Stabilised low-n amyloid-β oligomers induce robust NOR deficits associated with inflammatory, synaptic and GABAergic dysfunction in the rat

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Running title:

Aβ oligomer induced neuropathology in the rat.

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Abstract:

Background: With current treatments for Alzheimer's disease (AD) only providing temporary symptomatic benefits disease modifying drugs are urgently required. This approach relies on improved understanding of the early pathophysiology of AD. A new hypothesis has emerged, in which early memory loss is considered a synapse failure caused by soluble amyloid-β oligomers (Aβo). These small soluble Aβo, which precede the formation of larger fibrillar assemblies, may be the main cause of early AD pathologies.

Objective: The aim of the current study was to investigate the effect of acute administration of stabilised low-n amyloid-β_{1-42} oligomers (Aβo$_{1-42}$) on cognitive, inflammatory, synaptic and neuronal markers in the rat.

Methods: Female and Male Lister Hooded rats received acute intracerebroventricular (ICV) administration of either vehicle or 5 nmol of Aβo$_{1-42}$ (10µL). Cognition was assessed in the novel object recognition paradigm at different time points. Levels of inflammatory (IL-1β, IL-6, TNF-α), synaptic (PSD-95, SNAP-25) and neuronal (n-acetylaspartate, parvalbumin-positive cells) markers were investigated in different brain regions (prefrontal & frontal cortex, striatum, dorsal and ventral hippocampus).

Results: Acute ICV administration of Aβo$_{1-42}$ induced robust and enduring NOR deficits. These deficits were reversed by acute administration of donepezil and rolipram but not risperidone. Post-mortem analysis revealed an increase in inflammatory markers, a decrease in synaptic markers and parvalbumin containing interneurons in the frontal cortex, with no evidence of widespread neuronal loss.

Conclusion: Taken together the results suggest that acute administration of soluble low-n Aβo may be a useful model to study the early mechanisms involved in AD and

provide us with a platform for testing novel therapeutic approaches that target the early underlying synaptic pathology.

Keywords:

Alzheimer's disease; amyloid-β oligomers; cognition; parvalbumin interneurons.

Introduction:

Within the Alzheimer's disease (AD) brain, several species of soluble β-amyloid (Aβ) can be found, along with insoluble fibrils and plaques, and several attempts at identifying the toxic species of soluble oligomers have been made. It has been demonstrated that following intracerebroventricular injection (ICV) in mice, high molecular weight (HMW; ranging from ~50 to ~150 kDa), and low molecular weight (LMW; dimers-tetramers) oligomers act differently, with LMW oligomers causing long lasting, synaptic alterations, and HMW oligomers causing short term NMDA receptor associated cognitive disruption [1]. Other studies have demonstrated a role for LMW oligomers in synaptic loss or dysfunction [2-5]. LMW oligomers have also been shown to induce the collapse of the endoplasmic reticulum (ER) and destabilise microtubules in rat hippocampal cells [6]. ER dysfunction has also been shown in the APPSwe mutant mouse model and human AD brain [6,7].

The literature surrounding the use of different oligomerisations is vast. This may be down to the large variety of oligomer preparation methods, differences in using either synthetic or purified Aβ, or the tendency of Aβ to aggregate spontaneously. Whilst elucidating the role of each type of Aβ oligomer is important, it is also crucial to

understand how Aβ oligomers act when in a more physiological mixed form. Protocols also differ in the site, concentration and volume of administration used.

In the current study we focused our research on the administration of stabilised low-n amyloid-β_{1-42} oligomers (Aβo$_{1-42}$). This peptide is not only one of the main species found in AD, but its stabilised aggregation state facilitates the study of oligomeric forms, thought to have a more potent toxic effect than larger aggregates [8,9]. This model is furthermore supported by recent publications using the same species of Aβo$_{1-42}$ in a similar mouse model (SynAging, France), showing cognitive and neuropathological changes of relevance for AD research [10–13].

In the current study rats received an acute ICV injection of stabilised LMW Aβo$_{1-42}$ composed of dimers, trimers and tetramers. Novel object recognition (NOR) was performed to access any cognitive changes caused by the oligomers. Long lasting effects on cognition, effect of gender and pharmacological interventions assessed. Post-mortem studies focussed on neuropathological markers (including inflammatory, synaptic, general neuronal and GABAergic markers), commonly explored in AD models in regions related to cognitive function.

Materials and Methods

Animals

Adult female (n=100, 190 – 230 g) and male (n=20, 250 – 280 g) Lister Hooded rats (Charles River, UK) aged approximately 3-months old at the time of intracerebroventricular (ICV) administration of Aβo$_{1-42}$ were used in the studies. Rats were housed in groups of 4-5, in individually ventilated cages with two levels

(GR1800 Double-Decker Cage, Techniplast, UK) on a 12-h light-dark cycle, with free access to food and water. Cages were kept in a controlled environment (temperature 21±2°C and humidity 55±5%) in the Biological Services Facility at the University of Manchester. Experiments were conducted during the light cycle, in the morning. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) 1986 Act and University ethical guidelines. A free online power analysis software was used to calculate the sample size required for each group of animals. Calculations were based on the most restrictive test, behavioural tests usually requiring a larger effect size than biochemical analyses. The effect size and standard deviation were estimated from previous studies performed by our laboratory on similar studies. Power calculations were based on a Type 1 error of 5% (p=0.05). A power of 80% was set, the direction of the effect was two-tailed and statistical analysis was based on the Student's t-test and ANOVA. Results indicated that 10 animals were required per group in order to obtain relevant and significant results in the behavioural tests. The same calculations were made for the post-mortem analysis with effect size based on previous in house studies of similar markers.

Experimental design

Behavioural studies

For the time-course study *(Fig.1A)*, animals received 10 µL ICV administration of either vehicle (n=10 females) or $A\beta o_{1-42}$ 5 nmol (n=10 females). The novel object recognition (NOR) tasks were then performed 4, 14, 35 and 70 days later. These time points were chosen in order to allow enough time for animals to recover from surgery, to allow enough time in between each test so animals would complete the task and to assess the of the duration of the deficits observed.

The NOR test is extremely useful for identification of cognitive deficits, their neural basis, and for testing the efficacy of novel therapeutic agents in a number of disorders [41]. It is a two trial cognitive paradigm that assesses recognition memory. Recognition memory is disturbed in a range of human disorders and NOR is widely used in rodents for investigating deficits in a variety of animal models of human conditions where cognition is impaired. In the current study the NOR task was chosen as it possesses several advantages over more complex tasks that involve lengthy training procedures and/or food or water deprivation. It is quick to administer and allows animals to be retested. It is non-rewarded, provides data quickly and most importantly, ethologically relevant as it relies on the animal's natural preference for novelty.

For the gender study *(Fig.1B)*, animals received 10 µL ICV administration of either vehicle (n=10 females, n=10 males) or $A\beta o_{1-42}$ 5 nmol (n=10 females, n=10 males). The NOR task was then performed 4 days later.

For the pharmacological study *(Fig.1C)*, animals received 10 µL ICV administration of either vehicle (n=10 females) or $A\beta o_{1-42}$ 5 nmol (n=20 females). The NOR tasks were then performed after acute intraperitoneal (IP) administration of either saline, donepezil (1 mg/kg), rolipram (0.01 mg/kg), or risperidone (0.1 mg/kg), respectively 4, 8 and 14 days later. Pre-treatment times and doses used were determined from previous work in our laboratory in female Lister Hooded rats [42].

