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Investigating the impact of midlife obesity on Alzheimer's disease (AD) pathology in a mouse model of AD

The rising prevalence of Alzheimer's disease (AD), the most common form of dementia, distinguishes it as a worldwide healthcare concern. Recent epidemiological studies have identified modifiable lifestyle features such as diabetes, mid-life hypertension, mid-life obesity, smoking, late-life depression, low education, and physical inactivity as significant risk factors for AD (Barnes and Yaffee, 2011). Importantly, an accumulation of evidence has recognized that midlife obesity, in particular, can increase the risk of AD later in life, independently of other risk factors (Whitmer et al., 2007). Animal studies using a high-fat diet to induce obesity have found increases in amyloid-beta protein and soluble tau (Vandal et al., 2014; Barron et al., 2014; Julien et al., 2010), suggesting that obesity may accelerate the brain pathological changes implicated in AD. However, these findings are not consistent between groups, with some studies reporting no change in levels of amyloid-beta or tau (Knight et al., 2014). Moreover, no animal studies have used magnetic resonance imaging (MRI) to assess structural changes in the brain associated with obesity and AD. Since brain structural integrity, as measured by brain structure volumes, is a reasonable index of neurological health, it is vital to characterize the brain structural changes caused by obesity in order to clarify how obesity in midlife increases risk for AD later in life.

To elucidate the interplay between midlife obesity and AD-related neuropathophysiology, we evaluated the impact of midlife obesity on the brain morphology of the triple transgenic mouse model of AD (3xTg; harbouring mutations leading to both amyloid and tau accumulation) and a non-transgenic (non-Tg) control model of the same background strain (C57BL6/129s) using longitudinally acquired MRI. With regards to the current body of research, we

hypothesized that midlife obesity would accelerate the structural changes associated with AD and impair memory in 3xTg-AD and non-Tg control mice.

At 2 months of age, 3xTg and non-Tg animals were placed on either a high-fat diet (HFD) (3xTg-HFD n = 7; B6129s-HFD n = 4) or an ingredient-equivalent control diet (CD) (3xTg-CD n = 10; B6129s-CD n = 4) and were maintained on the diet for the remainder of the experiment. Animals were weighed daily for the first 5 days following dietary change to ensure that they were able to adhere to the new diet and were weighed weekly for the remainder of the experiment. Animals were scanned with MRI at 2 months, 4 months (midlife), and 6 months. After the third scan, animals underwent the novel object recognition (NOR) task and the Morris Water Maze (MWM) for assessment of short-term non-associative memory and spatial memory, respectively.

To assess whether HFD-induced obesity was associated with brain volume deficits in 3xTg-AD mice, MRI images were segmented into 159 structures per hemisphere using the Multiple Automatically Generated Templates (MAGeT) brain segmentation algorithm (Chakravarty et al., 2013; Pipitone et al., 2014) and volumes of brain structures were then extracted from these segmentations. Deformation-based morphometry (DBM) was also used to assess longitudinal voxel-wise changes in brain volume in all subjects. All measures were analyzed with linear mixed effects models to assess group differences in the rates of volumetric changes in MRI-derived brain morphology. Correction for multiple comparisons was carried out through the false discovery rate (FDR) technique.

We found that high-fat feeding induced robust effects of weight gain, regardless of genotype (Figure 1B). Results from the MWM and NOR tasks showed that the triple transgenic mice on the HFD had significantly impaired memory in comparison to non-Tg control mice on the CD (Figure 1C, D). For the brain volumetric and deformation-based morphometric results, a significant interaction between group and time point was observed for widespread brain regions, such as the hippocampus, entorhinal cortex, amygdala, thalamus, olfactory bulbs, and parietal

cortex. DBM results showed that high-fat feeding in the triple transgenic mouse model initially increases local brain volume by midlife, but dramatically decreases local brain volume by late life (Figure 1E). Notably, these decreases are more significant than decreases caused by genotype or diet alone, suggesting that it is only the additive combination of diet and AD-like pathology that results in the more dramatic and widespread atrophy seen in the triple transgenic high-fat fed mice. Taken together, our results suggest that midlife obesity may interact with the brain changes associated with AD-related pathology in the 3xTg mouse model to mediate alterations in the neuroanatomical trajectories of select brain regions and impair memory.

Though we show here that HFD-induced obesity alters the pathological changes associated with AD at the gross morphological level, an important limitation of our work is that no conclusions can be drawn concerning the fine-grained cellular changes that may mediate this neuroanatomical remodelling. Though such a mechanism remains unknown, several have been proposed, including insulin resistance, oxidative stress and chronic inflammatory processes (Freeman et al., 2013; Kang et al., 2017).

Overall, we found that obesity at midlife interacts with AD-like progression in the 3xTg mouse model of AD to mediate alterations in neuroanatomy that were associated with memory impairments in short-term non-associative and spatial memory. Since brain structural integrity is a useful indicator of neurological health, our work adds an important dimension to the portrait of a potentially synergistic relationship between midlife obesity and risk for AD. Ultimately, knowledge obtained from our study will lead to a better understanding of the pathological correlates of midlife obesity as a risk factor for AD, which will in turn help the development of preventative strategies such as diet and exercise for reducing the risk of developing Alzheimer's.

