● **PERSPECTIVE**

The impact of vitamin D deficiency on neurogenesis in the adult brain

There is increasing evidence from epidemiological studies indicating that vitamin D deficiency during adulthood is associated with adverse brain outcomes in humans (Ginde et al., 2009) and rodents (Groves et al., 2014), however, a causal relationship has not yet been established. We have previously shown in a mouse model that vitamin D deficiency during adulthood impacts on a range of brain functions (Groves et al., 2013) and should therefore be considered as a biologically plausible risk factor for the development of neuropsychiatric and neurodegenerative disorders.

Vitamin D is known to be anti-proliferative and have pro-differentiation effects, as shown by the addition of 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) to cultures of normal and malignant cell lines (Banerjee and Chatterjee, 2003). Moreover, vitamin D has been shown to regulate a variety of neurotrophic factors, including nerve growth factor (NGF), providing further evidence for its ability to influence neuronal proliferation, differentiation, survival and growth. Vitamin D plays an important role in development on cell proliferation, differentiation and apoptosis. For example, 1α-hydroxylase knockout mice that lack the ability to make the active form of vitamin D (1,25$(OH)_2D$) showed increased cell proliferation in the hippocampal dentate gyrus, a reduction in the survival of the newborn neurons and increased apoptosis in adult mice (reviewed in Groves et al., 2014). Following on from this support for vitamin D's role in neuronal cell proliferation, differentiation and apoptosis, we designed a study to test the effects of adult vitamin D (AVD) deficiency on hippocampal neurogenesis in BALB/c mice and we proposed that AVD deficiency would lead to increased proliferation and decreased survival of adult born hippocampal neurons (Groves et al., 2016). This was assessed by measuring the number of $Ki67^+$ cells as a marker of cell proliferation, DCX^+ cells as a marker of immature neurons, and $BrdU^+$/$NeuN^+$ cells as a marker of newborn mature neurons surviving in the dentate gyrus. Newborn mature neurons were measured at baseline and following voluntary wheel running, which is known to stimulate levels of neurogenesis. Furthermore, we proposed that behavioural outcomes following AVD deficiency would correlate with altered neurogenesis, by exploring the effect of wheel running on immobility in the forced swim test (FST), and assessing the correlation between neurogenesis and immobility time (Groves et al., 2016). The main finding from this study was that AVD deficiency did not affect proliferation or survival of adult hippocampal neurons at baseline or following voluntary wheel running, and did not alter the number of immature neurons following behavioural testing either. The study did show that voluntary wheel running reduced immobility time in the FST as expected, but that AVD deficiency also led to a reduction in immobility time. In control mice, immobility time was significantly correlated with the number of $BrdU^+$/$NeuN^+$ cells, however there was no such correlation in AVD-deficient mice. Overall, the study provides some evidence that adverse brain-related outcomes previously associated with AVD deficiency may not be mediated *via* altered rates of proliferation or survival of new hippocampal neurons in BALB/c mice.

Although AVD deficiency did not impact on these specific measures, there is evidence that AVD deficiency may instead impact on the function of the newly generated cells. For example, the extent of branching of immature neurons, differentiation, and protein expression within neurons may all be impacted by AVD deficiency, as well as neurotransmission between neurons and synaptic plasticity of neurons (Groves et al., 2013, 2016), although future studies need to address these issues.

We have previously shown that AVD deficiency in male mice leads to reduced glutamate and glutamine levels and increased gamma-amino butyric acid (GABA) levels, measured by high-performance liquid chromatography (HPLC) in whole brain; and a small but significant reduction in the GAD65/67 enzymes in whole brain, measured by western blot (Groves et al., 2013). Studies in knockout mice have shown that ablation of GAD67 results in neonatal lethality and reductions in GABA levels to 7% of wildtype mice. GABA is necessary during development to regulate neocortical neurogenesis. However, in GAD65 knockout mice, although there was no change to GABA levels, it was shown that GAD65 is not required for development but is important for modulating inhibitory neurotransmission in response to increased demand. GAD65 is localized to axon terminals and is reversibly bound to the membrane of synaptic vesicles. Further studies in the GAD65 knockout mice have shown that GAD65 mediates activity-dependent GABA synthesis but moreover, has a significant impact on GABA release during sustained activation, possibly through mobilization of vesicles or replenishment of vesicles at the synapse, reviewed by Muller et al. (2015). Therefore, it is feasible that the reduction in GAD65 protein expression associated with AVD deficiency could have an impact on GABAergic inhibitory neurotransmission.