Post-mortem analysis of inflammatory, synaptic and neuronal markers.

A separate cohort of animals received 10 µL ICV administration of either vehicle (n=15 females) or $A\beta o_{1-42}$ 5 nmol (n=15 females). 14 days later and following NOR testing, n=5 in each group were culled for high-performance liquid chromatography

(HPLC) analysis of the general neuronal marker, n-acetylaspartate (NAA). 35 days following ICV administration of Aβo$_{1-42}$, the remaining n=10 in each group were culled for ELISA analysis of inflammatory (IL-1β, IL-6 and TNF-α) and synaptic (PSD-95 and SNAP-25) markers *(Fig.1D)*.

Rats from experiment 1 were culled 70 days after ICV administration of Aβo$_{1-42}$, following NOR testing and brains were prepared for immunohistochemical staining for GABAergic parvalbumin-positive interneurons *(Fig.1A)*.

For post-mortem analysis regions of interest were defined according to the Atlas of Paxinos and Watson in relation to bregma. Brains were dissected on ice (or cut using a cryostat, immunohistochemistry) and the area of interest dissected from 1-2mm thick slices. Approximate coordinates of the regions investigated were - Frontal cortex, between bregma +5.20 – +3.20; prefrontal cortex, between bregma +3.2 and +1.7; striatum, between bregma +1.2 and -0.2; hippocampus, dorsal: between bregma -2.30 and -3.80, ventral: between bregma -4.8 and -6.0 and temporal cortex, between bregma -2.30 and -3.80. General neuronal dysfunction (N-acetylaspartate) was assessed in a number of regions of relevance to cognitive function and in relation to regions studied in previous rodent models [43, 44]. Neuroinflammatory, synaptic and parvalbumin studies were carried out in the frontal cortex and hippocampus (and the prefrontal cortex for parvalbumin). These areas were chosen in relation to previous studies using the same oligomer preparation in the mouse model work by SynAging [11, 12].

Amyloid-β$_{1-42}$ oligomer administration

Rats were randomly assigned into two groups and received 10 µL ICV administration of either vehicle or Aβo$_{1-42}$ (5 nmol, SynAging, France) into the left hemisphere.

Concentration of oligomers was adapted from previous work conducted by SynAging in mice (10-13). $A\beta o_{1-42}$ preparations largely consist of tetramers, some trimers and residual monomers. They are completely stable when stored frozen. Every preparation was validated in vitro. In vivo, $A\beta o_{1-42}$ were used at a dose of 5 nmol (equivalent to the constituent monomer peptide concentration). Briefly, rats were anaesthetised with 4% isoflurane in O_2 (maintained at 2-3% isoflurane) and placed into a stereotaxic frame. Animals then received a sub-cutaneous administration of 0.1 mg/kg of buprenorphine. Following an incision in the scalp, a hole was drilled in the skull at the following coordinates, AP: -0.8 mm (posterior) and DV:-1.5 mm (lateral) from Bregma. A needle (Hamilton[®] 701N) was gently inserted at -4.5 mm (ventral), to reach the left ventricle [14]. Vehicle (phosphate buffered saline) or $A\beta o_{1-42}$ was administrated at a rate of 2.5 µL/min. The scalp was sutured and an antibiotic was locally applied. Animals were left to recover in a heated chamber and closely monitored before being returned to their home cage.

Behaviour

Cognition was assessed by measuring short-term recognition memory in rats using the Novel Object Recognition (NOR) task [18, 24]. Briefly, animals were habituated to the testing arena with their cage mates on two consecutive days for 30 and 15 minutes respectively prior to the first experimentation. On the day of testing rats were individually placed in a 52x52 cm box and left free to explore two copies of the same object for 3 min (acquisition phase). After an inter-trial interval (ITI) of 2 min, the animal was placed back in same box and left free to explore an identical copy of the previously seen object (referred to as the familiar object) and a new object (referred to as the novel object) for another 3 min (retention phase). Time spent exploring both objects was scored in both the acquisition and retention phases, blind to treatment

and to the novelty or familiarity of the object. The location of the novel object in the retention trial was randomly assigned for each rat using a pseudo-random Gellerman schedule. Different objects, validated in house, were used for the each session. All experiments were filmed and video-recorded for subsequent behavioural analysis by an experimenter blind to the treatments. Object exploration was defined as animals sniffing, licking or touching the objects with forepaws, but not leaning against, turning around, standing or sitting on the objects. The objects used, their left/right position and their familiarity/novelty were balanced between animals. Inter-observer reliability of behavioural results was assessed for all experiments.

Parvalbumin immunohistochemistry

Rats were culled by overdose of anaesthetic (5% isoflurane in O_2), and brains were perfused with phosphate buffered saline (PBS) 0.1 M, and perfusion-fixed with 4% paraformaldehyde in PBS. Brains were rapidly extracted and incubated in 4% paraformaldehyde for 24 hs followed by 30% glucose for 48 hs at 4°C, before being stored at -80°C. The frontal cortex and prefrontal cortex were cut and free floating sections were stained for parvalbumin with a mouse monoclonal anti-parvalbumin antibody (Swant, PV235) and revealed by ABC and DAB kits (Vector Laboratories, PK-6100 and SK4100) as described previously [15]. Sections were mounted on slides, coded and analysed blind to treatment. Stained sections were scanned at 4x magnification using an Olympus BX51 microscope interfaced to an Image ProPlus (version 6.3) analysis system (Media Cybernetics, USA) via a JVC 3-CCD video camera. Estimations of neuronal density (cells/mm^2) were carried out in every 6th section per region, with a minimum of 6 sections per animal counted. The region of interest was highlighted and parvalbumin positive neurons were counted live at a

higher magnification (20x) using randomly generated points and a 2D counting frame. There was no staining in sections where the primary antibody was omitted.

HPLC analysis of N-acetylaspartate (NAA)

Rats were culled by overdose of anaesthetic (5% isoflurane in O_2), and brains were perfused with PBS 0.1 M, rapidly dissected and snap frozen in isopentane before being stored at -80˚C. The neuronal marker NAA was investigated in 6 brain regions (frontal cortex, prefrontal cortex, striatum, temporal cortex, dorsal hippocampus, ventral hippocampus). NAA levels were assessed as described previously [16]. Briefly, proteins in the tissue were precipitated with 0.1 M perchloric acid and NAA was extracted from the supernatant using strong anion exchange columns. The extracted sample was analysed by HPLC (Genesis, C18, 4μm, 4.6 mm x 250 mm), mobile phase 0.1% phosphoric acid, UV detection 215 nm. NAA levels in each sample were measured by peak height comparison with an external standard curve.

Neuroinflammatory and synaptic markers

Rats were culled by overdose of anaesthetic (5% isoflurane in O_2), and brains were perfused with PBS 0.1 M, rapidly dissected and snap frozen in isopentane before being stored at -80˚C. Neuroinflammatory (IL-1β, IL-6 and TNF-α) and synaptic (PSD-95 and SNAP-25) markers were investigated in the frontal cortex and the hippocampus. Briefly, samples were homogenised in a sample buffer (Trizma base .01 M, sucrose .03 M, EDTA .0025M, PMSF 0.1M, sodium orthovanadate 0.1 M, Protease inhibitor cocktail cOmplete (Roche) tablet), and then centrifuged at 800g for 15 min. The supernatant was further centrifuged at 12000g for 20 min. The final supernatant was used to measure inflammatory markers (ELISA kit for IL-1β/IL-1F2,

IL-6 and TNF-α, DuoSet), while the final pellet was suspended in PBS 0.1 M for synaptic marker analysis (ELISA kit for DG4 and SNAP-25, Cloud-Clone Corp).