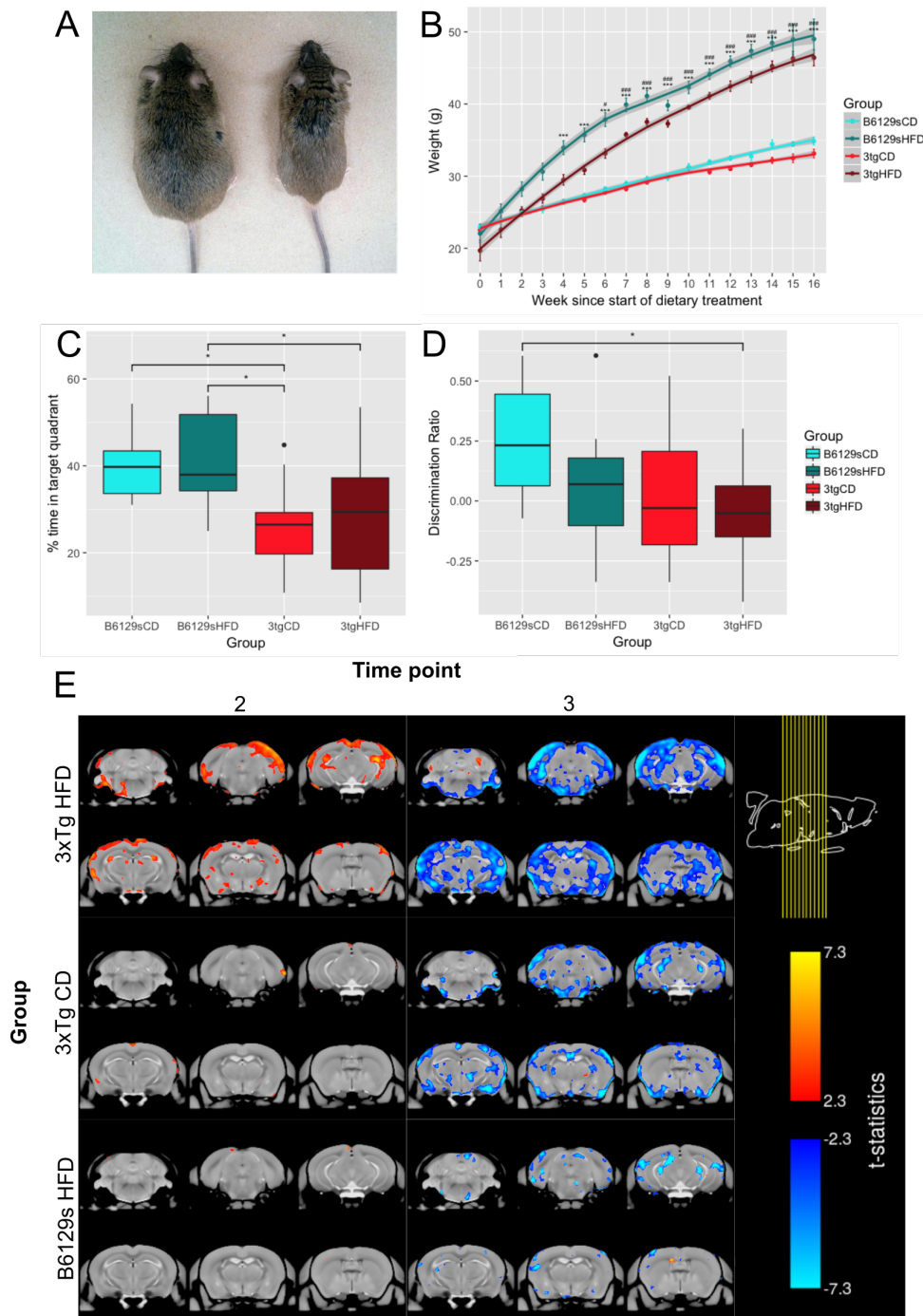


Figure 1. (A) 3xTg mice at midlife (4 months) on the HFD (left) and CD (right). (B) Mice maintained on the HFD had greater body weights than mice fed the CD from week 6 on the diet onwards ($p < 0.01$). (C) Discrimination ratios on the NOR task. Impaired performance in novel object recognition was observed in 3xTg mice fed the HFD in comparison to B6129s mice on the CD ($p < 0.05$). (D) Percent time in the target quadrant during the MWM probe trial. 3xTg-CD mice performed worse than all B6129s mice and 3xTg-HFD mice showed impaired memory compared to B6129s-HFD mice ($p < 0.05$ for all). (E) Visualization of the interaction of group by time point on local brain volume (FDR < 5%). 3xTg mice maintained on the HFD undergo significant increases in local brain volume in comparison to B6129s mice fed a CD from adolescence to midlife, followed by dramatic and widespread decreases from mid to later life.

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