

## Brain Iron Content and Smoking History in Healthy Older Individuals

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**Introduction:** While cigarette smoking has been demonstrated to increase iron accumulation in the lungs<sup>1,2</sup> and significantly alter systemic iron metabolism,<sup>2,3</sup> little is known about the relationship between smoking and brain iron accumulation. Magnetic Resonance Imaging (MRI) provides an in vivo method to assess brain iron content indirectly by measuring the water proton transverse relaxation rate constant ( $R_2$ ), which varies linearly with iron concentration. This study utilized  $R_2$  measurements from the MRI scans of 81 healthy elderly individuals to investigate the relationship between smoking history and MRI measures of iron content within basal ganglia structures.

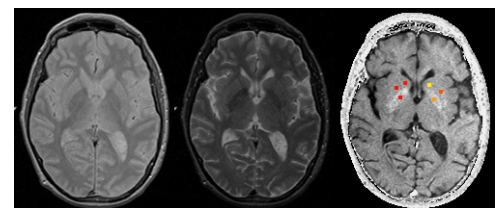
**Methods:** MRI scans and de-identified subject data were acquired through the Oregon Brain Aging Study (OBAS). Eighty-nine healthy subjects, independently living and cognitively intact, aged 62-102 y, who had comparable MRI scans and information about smoking history were identified. Of these, 51 subjects (18 men, 33 women) reported smoking fewer than 100 cigarettes in their lifetime and were classified as nonsmokers. Thirty-two subjects (16 men and 16 women) were identified as having a positive smoking history, including two individuals who continued to smoke and 30 exsmokers that had at least a 5 year period of regular smoking in their lifetime. These subjects were dichotomized into "long-term smokers" (n=16) and "short-term smokers" (n=16) using a median split of their years smoking. Six subjects who either smoked primarily cigars or pipes or smoked more than 100 cigarettes but for fewer than 5 years were excluded from the analysis. A Fast Spin Echo Double Echo sequence (FOV=24x24x16 cm, matrix=256x256x40, TE=32, 80ms, TR=3s) was used to generate voxelwise  $R_2$  maps using a mono-exponential decay function. Square regions of interest (14 mm<sup>2</sup>) were placed bilaterally in the caudate, putamen and pallidum (see **Figure 1**). The effect of smoking history on  $R_2$  values was analyzed with analysis of variance, followed by Tukey's HSD to compare individual groups. Additional linear models adjusting for sex and age were also analyzed. Pack year estimates were determined in 29 of the subjects with positive smoking history and additional correlations were run between ranked pack year estimates and  $R_2$  values.

**Results:** The groups did not differ in age ( $F(2,80)=0.47$ ,  $p=0.63$ ) or gender distribution ( $\chi^2(1)=1.76$ ,  $p=0.42$ ). Significant main effects of smoking history were found bilaterally in the caudate (Left:  $F(2,80)=4.54$ ,  $p=0.014$ ; Right:  $F(2,80)=6.24$ ,  $p=0.0030$ ) and putamen (Left:  $F(2,80)=6.85$ ,  $p=0.0018$ ; Right:  $F(2,80)=8.58$ ,  $p=0.00042$ ), but not in the pallidum. Post hoc analyses revealed that "long-term smokers" (>23 years of smoking) had higher  $R_2$  values than both "short-term smokers" (5-22 years) and nonsmokers in these regions (**Figure 2**). Linear models adjusting for age and gender resulted in similar main effects of smoking status for the caudate and putamen, with "long-term smokers" displaying higher  $R_2$  values compared to the other groups. Small age effects were seen in left pallidum ( $F(1,79)=5.14$ ,  $p=0.026$ ) and left caudate ( $F(1,79)=4.73$ ,  $p=0.033$ ) with increasing age associated with decreasing  $R_2$  values. No gender effects were detected. Positive correlations were found between ranked pack years and  $R_2$  values in the right and left caudate ( $r(27)=0.45$ ,  $p=0.015$ ;  $r(27)=0.48$ ,  $p=0.009$ ), respectively). Similar positive correlations were seen in the right ( $r(27)=0.36$ ,  $p=0.053$ ) and left putamen ( $r(27)=0.30$ ,  $p=0.12$ ) and in the left pallidum ( $r(27)=0.30$ ,  $p=0.11$ ), but these failed to reach significance.

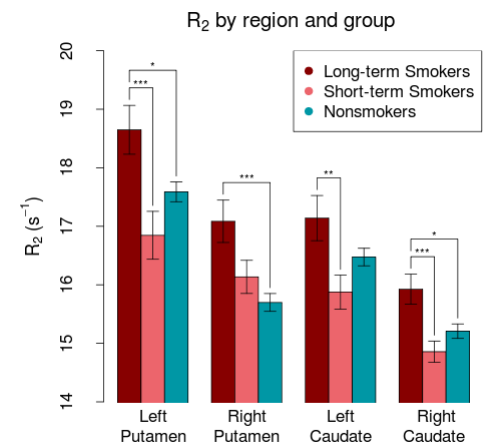
**Discussion and Conclusions:** Individuals with "long-term" smoking histories had increased  $R_2$  values in the caudate and putamen compared to aged-matched nonsmokers and individuals with short-term smoking histories. Increased  $R_2$  values were correlated with pack year history in the caudate. In the aging brain, there are two major changes in tissue composition that affect  $R_2$  measures: increased water content which decreases  $R_2$  and increased iron content which increases  $R_2$ . While both of these processes could contribute to the current results, it seems unlikely that smoking would be associated with decreased tissue water content in these regions, as smoking has been repeatedly associated with increased brain water content in large white matter regions.<sup>4</sup> This suggests that our results are consistent with the hypothesis that smoking cigarettes increases lifetime brain iron accumulation in basal ganglia structures in a dose dependent manner. If verified and replicated, these findings would complement previous studies demonstrating regional and systemic alterations in iron metabolism in smokers<sup>1,2,3</sup> as well as findings in rodent models showing increased brain iron levels in response to cigarette smoke exposure.<sup>5</sup> Increased brain iron levels have been associated with age-associated cognitive impairment and risk of dementia.<sup>6,7,8</sup> The current study suggests that cigarette smoking increases brain iron content and this effect may provide some insight into the associations of smoking and cognitive decline in old age.<sup>9</sup>

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**Figure 1:  $R_2$  maps and region of interest placement.** Examples of the proton density (left) and  $T_2$  weighted (middle) images that were used to generate  $R_2$  maps (right). Regions of interest were placed bilaterally in the caudate, putamen and pallidum.



**Figure 2: Effect of smoking history on striatal  $R_2$  values.** \*  $p<0.05$ ; \*\*  $p<0.01$ ; \*\*\*  $p<0.005$