Data analysis

Results are expressed as mean ± standard error of the mean (SEM). NOR data were analysed by ANOVA on repeated measures with object as within-subject factor and group as in-between subject factor. In cases of significance ($p < 0.05$), individual paired samples Student's t-tests were run in each group, comparing the exploration time of both objects. Total exploration times were analysed by ANOVA and Bonferroni post-hoc. NAA levels were analysed by independent samples Mann-Whitney test. Inflammatory markers, synaptic markers and parvalbumin-positive cell density were analysed by independent samples Student's t-tests. All the statistical analyses have been run using IBM SPSS (version 20).

Results

Stabilised Aβo$_{1-42}$ induce a long lasting NOR deficit in the rat *(Fig. 2)*

In the time course study, on days 4, 14, 35 and 70 after ICV administration of Aβo$_{1-42}$, both groups explored both objects equally, in each of the acquisition phases. There was no difference in left/right object exploration [$F_{(1,18)}=2.326$ $p>0.05$] regardless of the day of testing [$F_{(3,16)}=0.496$ $p>0.05$]. Total object exploration was not different between groups [$F_{(1,18)}=1.425$ $p>0.05$] but was however affected by the day of testing [$F_{(3,16)}=47.998$ $p<0.001$], and was decreased from day 4 to day 14 [$p<0.001$] but not on the following sessions [$p>0.05$].

In the retention phases, there was a significant difference of exploration between the familiar and novel object [$F_{(1,16)}=89.046$ $p<0.001$] with an effect of group

$[F_{(1,16)}=50.249$ p<0.001]. The vehicle group spent more time exploring the novel object on day 4 $[t_{(9)}=-6.244$ p<0.001], day 14 $[t_{(9)}=-4.923$ p<0.001], day 35 $[t_{(9)}=-3.180$ p<0.05], and day 70 $[t_{(9)}=-2.372$ p<0.05]. There was however no difference in exploration for the $A\beta o_{1-42}$ group on any day: day 4 $[t_{(8)}=-0.715$ p>0.05], day 14 $[t_{(8)}=-0.890$ p>0.05], day 35 $[t_{(9)}=-0.251$ p>0.05], and day 70 $[t_{(9)}=-1.419$ p>0.05]. Total object exploration was not different between groups $[F_{(1,16)}=2.528$ p>0.05] but was again affected by the day of testing $[F_{(3,14)}=11.216$ p<0.001], and was decreased from day 14 to day 35 only [p<0.05].

In summary, independent of the day of testing, the vehicle group significantly spent more time exploring the novel over the familiar object, an effect that was abolished in the group receiving ICV administration of $A\beta o_{1-42}$.

Stabilised $A\beta o_{1-42}$ induce NOR deficits in both female and male rats *(Fig.3)*

In the acquisition phase, all female and male groups explored both objects equally. There was no difference in left/right object exploration $[F_{(1,36)}=0.005$ p>0.05]. Total object exploration was not different between vehicle and $A\beta o_{1-42}$ groups in each gender sub-group $[F_{(1,36)}=0.663$ p>0.05]. However, total object exploration was higher in the female groups when compared to the male groups $[F_{(1,36)}=21.342$ p<0.001].

In the retention phase, there was a significant difference of exploration between the familiar and novel object $[F_{(1,35)}=40.563$ p<0.001] with a significant effect of group $[F_{(1,35)}=34.649$ p<0.001]. Both vehicle groups spent significantly more time exploring the novel object, female $[t_{(9)}=-6.244$ p<0.001] and male $[t_{(9)}=-4.927$ p<0.001]. There was however no difference of exploration in both $A\beta o_{1-42}$ treated groups, female $[t_{(9)}=-0.715$ p>0.05] and male $[t_{(9)}=0.162$ p>0.05]. Total object exploration was not

different between animals, regardless of group [$F_{(1,35)}=0.051$ p>0.05] or gender [$F_{(1,35)}=1.042$ p>0.05].

In summary, independent of the gender of the rats, vehicle groups spent significantly more time exploring the novel over the familiar object, an effect that was abolished in the groups receiving ICV administration of Aβo$_{1-42}$.

Acute treatment with donepezil and rolipram, but not risperidone, rescues the NOR deficit. *(Fig.4)*

In the acquisition phases all groups explored both objects equally. There was no difference in left/right object exploration in any group, on each day of testing; respectively [$F_{(1,25)}=0.0001$ p>0.05] on day 4, [$F_{(1,22)=}0.935$ p>0.05] on day 8, and [$F_{(1,22)=}0.935$ p>0.05] on day 14 after ICV administration of Aβo$_{1-42}$.

In the retention phases, ICV administration of Aβo$_{1-42}$ induced a NOR deficit on each day of testing; an effect that was rescued by acute IP treatment with donepezil (1 mg/kg) and rolipram (0.01 mg/kg), but not risperidone (0.1 mg/kg). All statistical test results are summarised in *Table 1*.

In summary, vehicle groups spent significantly more time exploring the novel over the familiar object, an effect that was abolished in the groups receiving ICV administration of Aβo$_{1-42}$ and restored by acute IP treatment with donepezil and rolipram but not risperidone.

Stabilised Aβo$_{1-42}$ induces a deficit in parvalbumin-containing interneurons, with no change in the general neuronal marker N-acetylaspartate *(Fig.5)*

Rats from the time-course study were culled following the last NOR session, 70 days after ICV administration of $A\beta o_{1-42}$. Parvalbumin positive staining was found throughout the frontal and prefrontal regions with no staining in sections where the primary antibody was omitted (Figure 6). Parvalbumin-positive cell density was significantly reduced in both the frontal cortex $[t(16)=3.365\ p<0.01]$ and prefrontal cortex $[t(10.89)=4.008\ p<0.01]$ of the $A\beta o_{1-42}$ treated animals *(Fig.5A)*.

In a separate experiment and following confirmation of the NOR deficit (data not shown), levels of the neuronal marker NAA were investigated in 6 regions, 14 days after ICV administration of $A\beta o_{1-42}$. There was no significant difference in the levels of NAA between groups in each of the 6 brain regions investigated *(Fig.5B)*.

Stabilised $A\beta o_{1-42}$ result in raised levels of the inflammatory markers IL-1β and TNF-α in the frontal cortex. *(Fig.7)*

Inflammatory markers IL-1β, IL-6 and TNF-α levels were investigated in two regions, frontal cortex and hippocampus, 35 days after ICV administration of $A\beta o_{1-42}$, and after confirmation of the NOR deficit (data not shown). There was no difference in the levels of IL-1β in the hippocampus $[t_{(18)}=-0.451\ p>0.05]$. In contrast, the level of IL-1β in the frontal cortex was significantly higher in the group administrated with $A\beta o_{1-42}$ $[t_{(15)}=-2.694\ p<0.05]$. There was no difference in the levels of IL-6 in the hippocampus $[t_{(17)}=0.862\ p>0.05]$ or the frontal cortex $[t_{(16)}=-1.264\ p>0.05]$. There was no difference in levels of TNF-α in the hippocampus $[t_{(17)}=0.752\ p>0.05]$. In contrast, levels of TNF-α in the frontal cortex were significantly higher in the group administrated with $A\beta o_{1-42}$ $[t_{(16)}=-2.299\ p<0.05]$.

