Intrahippocampal administration of a domain antibody that binds aggregated amyloid- β reverses
cognitive deficits produced by diet-induced obesity

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General Significance and Interest

Our work integrates the engineering of domain antibodies with conformational- and sequence-specificity for oligomeric amyloid beta with a clinically relevant model of diet-induced obesity in order to demonstrate not only the pervasive effects of obesity on several aspects of brain biochemistry and behavior, but also the bioengineering of a successful treatment against the long-term detrimental effects of a pre-diabetic state on the brain. We show for the first time that cognitive impairment linked to obesity and/or insulin resistance may be due to early accumulation of oligomeric beta-amyloid, and hence may represent a pre-Alzheimer's state.

Abstract

Background: Prevalence of high fat diets (HFD), diet-induced obesity (DIO) and Type 2 diabetes continues to increase, associated with cognitive impairment in both humans and rodent models.

Mechanisms transducing these impairments remain largely unknown: one possibility is that a common mechanism may be involved in the cognitive impairment seen in obese and/or diabetic states and in dementia, specifically Alzheimer's disease (AD). DIO is well established as a risk factor for development of AD. Oligomeric amyloid- β (A β) is neurotoxic, and we showed that intrahippocampal oligomeric A β produces cognitive and metabolic dysfunction similar to those seen in DIO or diabetes. Moreover, animal models of DIO show elevated brain A β , a hallmark of AD, suggesting that this may be one source of cognitive impairment in both conditions.

Methods: Intrahippocampal administration of a novel anti-A β domain antibody for aggregated A β , or a control domain antibody, to control or HFD-induced DIO rats. Spatial learning measured in a conditioned contextual fear (CCF) task after domain antibody treatment; postmortem, hippocampal NMDAR and AMPAR were measured.

Results: DIO caused impairment in CCF, and this impairment was eliminated by intrahippocampal administration of the active domain antibody. Measurement of hippocampal proteins suggests that DIO causes dysregulation of hippocampal AMPA receptors, which is also reversed by acute domain antibody administration.

Conclusions: Our findings support the concept that oligomeric A β within the hippocampus of DIO animals may not only be a risk factor for development of AD but may also cause cognitive impairment before the development of dementia.

Keywords: Amyloid; AMPA; Obesity; Cognition; Diet, High-Fat; Antibody

Introduction

The incidence of both obesity and the common corollary of Type 2 Diabetes (T2D), continues to rise (1-3). T2D can cause cognitive impairment in patients of all ages (4), but particularly in older individuals (5). People with T2D have deficits in information processing (6), compromised executive function (7), and hippocampal volume loss has also been reported (8). Rodent models of diet-induced obesity (DIO), insulin resistance and/or T2D are also linked to decreased cognitive performance in hippocampally-dependent tasks (9-13) as well as to reduced hippocampal metabolism (14).

A relevant clinical finding of increasing interest is the link between DIO and Alzheimer's disease (AD). DIO increases the risk of developing sporadic AD (15) and is linked to elevated brain accumulation of beta-amyloid ($A\beta$), a hallmark of AD. $A\beta$ is increased in the brains of both human and animal models of DIO (16-18). Additionally, we have shown that acute intrahippocampal delivery of oligomeric $A\beta$ causes rapid cognitive impairment that is accompanied by a reduction in hippocampal insulin signaling, in task-associated hippocampal metabolism, and in translocation of the insulin-regulated glucose transporter GluT4 (19). Neuroimaging studies suggest that there are parallels in the structural brain impact of T2D and AD, for example in hippocampal and subcortical atrophy (20, 21). Furthermore, DIO alone, even in the absence of T2D, decreases cognitive performance in spatial water-maze testing as well as reducing expression of markers of hippocampal learning and plasticity such as BDNF and CREB, and alters glial morphology and transporters vital for glutamate homeostasis (22, 23). Similarly, hyperleptinemia without insulin resistance increases several markers of AD and pro-inflammatory cytokines (24). Overall, these studies suggest a causal link between DIO and early abnormal, deleterious accumulation of $A\beta$ in the human brain. Elevated brain $A\beta$ may thus

represent a possible mechanistic link between obesity and cognitive impairment, including risk of dementia.

