Touching BACE. How Inhibiting β-Amyloid Production by Steric Hindrance with a Novel Immunotherapy Improves Memory in PDAPP Mice

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Introduction

- We have developed a novel monoclonal antibody, 2B3, which targets APP at the β-secretase cleavage site, inhibiting Aβ production by steric hindrance(1)(Figure 1).
- The PDAPP mouse model of AD overexpresses a familial *APP* mutation is reported to show an age-dependent rise in levels of Aβ and cognitive deficits(2).
- Aβ has been observed to increase NMDA receptor phosphorylation leading to excitotoxicity and cognitive impairment in *hAPP* mouse models of AD(3) (Figure 2).

Figure 1

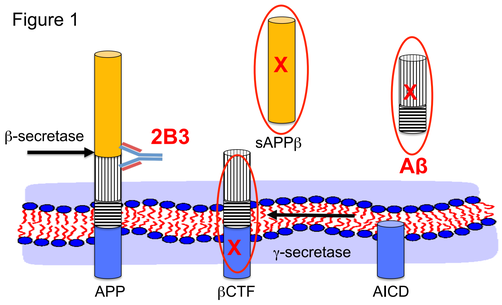


Figure 2

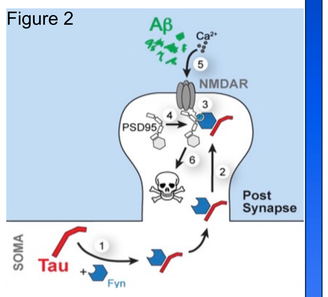


Figure 1. 2B3 binds APP at the β-secretase cleavage site, inhibiting cleavage of APP by β-secretase and preventing the production of Aβ.

Figure 2. Src kinase Fyn interacts with tau protein, translocating to the post synapse (1-2). Fyn phosphorylates the NMDA NR2B subunit, stabilizing its interaction with PSD95 and synaptic localisation (3-4). Aβ activates NMDA receptors and, following enhanced synaptic localisation of NR2B containing NMDARs, causes disproportionate influx of Ca^{2+} and excitotoxicity (5-6)(3).

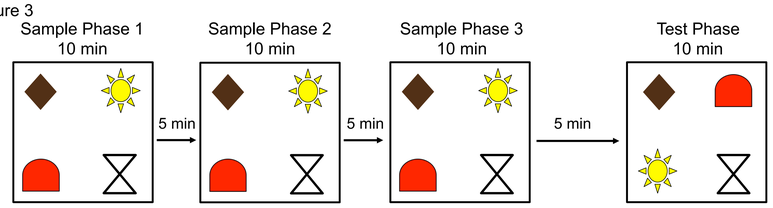
Aims

1. Assess whether intracerebroventricular (ICV) administration of 2B3 alleviated age-dependent memory deficits in PDAPP mice.
2. Determine whether 2B3 altered APP metabolism and NMDA receptor phosphorylation *ex vivo*.

Methods

- At 17-18 months of age PDAPP mice were administered 2B3 (n=10), at a concentration of 2mg/ml, or vehicle (n=11). WT mice were treated with vehicle (n=10) or received no treatment (n=11). 2B3 and vehicle were delivered via ICV administration using osmotic mini-pumps for a period of 14-days.
- On treatment days 13 and 14, object-in-place (OiP) memory (Figure 3) was assessed and discrimination ratios calculated to determine the effects of 2B3 *in vivo* on memory function.
- Immediately after behavioural training on day 14, mice were culled and the hippocampus was dissected and snap frozen. Soluble proteins were extracted and protein levels were quantified by Western blot and ELISA analysis.

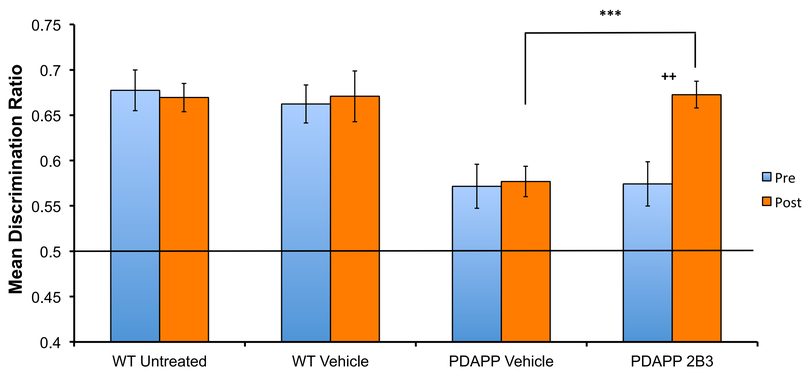
Figure 3



Discrimination ratios were calculated as follows:
Contact time with objects in novel locations / total contact time with objects