Stabilised Aβo$_{1-42}$ induce a deficit in the postsynaptic marker PSD-95 in the frontal cortex. (Fig. 8)

Pre- (SNAP-25) and post- (PSD-95) synaptic markers were investigated in two regions, frontal cortex and hippocampus, 35 days after ICV administration of Aβo$_{1-42}$ and after confirmation of the NOR deficit (data not shown). There was no difference in levels of SNAP-25 in the hippocampus [$t_{(17)}$=1.186 p>0.05] or the frontal cortex [$t_{(16)}$=1.908 p>0.05]. There was no difference in levels of PSD-95 in the hippocampus [$t_{(14)}$=0.752 p>0.05]. In contrast, levels of PSD-95 in the frontal cortex were significantly lower in the group administrated with Aβo$_{1-42}$ [$t_{(16)}$=3.298 p<0.01].

Discussion

The current study demonstrated the appearance of a robust and lasting NOR deficit as early as day 4 and up to day 70, and associated with neuropathological changes, following an acute ICV administration of 5 nmol of Aβo$_{1-42}$, in both female and male Lister Hooded rats. Post-mortem analysis revealed an increase in inflammatory markers, a decrease in synaptic markers and parvalbumin containing interneurons in the frontal cortex, with no evidence of widespread neuronal loss.

In successive NOR testing – 4, 14, 35 and 70 days following ICV administration of Aβo$_{1-42}$ – both treatment groups showed no difference of exploration time between the identical objects presented during the acquisition phase, a finding which underlines that Aβo$_{1-42}$.did not induce non -specific motor disturbances. In contrast, during the retention phase only the group administrated with vehicle could perform the task, showing a clear preference for the novel object over the familiar one *(Fig.2A-D).* It is however noteworthy to observe that in both groups there was an

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overall decrease in total exploration time over the sessions. In line with previous reports [17], we found that the novelty of the task decreases over time for the rats, lowering their willingness to explore the environment and the objects. We also demonstrated that the $A\beta o_{1-42}$ induced NOR deficits appeared to affect both female and male Lister Hooded rats (Fig.3). Our data support the hypothesis of short-term recognition memory being one of the early cognitive domains affected in our model. These results supplement and confirm cognitive data from a similar mouse model using ICV administration of the same preparation of $A\beta o_{1-42}$ (SynAging, France) [10–13]. The SynAging laboratory has found that an ICV injection of the same $A\beta o_{1-42}$ in wild type C57BL/6 mice induced an impairment in the NOR task (with no effect following administration of the vehicle or the reverse sequence $A\beta o_{42-1}$).

The pharmacological experiments demonstrated a reversal of the $A\beta o_{1-42}$ induced NOR deficit following acute IP treatment with donepezil (1 mg/kg) and rolipram (0.01 mg/kg) but not risperidone (0.1 mg/kg) *(Fig.4)*.

Donepezil is currently used for the symptomatic treatment of Alzheimer's disease as an acetylcholinesterase inhibitor. Its effect in our model may highlight the presence of a deficit in acetylcholine that the drug could compensate by acutely increasing local levels. We recently demonstrated the ability of donepezil to improve object recognition deficits in a test of "natural forgetting" following a 6 h inter-trial interval in female Lister Hooded rats [18].

Rolipram is a phosphodiesterase-4 (PDE-4) inhibitor, a family of enzymes that regulate the hydrolysis of cAMP and cGMP. These two second messengers are involved in controlling levels of phospho-cAMP response element-binding (pCREB) in the brain and indirectly playing a role in the modulation of LTP, synaptic plasticity

and memory [19]. Inhibiting PDEs that hydrolyse cGMP could have beneficial effects in dementia [20] and opens new therapeutic possibilities. In line with our findings other studies in preclinical models with rolipram, have shown promising results on restoring cognition in both a mouse transgenic model [21] and a rat model of intra-hippocampal Aβo administration [22,23].

Risperidone is an atypical antipsychotic used for the treatment of schizophrenia. At lower doses, where you reduce dopamine D2 receptor blockade, we have consistently demonstrated the ability of risperidone to improve cognitive deficits induced by sub-chronic phencyclidine (PCP) in female Lister Hooded (preclinical model of relevance to the cognitive deficits in schizophrenia). We reported improvements in a number of domains including object recognition memory [24], reversal learning [25] and attentional set shifting [26]. In the current study risperidone was unable to reverse the deficit in NOR following administration of $Aβo_{1-42}$. In line with the finding in the current study we previously showed that risperidone did not reverse the deficit in NOR in "normal" rats following a 6 h inter-trial interval [18]. Taken together these studies highlight the different mechanisms that underlie the deficits induced by sub-chronic PCP and $Aβo_{1-42}$.

As a result of the study design different drugs were tested at different, successive time points and we cannot fully rule this out as a potential confound. However it is of interest to note that the reversal of the NOR deficit in $Aβo_{1-42}$ group was only apparent when the compound was "on-board". The cross over design of these experiments demonstrated that the behavioural deficit was seen once more in the $Aβo_{1-42}$ group when the compound (donepezil or rolipram) was washed out, demonstrating no carry over effect on subsequent testing.

These studies highlight the potential benefit of this approach in detecting symptomatic treatments for cognitive deficits in AD but also demonstrate the lack of effect on the underlying pathology responsible for these deficits, indicating the lack of a disease modifying effect.

The density of parvalbumin interneurons was significantly reduced in both the prefrontal and frontal cortices 70 days after acute administration of LMW $A\beta o_{1-42}$ *(Fig.5A)*. The reduction of these interneurons is in accordance with studies of human AD brains, and mouse models of AD, where parvalbumin neurons are known to be reduced [27, 28,30]. It is plausible that these parvalbumin reductions could be caused by the LMW $A\beta$ oligomers in AD. This would support data that has shown that the altered oscillations in AD, thought to be caused by disrupted inhibitory signalling via parvalbumin interneurons [29, 30] occur early in AD [31] at a similar time that LMW oligomers, notably trimers are found to be elevated [32, 33], suggesting this model may present the potential to study the $A\beta o$ mechanisms of relevance to early AD.

N-acetylaspartate (NAA) is utilised as a non-specific neuronal marker, with changes indicating neuronal loss or dysfunction [34]. The lack of NAA deficits in any of the brain regions investigated, 14 days following ICV administration of $A\beta o_{1-42}$ *(Fig.5)*, highlights a lack of widespread non-specific neuronal death, at the concentration of oligomers administered. One conclusion could be that these oligomers are toxic to only parvalbumin interneurons, or at least only specific neurons, in this model, and that the overall neuronal population is not decreased. However, the difference in time points means any conclusions must be made with caution.