The higher order structure of A β plays an important role in determining its effects on the brain. Most research has focused on three forms of A β (monomers, oligomers, fibrils; (25, 26)), although other forms have been reported (27). Fibrillar, insoluble A β is the form found in plaques and was initially believed to be the toxic cause of AD. However, several studies now suggest that soluble, oligomeric A β is the most toxic form of amyloid (28, 29), which has been shown to directly impair cognitive processes (19). Soluble oligomers are linked to learning and memory impairments in AD (30, 31), and also to dysregulation of AMPA (32, 33), NMDA (34), and insulin (35) receptors. Conversely, monomeric A β - the predominant form in healthy brains - appears to be benign (31, 36) and may have an endogenous role in several aspects of learning and memory. In rodents, picomolar doses of monomeric A β enhanced both *in vitro* hippocampal long-term potentiation (LTP) and *in vivo* performance on memory tasks (37), while blockade of amyloid precursor protein (APP) production impaired both LTP and memory (38). The fact that differences in tertiary structure can transform A β from a procognitive molecule to one intrinsically-linked to cognitive impairment and neurodegeneration shows that measurement of cognitive performance after direct, acute manipulation of specific amyloid conformers *in vivo* is essential for understanding their physiological effects, but this has not previously been attempted.

Several mechanisms have been suggested to transduce the link between DIO and AD pathology: these include increased inflammation (39, 40), increased blood-brain barrier permeability (41), and decreased expression of brain growth factors (42, 43). One further suggested mechanism, altered glutamate receptor activity (44), may represent a convergence

point for several pathways. Dysregulation of the glutamatergic AMPA and NMDA receptors has been suggested to underlie cognitive impairment in both DIO and AD (9, 45-47), although the specifics of this dysregulation vary between studies. Glutamate-induced excitotoxicity has been linked to neuroinflammation-induced AMPAR trafficking and decreased glutamate uptake (48, 49), as well as loss of growth factor-mediated neuroprotection (50, 51). HFD consumption affects astrocytic glutamate recycling by upregulating both glutamate transporter type-1 and astrocyte glutamate transporter (23). Diabetes-induced cognitive dysfunction is reversed by administration of a NMDAR antagonist (9). Hippocampal glutamate signaling also shows early perturbation in AD (52) including increased NMDAR2B expression (53), consistent with an adaptive response to the decreased glutamate levels in the hippocampus caused by DIO (54). Overall, several studies that provide mechanistic insight into the etiology of AD following DIO converge on changes in hippocampal glutamate signaling as a likely causal mechanism for progression towards dementia, and such changes would be consistent with abnormally elevated oligomeric A β .

Taken together, the body of work reviewed here suggests the hypothesis that DIO results in abnormal elevation of oligomeric A β in the brain, and specifically within the hippocampus, which may cause cognitive impairment, and that this impairment may be linked to hippocampal glutamate dysregulation. We tested this hypothesis in our DIO rat model using direct intrahippocampal administration of a novel domain antibody engineered to preferentially bind to A β oligomers and fibrils, and thus block downstream deleterious effects of A β but not prevent any procognitive role of A β monomers. These domain antibodies are referred to as Grafted AMyloid-Motif AntiBODIES (gammabodies; (55, 56)), and they are single V_H domains. Their small size relative to full-length IgGs enables them to better diffuse through the hippocampus,

while their binding specificity and effectiveness has been well-characterized (55-58). The fact that rat A β does not form amyloid fibrils suggests that in our model, A β gammabodies will primarily affect oligomeric A β .

Full-length antibodies that bind all A β isoforms are effective at restoring synaptic plasticity in a hippocampal slice preparation or when given in very large volumes over several hours to anesthetized animals (59). This further supports the effectiveness of using antibodies to block A β in the hippocampus. Our specific hypothesis in this study was that blocking oligomeric hippocampal A β would attenuate or reverse cognitive impairment seen in the DIO animals.

