.011	25.86 ± 6.07	−0.246	0.103	32.73 ± 7.52	−0.062	0.687
Occipital	23.80 ± 5.74	−0.268	0.075	27.11 ± 6.53	−0.257	0.088	39.89 ± 9.66	−0.016	0.915
Paracentral Lobule	19.65 ± 5.01	−0.264	0.080	22.95 ± 5.89	−0.252	0.095	37.67 ± 9.26	−0.078	0.612
Postcentral Gyrus	22.13 ± 5.55	−0.303 [a]	0.043	26.27 ± 6.50	−0.253	0.094	43.64 ± 10.89	−0.091	0.551
Posterior Cingulum	19.11 ± 5.15	−0.478 [a]	0.001	24.05 ± 6.23	−0.282	0.060	35.17 ± 9.06	−0.026	0.867
Precentral Gyrus	20.65 ± 5.52	−0.372 [a]	0.012	25.49 ± 6.74	−0.361 [a]	0.015	40.38 ± 10.00	−0.128	0.401
Precuneus	24.79 ± 6.18	−0.308 [a]	0.040	28.04 ± 6.80	−0.272	0.071	42.41 ± 10.08	−0.079	0.606
Putamen	27.33 ± 6.35	−0.186	0.222	32.07 ± 7.00	−0.115	0.450	42.43 ± 9.72	−0.189	0.215
Thalamus	17.55 ± 3.96	−0.382 [a]	0.010	21.41 ± 4.68	−0.301 [a]	0.044	28.53 ± 5.95	−0.076	0.620

Data are represented as mean ± standard deviation. ROI = region of interest, V_T = volume of distribution

[a] $P < 0.05$ for Pearson's correlation; uncorrected for multiple comparisons.