We found increased levels of the neuroinflammatory markers IL-1β and TNF-α in the frontal cortex, 35 days after ICV administration of Aβo$_{1-42}$ *(Fig.7)*. IL-1β is known to be one of the earliest pro-inflammatory cytokines released following central nervous system insult [35]. Moreover, IL-1β is believed to be an initiator of inflammation, following acute injury [36], with IL-6 and TNF-α release being delayed in time [37]. However, kinetics data on the mid- and long term changes in rodent in vivo models of Aβo administration remain an unmet need. In the current study, the presence of increased levels of IL-1β and TNF-α, 35 days after administration of Aβo$_{1-42}$, could highlight an underlying phenomenon of constant and lasting inflammation, contributing to the NOR deficit observed at this time point. We also found reduced levels of the postsynaptic marker PSD-95 in the frontal cortex (Fig. 8). Aβo$_{1-42}$ are known to specifically bind to PSD-95 positive sites [38]. Decreased levels of PSD-95 are indicative of a disruption of synaptic activity in this area, mirroring the inflammatory changes seen in this region. Taken together these results suggest decreased synaptic activity associated with neuroinflammation, in the frontal cortex, following ICV administration of Aβo$_{1-42}$ may be contributing to the NOR deficits observed. In support of this is a recent study where we demonstrated the ability of the anti-inflammatory Mefenamic acid to prevent the NOR deficits following Aβo$_{1-42}$ administration [39].

We found no changes in inflammatory or synaptic markers in the hippocampus in this study, indicating that ICV administration of LMW Aβo$_{1-42}$ does not have an effect in this region. In line with these findings, spatial memory (as assessed by Y-maze and dependent on hippocampal input) was preserved at day 35 in this model (data not shown). In the current study we have not been able to track the distribution of the

LMW $A\beta o_{1-42}$ following administration and so cannot fully explore the specificity of the deficits observed in the frontal cortex over the hippocampus. Brouillette et al., [40] demonstrated that repeated hippocampal injections of small soluble $A\beta o_{1-42}$ in awake, freely moving mice were able to induce behavioural (deficits in hippocampus dependent memory) and pathological (marked neuronal loss and tau hyperphosphorylation) deficits or relevance to AD. In acute administration models the site of injection may play an important role in the regions affected.

Conclusion

Taken together these data provide a characterisation of the effects of an acute administration of LMW $A\beta o_{1-42}$ on cognitive, inflammatory, synaptic and neuronal markers in Lister Hooded rats. This study adds to the evidence implicating $A\beta o$ in Alzheimer's disease and may provide a platform for assessing symptomatic and / or neuroprotective effects of disease modifying drug candidates.

Conflict of Interest/Disclosure Statement

Nicolas Fischer, A Allouche, Violette Koziel & Thierry Pillot are employees of SynAging.

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Figure 1: Outline of the experiments. On the day of surgery (day 0), rats were administrated with 10 µL of either vehicle or $A\beta o_{1-42}$ (5 nmol). **(A, B and C)** Experiment 1: Characterisation of the cognitive deficit. NOR tasks were performed on days 4, 14, 35 and 70. In study C, rats received an acute IP treatment of vehicle or: donepezil 1 mg/kg (day 4), rolipram 0.01 mg/kg (day 8) and risperidone 0.1 mg/kg (day 14), the $A\beta o_{1-42}$ group receiving the treatment was reversed at each time point, allowing a washout of the previous treatment. **(A and D)** Experiment 2: Neuropathological markers. (A) Rats from Experiment 1 were culled on day 70 and frontal and prefrontal cortices stained for parvalbumin-positive cells. (D) Neuropathological markers. On day 14, n=5 in each group were culled for N-acetylaspartateanalysis. On day 35, n=10 in each group were culled for inflammatory (IL-1β, IL-6 and TNF-α) and synaptic (SNAP-25, PSD-95) marker analysis.

Figure 2: Experiment 1 – Time course. NOR exploration times on day 4 (A), 14 (B), 35 (C) and 70 (D) after ICV administration of vehicle or $A\beta o1-42$. Data are presented as mean+SEM, n=9-10 per group. Paired ANOVA and Student's t-test, *$p<0.05$ ***$p<0.001$ Novel vs. Familiar.

Figure 3: Experiment 1 – Gender specificity. NOR exploration times in both female and male rats, on day 4 after ICV administration of vehicle or $A\beta o1-42$. Data are presented as mean+SEM, n=9-10 per group. Paired ANOVA and Student t-test, ***$p<0.001$ Novel vs. Familiar.

Figure 4: Experiment 1 – Acute pharmacological treatment. NOR exploration times following ICV administration of vehicle or Aβo1-42 and acute treatment with donepezil (A), rolipram (B) and risperidone (C). Data are presented as mean+SEM, n=7-10 per group. Paired ANOVA and Student t-test, *p<0.05 **p<0.01 Novel vs. Familiar.

Figure 5: Experiment 2 – (A) Results from parvalbumin-positive cell counting in the frontal cortex and prefrontal cortex, 70 days after ICV administration of either vehicle or Aβo$_{1-42}$. Data are presented as mean±SEM, n=8-9 per group, independent samples Student's t-test , ** p<0.01 vs. Vehicle. (B) NAA levels as measured by HPLC across 6 brain areas, 14 days after ICV administration of vehicle or Aβo$_{1-42}$. Data are presented as individual plots, mean±SEM, n=4-5 per group. Mann-Whitney test, no significant difference between the two groups.

Figure 6: (A) Parvalbumin immunoreactivity in the rat prefrontal cortex. Brightfield photomicrograph of a coronal section showing the distribution of parvalbumin immunoreactivity throughout the hippocampus. 4× magnification. (B) Selected area from (A) of high power (20× magnification) brightfield photomicrograph of parvalbumin-immunopositive interneurons in prefrontal cortex. Scale bar = 50 µm.
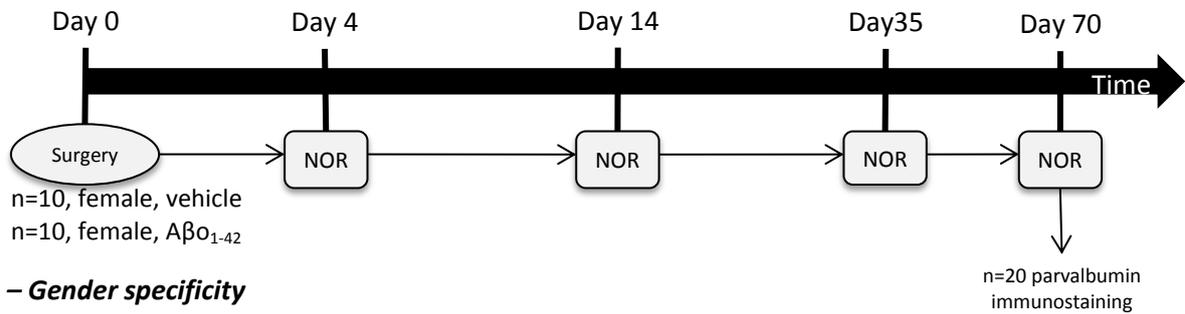
Figure 7: Experiment 2 – Results from the ELISA on neuroinflammatory markers in the hippocampus (left panel) and frontal cortex (right panel), 35 days after ICV administration of vehicle or Aβo$_{1-42}$. Data are presented as individual plots, mean±SEM, n=8-10 per group. Independent samples Student's t-test, * p<0.05 vs. Vehicle.

Figure 8: Experiment 2 – Results from the ELISA on synaptic markers in the hippocampus (left panel) and frontal cortex (right panel), 35 days after ICV administration of vehicle or $A\beta o_{1-42}$. Data are presented as individual plots, mean±SEM, n=8-10 per group. Independent samples Student's t-test, ** $p<0.01$ vs. Vehicle.