Methods

Animals

All procedures were approved by the Institutional Animal Care and Use Committee at the University at Albany. Male Sprague-Dawley rats (Charles River, Wilmington, MA) were purchased at six weeks of age and group housed with a 12-hour light/dark schedule (lights on at 7 am). After one week of acclimation, rats were randomly selected to receive either regular Chow (Laboratory Rodent Diet 5001, LabDiet, St. Louis, MO) diet or a high-calorie diet (D12492, Research diets, Inc.) and given *ad libitum* access to food and water for 6 months. By percent calories, the regular chow contained ~28% protein, ~13% fat, and ~58% carbohydrate while the high-calorie diet contained 20% protein, 60% fat, and 20% carbohydrate. Prior to surgery rats were housed two per cage. Following surgery rats were single-housed to prevent damage to the infusion cannulae.

Surgical Procedures

Rats were anesthetized with 5% isoflurane. Standard sterile stereotaxic surgical procedures were used as described previously (14, 19, 60) to implant a single guide cannula into the left dorsal CA1 region of the hippocampus, with coordinates of -5.6 mm posterior, +5.0 mm lateral, and -3.3 mm ventral to bregma. The left dorsal hippocampus was targeted for maximum comparability to previous related studies which used unilateral left hippocampal manipulations (14, 19); studies also suggest lateralization of spatial functions in rodents to the left dorsal hippocampus (61, 62). This approach also allowed for within-subjects comparison of protein measures. The 6 mm cannula (Plastics One) was secured in place with acrylic cement and two screws, and a dummy stylet was inserted. Rats recovered in a heated chamber and were returned to their home cages once they had regained consciousness and full motor control. Animal recovery was monitored for three days. Rimadyl once daily was used for post-surgical analgesia. Rimadyl was provided in the form of a bacon-flavored tablet. A new tablet was placed in each rat's cage every day for three days beginning the day of surgery. Each tablet provided 2mg of Rimadyl. Correct cannula placement was confirmed visually in all animals at the time of tissue extraction by locating the tract path created by the cannula which terminated in the CA1 region of the hippocampus.

A β domain antibodies and infusion procedures

Rats under both diet conditions were randomly assigned to either receive one of two different gammabody preparations. Gammabodies were prepared in PBS and infused at a rate of 0.125 μ l/min over 4 min into the left dorsal hippocampus. The infusion cannula was left in place for a further 6 min to ensure diffusion of each treatment. The active treatment group received a V_H domain that has A β residues 33-42 in CDR3, which is identified as the A β 33-42 gammabody. This domain antibody preferentially interacts with A β aggregates including

oligomers ($K_D \sim 400$ nM) with only weak binding to monomeric A β (56). The other antibody fragment was a V_H domain with A β residues 1-10 in CDR3, which is identified as the control gammabody. This domain antibody has negligible affinity for A β (56). Both gammabodies were administered as obtained, which resulted in a slightly higher protein concentration in the control group (9.2 mg/mL for the control gammabody relative to 5.7 mg/mL for the A β 33-42 gammabody) in order to control for any possibility of non-specific effects in the active treatment group. Immediately following treatment, rats were brought to the testing room for training in contextual conditioned fear (CCF), followed 24h later by CCF testing. Rats received a second infusion of their assigned gammabody immediately prior to tissue collection, one week after completion of behavioral testing.

Contextual Conditioned Fear

CCF was used to measure the impact of diet and gammabody treatment on hippocampal cognitive function. CCF testing specifically measures hippocampal-dependent, long-term spatial memory (63-68). Methods reported previously (69) were adapted and began with rats being placed in a chamber with distinct spatial cues. Following habituation, a tone sounded (80db) for 10 s followed immediately by five seconds of a 0.8 mA foot shock. This was repeated three times with one minute inter-trial intervals. Rats were returned to their home cage for 24 h and then placed in the shock chamber for a second time. During this second shock chamber exposure, freezing was measured for 3 min in the absence of any tones. Freezing was defined as an absence of movement, with the exception of those motions related to breathing (70). Rats were trained and tested twice in CCF. The first was to measure any effect of diet on CCF performance. One week after this baseline testing, rats underwent the cannulation surgery and were then given a second week to recover (with extensive handling) prior to gammabody

treatment and a second CCF measurement. Our results show that CCF performance was not different in animals at baseline and following control gammabody treatment, suggesting no attenuation of performance in the CCF task across the two trials.