AVD-deficient male mice were also shown to have mild cognitive impairments in attentional processes, measured using the 5 choice-serial reaction time (5C-SRT) task (Groves and Burne, 2016). Lesion studies of the cortico-striatal pathways involved in attentional processes have shown that damage to the medial prefrontal cortex (PFC), using quinolinic acid, replicates the findings in AVD-deficient mice with deficits in accuracy and lengthening of choice latencies (Robbins, 2002). Lesions of other cortico-striatal regions produce other deficits including impulsive responding or altered motivation, which we did not see with AVD-deficiency (Robbins, 2002).

Impairments in attentional processes have previously been shown to be associated with impairments in GABAergic inhibitory neurotransmission. For example, the phenotype seen with AVD deficiency in males is similar to that produced following an infusion of picrotoxin, a $GABA_A$ receptor antagonist that dose-dependently reduces GABA currents, into the medial PFC of rats. Picrotoxin infusion led to deficits in accuracy and omissions, without altering impulsive or motivational behaviours on the 5C-SRT task (Pezze et al., 2014).

In Sprague-Dawley rats tested on a modified 3C-SRT in lieu of the 5C-SRT task, using two different $GABA_A$ receptor antagonists, researchers showed consistent results with that of picrotoxin effects; reduced accuracy and increased omissions, with no other alterations (Pehrson et al., 2013). There is consistent evidence from these previous rodent studies that disruption of GABAergic neurotransmission within the medial PFC and other areas of the cortico-striatal pathways does impact on aspects of the 5C-SRT.

AVD deficiency was associated with reduced immobility time on the FST and this is usually indicative of a reduced depressive-like state, or learned helplessness. However, drugs that impact on glutamate neurotransmission have been shown to reduce immobility in the FST, similar to that observed in our study (Groves et al., 2016). Therefore, this result may be more suggestive of altered glutamatergic neurotransmission, than a specific antidepressant-related outcome and is consistent with previous findings

in AVD-deficient BALB/c mice (Groves et al., 2013).

As well as altered glutamatergic and GABAergic neurotransmission exhibited with AVD deficiency, previous *in vivo* and *in vitro* studies have shown that the addition of $1,25(OH)_2D$ regulates proteins involved in glutathione biosynthesis and leads to increased glutathione levels, reviewed by Groves et al. (2014). Oxidants are formed as a normal part of aerobic metabolism but can be formed at an accelerated rate during pathophysiological conditions or when the body's natural antioxidant mechanisms fail. Oxidative stress occurs when there is an imbalance between antioxidants and oxidants in favour of the oxidants, and if AVD-deficiency leads to a reduction in glutathione, it is likely that the AVD-deficient mice are exposed to chronic oxidative stress.

Oxidative stress can lead to cell membrane dysfunction, DNA damage and inactivation of proteins, eventually leading to cell death. It has been shown that people with mild cognitive impairment have higher levels of oxidative stress compared to healthy controls (Pratico et al., 2002). Furthermore, a study performed in rats showed that even in young animals (3 months of age), induction of oxidative stress led to impairments in spatial learning and memory (Fukui et al., 2002).

Induced oxidative stress, *via* exposure to 100% oxygen for 48 hours, produced an abnormal accumulation of synaptic vesicles in swollen nerve terminals and a reduction in neurotransmission (Fukui et al., 2002). In addition, following the induction of oxidative stress, synaptic membranes were shown to have elevated markers of oxidative stress including lipid peroxides and protein carbonyls, and a change in membrane surface potential. It was proposed that this would result in reduced membrane fusion between nerve terminal membranes and synaptic vesicles and produce the subsequent decline in neurotransmission shown previously (Fukui et al., 2002). Exposure to chronic oxidative stress with AVD deficiency could therefore result in the cognitive impairments seen in attentional processes using the 5C-SRT task (Groves and Burne, 2016).