Table 1: Experiment 1: Statistical test results from the NOR retention phases after IP treatment with donepezil, rolipram and risperidone. Paired ANOVA and Student t-test.
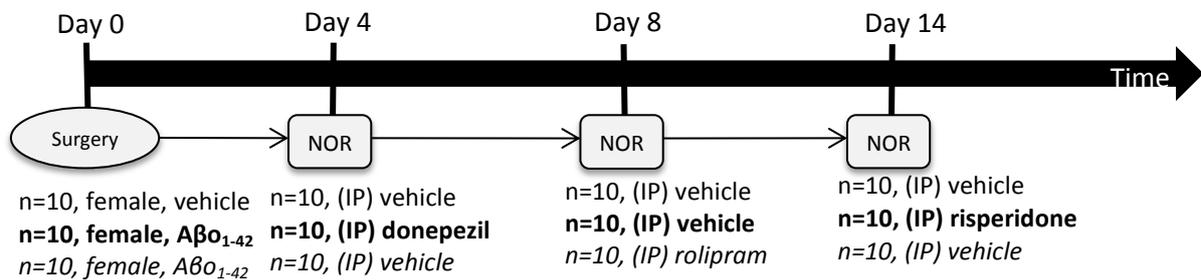
Figure 1

A – Time course + Parvalbumin immunostaining

Day 0   Day 4   Day 14   Day35   Day 70   Time

Surgery

n=10, female, vehicle
n=10, female, $A\beta o_{1-42}$

NOR   NOR   NOR   NOR

n=20 parvalbumin immunostaining

B – Gender specificity

Day 0   Day 4   Time

Surgery   NOR

n=10, female, vehicle
n=10, male, vehicle
n=10, female, $A\beta o_{1-42}$
n=10, male, $A\beta o_{1-42}$

C – Pharmacological treatment

Day 0   Day 4   Day 8   Day 14   Time

Surgery   NOR   NOR   NOR

n=10, female, vehicle
n=10, female, $A\beta o_{1-42}$
n=10, female, $A\beta o_{1-42}$

n=10, (IP) vehicle
n=10, (IP) donepezil
n=10, (IP) vehicle

n=10, (IP) vehicle
n=10, (IP) vehicle
n=10, (IP) rolipram

n=10, (IP) vehicle
n=10, (IP) risperidone
n=10, (IP) vehicle

D – Neuropathological markers

Day 0   Day 14   Day 35   Time

Surgery   NOR   NOR

n=15, female vehicle
n=15, female $A\beta o_{1-42}$

n=10, NAA levels

n=20, inflammatory and synaptic markers levels

Figure 2

A - NOR day 4

B - NOR day 14

C - NOR day 35

D - NOR day 70

Figure 3

NOR day 4 - Female and male rats

Acquisition

Retention

Figure 4

A - NOR day 4 - Donepezil 1 mg/kg

B - NOR day 8 - Rolipram 0.01 mg/kg

C - NOR day 14 - Risperidone 0.1 mg/kg
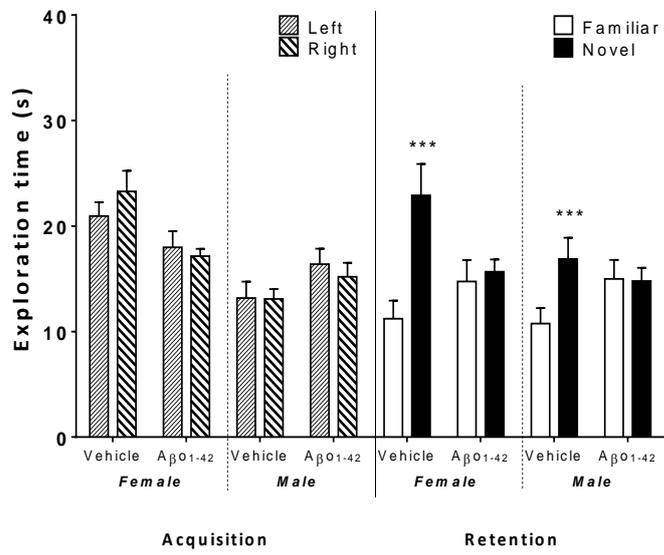
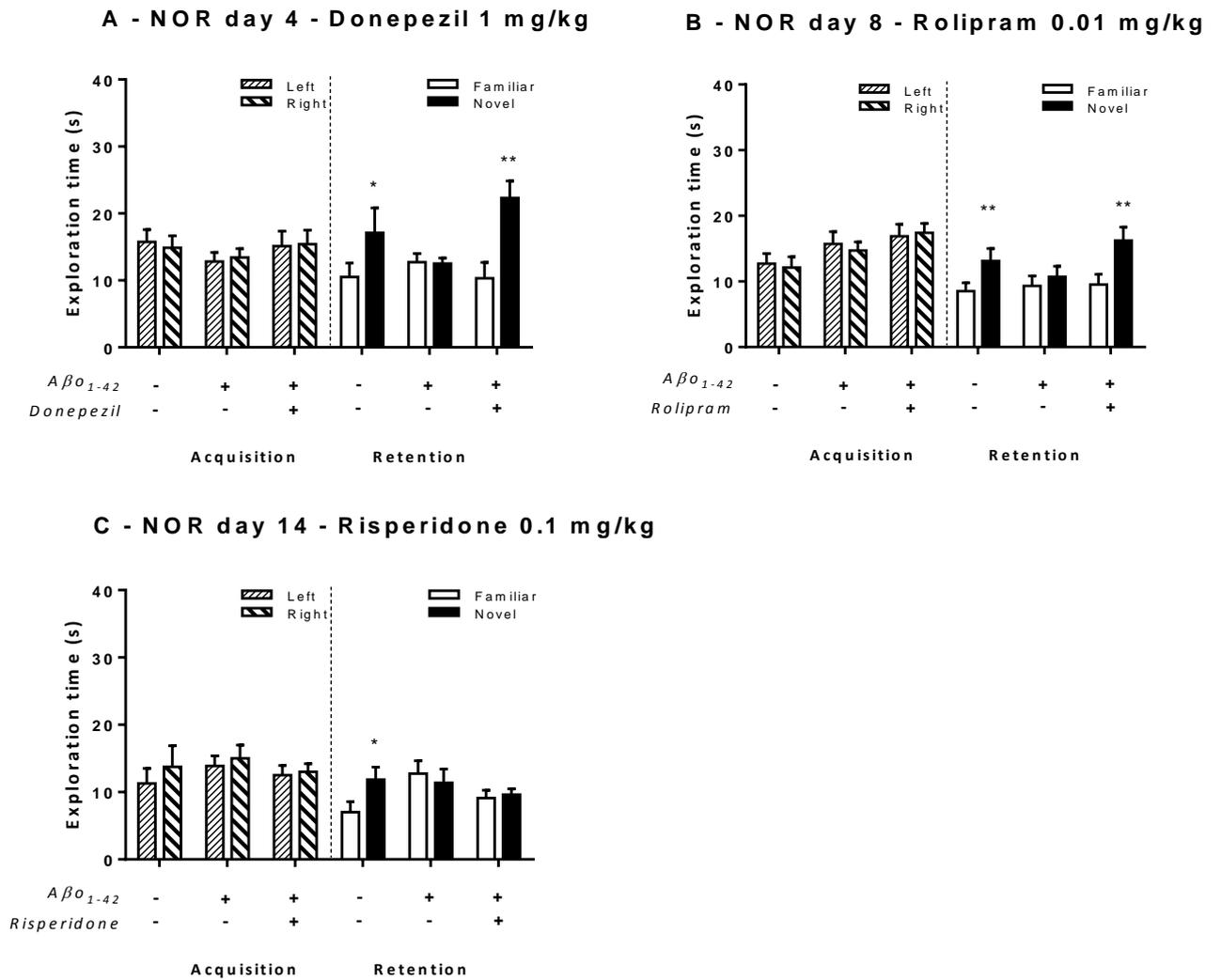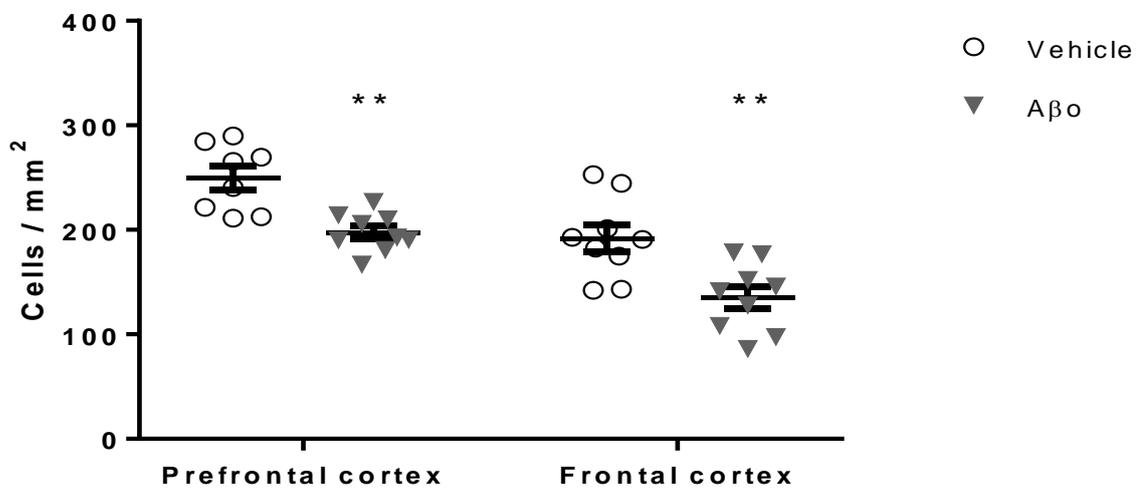**Figure 5**