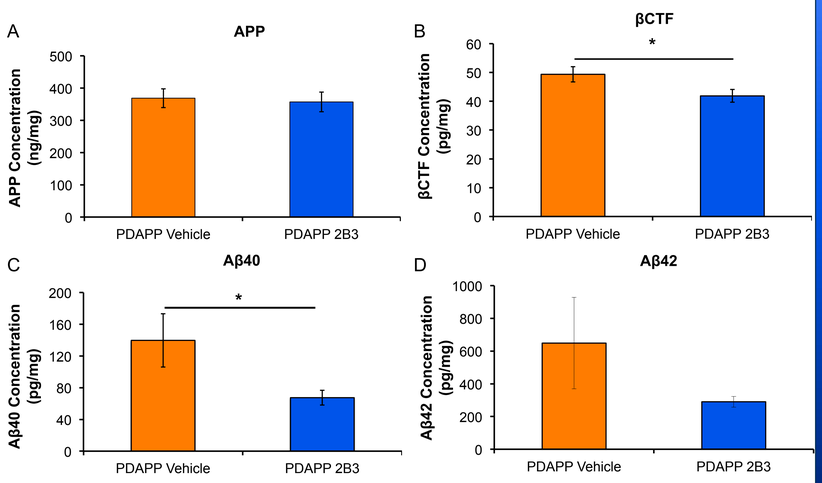
Results

Results 1: 2B3 reverses cognitive deficits in PDAPP mice



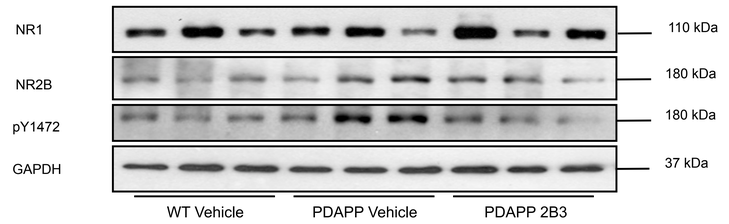
- PDAPP mice administered 2B3 showed a significant improvement in OiP memory performance when compared to vehicle treated PDAPP mice.
- PDAPP mice showed a significant improvement in OiP memory performance following 2B3 administration compared to pre-treatment performance scores.
- There were no significant differences between 2B3 treated PDAPP mice and either WT groups.
- Data were analysed using 3x2* ANOVA to determine 2B3 intervention on memory performance in PDAPP mice. (*Data were collapsed across both WT control groups as no effect of vehicle or minipump were observed (p>0.5)). ***p<0.001 (between-subject analysis), ##p<0.01 (within-subject analysis). Error bars show S.E.M.

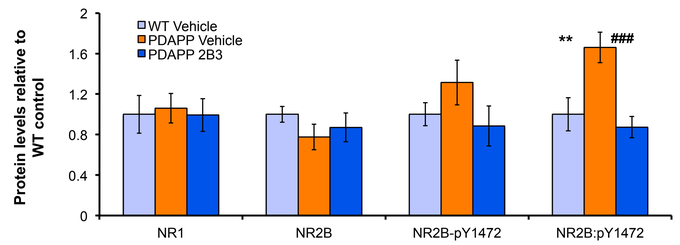
Results 2: 2B3 inhibits APP metabolism *ex vivo*



- PDAPP mice administered 2B3 showed a significant reduction in levels of soluble βCTF (B), Aβ40 (C), but not Aβ42 (D). Changes were not an effect of reduced APP (A).
- Protein levels were determined by ELISA. Data were analysed by independent samples t-test. *p<0.05 significant reduction in soluble Aβ40 and βCTF. Error bars show S.E.M.

Results 3: 2B3 reversed NR2B phosphorylation *ex vivo*





- No overall significant difference in total levels of NR1 (total NMDARs), NR2B or phospho-NR2B tyrosine 1472 (pY1472) were observed when comparing WT and PDAPP mice (p>0.1).
- However, when NR2B pY1472 was expressed as a ratio of total NR2B, 2B3 administered PDAPP mice showed a significant reduction in NR2B:pY1472 ratio compared to PDAPP vehicle treated mice.
- Protein levels were determined by Western blot. **p<0.01 significantly greater NR2B:pY1472 in PDAPP vehicle administered mice compared to WT vehicle controls and ###p<0.001 compared to PDAPP 2B3 administered mice. Error bars show S.E.M.

Summary & Conclusions

- Following ICV administration of 2B3 for 14-days, aged PDAPP mice showed a full recovery of OiP memory compared to vehicle treated PDAPP mice.
- *Ex vivo* tissue analysis showed evidence of reduced APP metabolism as determined by a significant reduction of βCTF and Aβ40.
- Further investigation revealed that vehicle treated PDAPP mice showed an increased phosphorylation of the NMDAR NR2B subunit when expressed as a ratio of total NR2B. This effect has previously been associated with excitotoxicity and cognitive impairments in APP mutant mice(3).
- 2B3 administration in PDAPP mice significantly reduced NR2B phosphorylation when observed as a ration to total NR2B levels. It is likely that this effect, at least in part, contributed to the improved memory performance in PDAPP mice.
- To date, no previous immunotherapy treatment has reported altered NMDA phosphorylation *ex vivo*.
- Collectively, these data provide evidence that inhibition of β-secretase cleavage of APP using an immunotherapy *in vivo* may rescue early stage memory deficits in AD.

References
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2. Games D, et al., *Nature*. 1995;**373**:523-527
3. Ittner L, et al., *Neuron*. 2010;**142**:387-397

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