There are currently no animal studies looking directly at the effects of oxidative stress on the 5C-SRT task. However, human studies have shown significant associations between cognitive impairments and cognitive decline with systemic oxidative stress and lower antioxidant status (Berr et al., 2000). Although many different tests are used to examine cognition in humans, the MMSE is used frequently, which measures global cognition including aspects of attention and hippocampal-dependent learning (Berr et al., 2000).

The active from of vitamin D, $1,25(OH)_2D$, is highly pleotropic with over 2,700 VDREs within the genome that are able to alter the transcription of wide range of genes. This most likely includes genes involved in glutamatergic and GABAergic neurotransmission, and calcium regulation, as well as neurotrophic factors and genes involved in neuroprotection such as γ-glutamyl transpeptidase and inducible nitric oxide synthase, as reviewed by Groves et al. (2014) and therefore, vitamin D deficiency may lead to a lack of neuroprotection. Previous studies have suggested that vitamin D deficiency may contribute to vulnerability to secondary insults or disorders, such as stroke or Parkinson's disease symptoms, which is consistent with a lack of neuroprotection, reviewed by Groves et al. (2014).

We propose that a combination of factors including increased oxidative stress, a reduction in neuroprotection, and dysregulation of a range of neurotrophic factors all contribute to the mild cognitive impairments seen with AVD deficiency as well as an increased vulnerability to adverse brain-related outcomes. Vitamin D deficiency may be impacting on a range of critical processes necessary for optimal brain function including neurotransmission, synapse formation and synaptic plasticity and dendritic arborisation. In conclusion, AVD deficiency does not impact on cell proliferation or the survival of newborn neurons within the adult hippocampus, providing a disparate finding compared to that during developmental vitamin D deficiency. However, exposure to vitamin D deficiency during adulthood in mice is sufficient to impair cognition and alter brain function, most likely by affecting pathways involved in neuroprotection and neurotransmission rather than a direct regulation of neuronal cell proliferation, differentiation and apoptosis.

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References

Berr C, Balansard B, Arnaud J, Roussel AM, Alperovitch A, Grp ES (2000) Cognitive decline is associated with systemic oxidative stress: The EVA study. J Am Geriatr Soc 48:1285-1291.

Fukui K, Omoi NO, Hayasaka T, Shinnkai T, Suzuki S, Abe K, Urano S (2002) Cognitive impairment of rats caused by oxidative stress and aging, and its prevention by vitamin E. Ann N Y Acad Sci 959:275-284.

Ginde AA, Liu MC, Camargo CA (2009) Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. Arch Intern Med 169:626-632.

Groves NJ, Burne TH (2016) Sex-specific attentional deficits in adult vitamin D deficient BALB/c mice. Physiol Behav 157:94-101.

Groves NJ, McGrath JJ, Burne TH (2014) Vitamin D as a neurosteroid affecting the developing and adult brain. Annu Rev Nutr 34:117-141.

Groves NJ, Kesby JP, Eyles DW, McGrath JJ, Mackay-Sim A, Burne TH (2013) Adult vitamin D deficiency leads to behavioural and brain neurochemical alterations in C57BL/6J and BALB/c mice. Behav Brain Res 241:120-131.

Groves NJ, Bradford D, Sullivan RK, Conn KA, Aljelaify RF, McGrath JJ, Burne TH (2016) Behavioural effects of adult vitamin D deficiency in BALB/c mice are not associated with proliferation or survival of neurons in the adult hippocampus. PLoS One 11:e0152328.

Muller I, Caliskan G, Stork O (2015) The GAD65 knock out mouse - a model for GABAergic processes in fear- and stress-induced psychopathology. Genes Brain Behav 14:37-45.

Pehrson AL, Bondi CO, Totah NK, Moghaddam B (2013) The influence of NMDA and GABA(A) receptors and glutamic acid decarboxylase (GAD) activity on attention. Psychopharmacology (Berl) 225:31-39.

Pezze M, McGarrity S, Mason R, Fone KC, Bast T (2014) Too little and too much: hypoactivation and disinhibition of medial prefrontal cortex cause attentional deficits. J Neurosci 34:7931-7946.

Pratico D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ (2002) Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch Neurol 59:972-976.

Robbins TW (2002) The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl) 163:362-380.