A - Parvalbumin-positive cells density
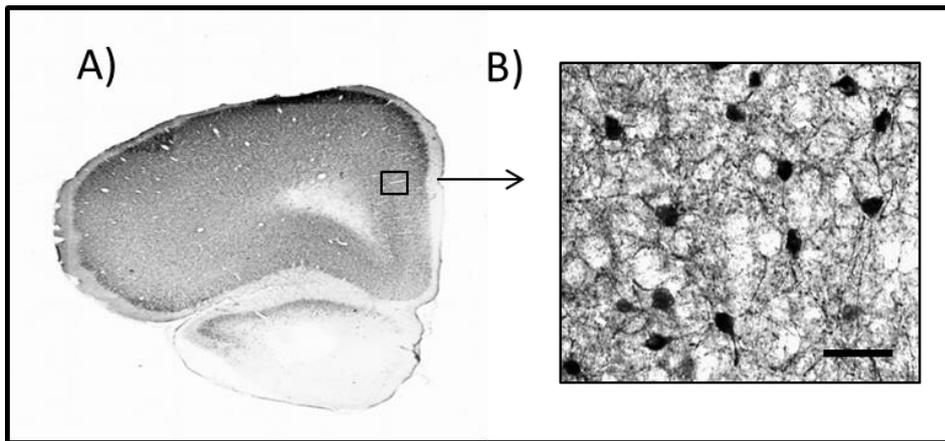

B - NAA level

Figure 6

Figure 7

IL-1β - Hippocampus

IL-1β - Frontal cortex

IL-6 - Hippocampus

IL-6 - Frontal cortex

TNF-α - Hippocampus

TNF-α - Frontal cortex

Figure 8

SNAP-25 - Hippocampus

SNAP-25 - Frontal cortex
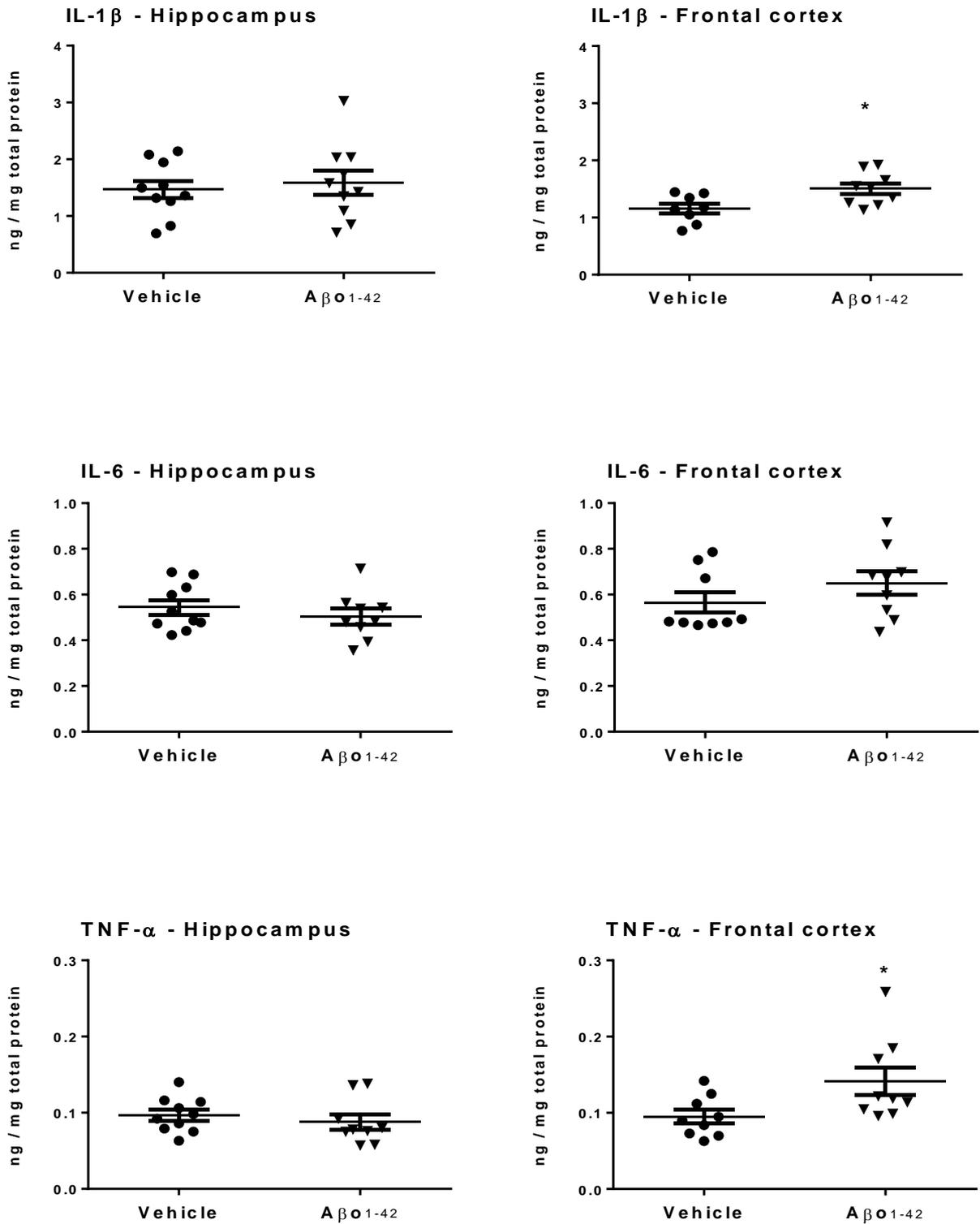
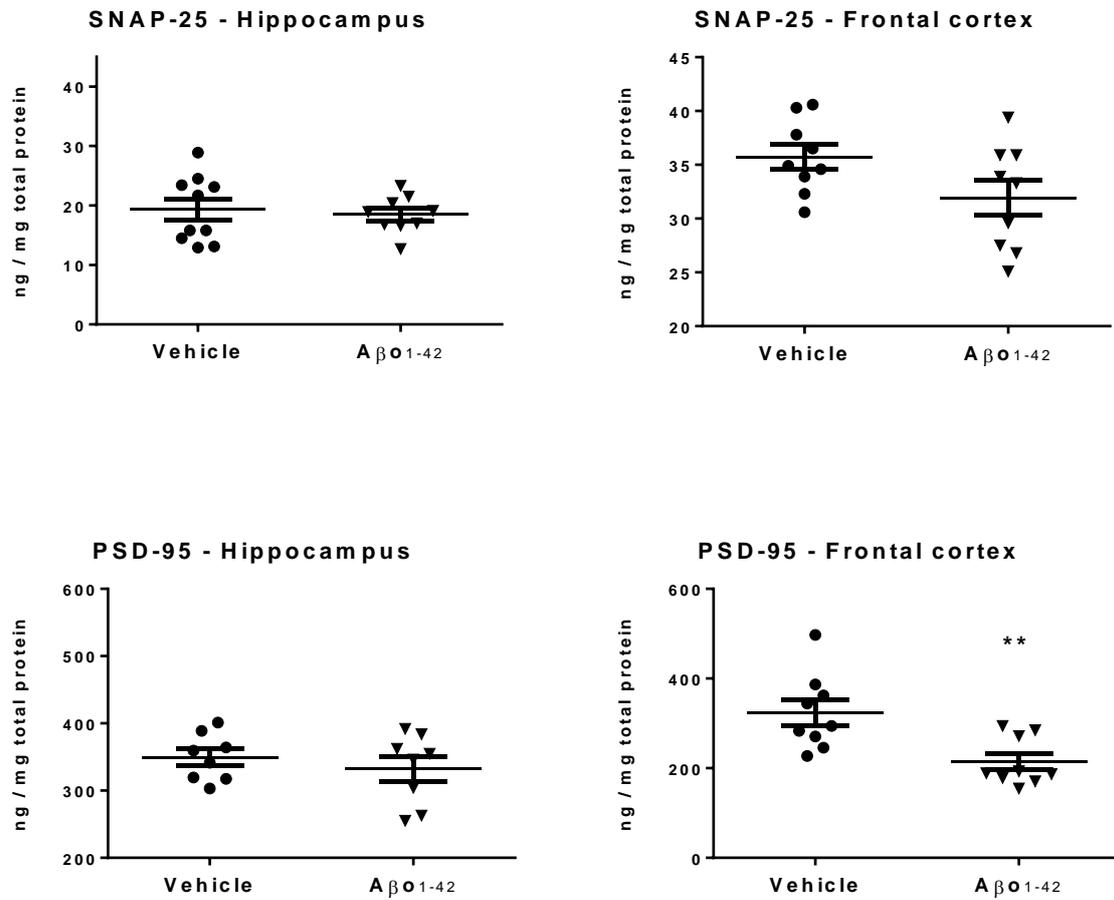PSD-95 - Hippocampus