Western Blots

Rats were euthanized using deep isoflurane anesthesia followed by rapid decapitation. Hippocampi were bilaterally dissected out and immediately processed for plasma membrane (PM) extraction and western blotting. The left (treated) side was used as a treatment sample, while the right (untreated) side was used as a control. Each sample was homogenized with 150 μ L of homogenization buffer containing phosphate inhibitor (1:500), from which 15 μ L of homogenate was removed and combined with 100 μ L of RIPA buffer containing protease and phosphatase inhibitors (1:200). Initial homogenate was processed for plasma membrane (PM) isolation per manufacturer's instructions (BioVision). Protein quantification was done with BCA per manufacturer's instructions (Pierce Scientific). Samples were combined with 2x Laemmli sample buffer (BioRad) and 2-mercaptoethanol to desired concentration for total (20 μ g) and plasma membrane (PM, 5 μ g) samples. Total samples were heated for 5 min in boiling water. Protein was loaded into SDS-PAGE 10% gels (BioRad) with ladder. Gels were transferred to PVDF membranes for immunoblotting. Membranes were blocked in 5% milk in tris-buffered saline with 5% tween 20. Primary antibodies for AMPAR GluR1 (Cell Signal; 1:1,000), actin (Sigma-Aldrich; 1:10,000), or NMDAR2B (Cell Signal; 1:1,000) were left to incubate overnight at 4° C. Biotinylated secondary antibodies were used at 1:10,000 in TTBS. Blots were incubated in tertiary streptavidin-HRP added to 5% milk in TTBS. Chemiluminescent (Thermo Scientific) was applied and bands were analyzed using Image J (NIH).

Data Analysis

Statistical analyses were performed with GraphPad Prism 6 software. Results are depicted as mean $\pm$ standard error with alpha set at $p < 0.05$. Planned comparisons were made with additional comparisons using multiple comparisons tests correcting for multiple comparisons. Data points more than three standard deviations from the mean were considered outliers and not included in analyses. Three baseline and two post-treatment data points across the four groups met this criterion and were hence excluded. In addition, four animals died from unrelated causes after baseline testing and before treatment.

Results

Consumption of a high-fat diet caused obesity, hyperglycemia and cognitive impairment

DIO rats had significantly increased body weight ($t_{32}=4.823$, $p < 0.0001$; Figure 1A) and fat pad weight ($t_{32}=4.398$, $p < 0.0001$; Figure 1B) compared to chow-fed control animals. DIO rats also had significantly higher fasting plasma blood glucose levels ($t_{32}=2.551$, $p = 0.0157$; Figure 1C). DIO resulted in significantly impaired CCF memory ($t_{31}=2.272$, $p < 0.05$; Figure 2A, left panel).

Treatment with A β 33-42 gammabody reversed cognitive impairment caused by a high-fat diet

Consistent with our hypothesis, the impairment in CCF performance seen in DIO animals was reversed by treatment with the A β 33-42 gammabody. CCF performance of DIO animals given the A β 33-42 gammabody did not differ from that of chow-fed, control-treated animals (62.6 $\pm$ 13.2 s freezing time vs. 60.6 $\pm$ 12.2 s, *n.s.*). This reversal of impairment in the DIO animals by treatment with the A β 33-42 gammabody was significant (paired within-animal comparison, $t_6=3.806$, $p < 0.01$; Figure 2B). Treatment with the A β 33-42 gammabody did not

affect performance in animals fed a chow diet, nor did treatment with the control gammabody affect performance in either diet condition (Figure 2B, all n.s.).

Reversal of cognitive impairment in HFD-fed rats is accompanied by restoration of plasma membrane AMPAR levels to normal

Plasma membrane (PM) levels of AMPAR (GluR1) in the hippocampus were significantly increased by DIO ($F_{1,53}=5.242$, $p<.05$) and were also affected by the interaction between diet and gammabody treatment ($F_{2,53}=3.415$, $p<.05$, Figure 3). Post hoc analysis confirmed that consumption of a HFD significantly increased levels of AMPA receptor at the plasma membrane of hippocampal neurons, and that this effect was fully reversed by hippocampal infusion of A β 33-42 gammabody (Figure 3). PM AMPAR group differences were not due to changes in total AMPAR protein, which did not differ across groups (Figure 4): there was no main effect of diet ($F_{1,26}=2.796$, $p>0.05$), treatment ($F_{2,26}=3.105$, $p>0.05$) nor an interaction ($F_{2,26}=0.516$, $p>0.05$).