PSD-95 - Frontal cortex
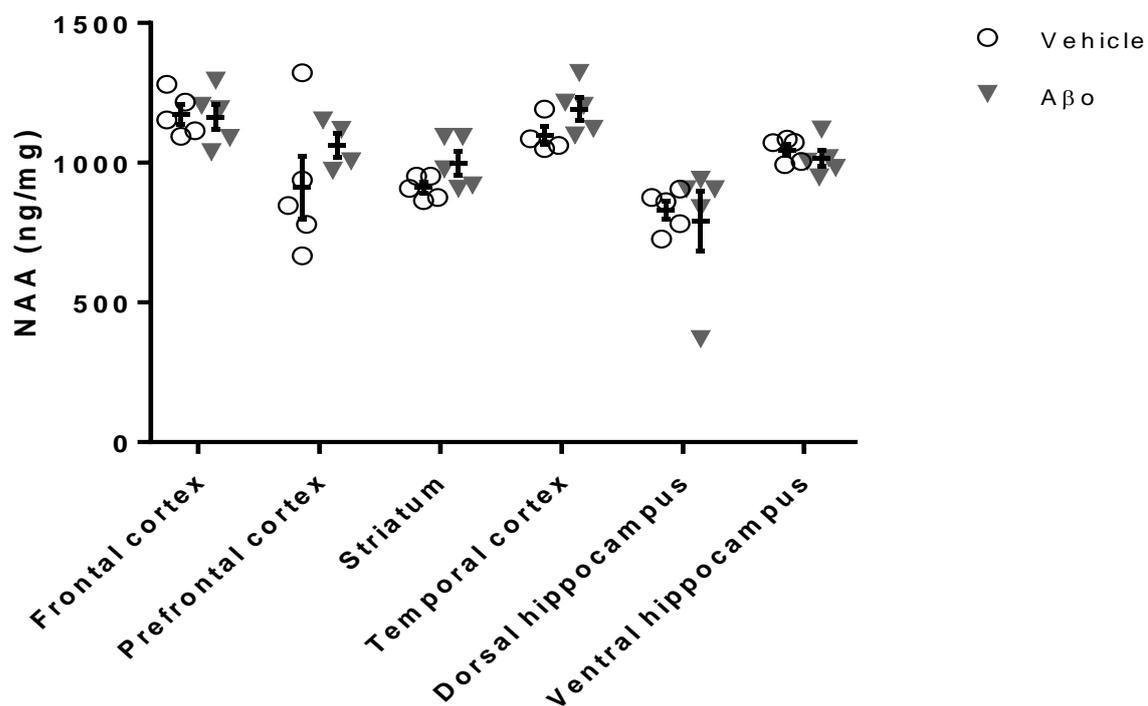
Table 1

		Overall difference in Familiar/Novel object exploration	*Overall group effect*	*Individual group difference in Familiar/Novel object exploration*		
				Vehicle + Vehicle	*Aβo + Vehicle*	*Aβo + Drug*
Drug	Donepezil 1 mg/kg	$F_{(1,25)}=24.147$ p<0.001	$F_{(2,25)}=8.638$ p<0.01	YES $t_{(7)}=-2.391$ p<0.05	NO $t_{(9)}=0.210$ p>0.05	YES $t_{(9)}=-4.768$ p<0.01
	Rolipram 0.01mg/kg	$F_{(1,27)}=24.818$ p<0.001	$F_{(2,25)}=3.288$ p=0.053	YES $t_{(9)}=-3.657$ p<0.01	NO $t_{(9)}=-0.924$ p>0.05	YES $t_{(9)}=-4.137$ p<0.01
	Risperidone 0.1 mg/kg	$F_{(1,22)}=2.262$ p>0.05	$F_{(21,22)}=3.973$ p<0.05	YES $t_{(6)}=-3.501$ p<0.05	NO $t_{(7)}=0.870$ p>0.05	NO $t_{(9)}=-0.337$ p>0.05