The impact of diet and/or gammabody treatment on PM NMDAR2B was less marked than that on PM AMPAR (Figure 4). The interaction between gammabody treatment and diet approached significance ($F_{2,52}=3.044$, $p=0.0562$; Figure 5), but there was no significant effect for either gammabody treatment ($F_{2,52}=0.9163$, $p=0.4063$) or diet group ($F_{1,52}=1.238$, $p=0.2711$) alone. Post hoc analysis confirmed that PM NMDAR2B levels were significantly higher in DIO animals treated with the Control gammabody than in those given the A β 33-42 gammabody (Tukey multiple comparison test). Total NMDAR levels were reduced by DIO (Figure 6), with a significant main effect of diet ($F_{1,62}=7.063$, $p<0.05$); however, no significant post hoc differences due to treatment were detected. There was no significant difference due to gammabody treatment ($F_{2,62}=2.865$, $p>0.05$) or interaction ($F_{2,62}=0.515$, $p>0.05$).

Discussion

Our results provide novel insight into mechanisms causing cognitive impairment in DIO. Six months on a HFD, resulting in DIO, caused severe cognitive impairment (accompanied by elevation in body mass, fat mass, and fasting glucose level, as expected). The main, novel finding here is that this cognitive impairment was reversed by a single acute intrahippocampal infusion of an A β 33-42 gammabody, a single-domain antibody with preferential affinity for aggregated A β including oligomers (56). This treatment had no effect on contextual fear memory in animals fed a chow diet; moreover, a control treatment that does not bind A β did not alter cognitive performance under either diet condition. It should be noted that the A β 33-42 gammabody binds both fibrillar and oligomeric A β (55, 56). However, rat amyloid does not form fibrils, so that the effect of this treatment in our model is, by design, likely due primarily to blockade of oligomeric A β . Although our data support our hypothesis of a causal role for hippocampal A β in DIO-associated cognitive impairment, we cannot entirely exclude a non-specific effect of gammabody treatment on e.g. motor performance or anxiety. However, gammabodies were administered 24 hours prior to behavioral testing, and produced behavioral changes only in DIO animals; we found no indication of any non-specific effect of treatment in any group.

These results enhance our understanding of the basis for cognitive impairment caused by DIO. Obesity and T2D are now well-established as primary risk factors for AD; several studies have shown that DIO is associated with both cognitive impairment and pathological changes similar to those seen in AD (10-13, 15, 46, 71). However, as far as we are aware these are the first data to show that elevated levels of hippocampal oligomeric A β caused by DIO may cause

cognitive and neural dysfunction prior to development of T2D, appearance of dementia, or advanced aging.

In addition to our behavioral findings, we identified a mechanism by which oligomeric A β may cause hippocampal dysfunction. DIO rats had a dramatic increase in AMPA receptors at the plasma membrane: treatment with the A β 33-42 gammabody not only prevented behavioral impairment in these animals but also returned PM AMPAR levels to those seen in chow-fed animals. Paralleling the behavioral data, treatment with the A β 33-42 gammabody had little effect on PM AMPAR in chow-fed animals, a result that further supports the selectivity of this treatment, and the control gammabody did not significantly affect either group. The results for PM NMDAR were more difficult to interpret. DIO decreased total NMDAR levels, which was not affected by gammabody treatment. However, PM NMDAR levels were significantly reduced in DIO animals receiving the A β 33-42 treatment compared to DIO-Control animals, and the A β 33-42 treatment restored PM NMDAR to Chow diet levels. Downregulation of total NMDAR levels in response to DIO has been reported in similar studies examining HFD without induction of T2D (45, 72). These results suggest that the decrease in total NMDAR may, perhaps, serve to attenuate overexcitation produced from elevated PM localization of NMDAR. The decrease in PM NMDAR with A β 33-42 infusion further suggests that increased NMDAR trafficking may be a result of A β -induced perturbation of glutamate signaling. However, these suggestions are speculative, as mechanisms driving alterations in NMDAR localization cannot be determined from this study.

AMPA and NMDARs are important for synaptic transmission and essential for learning-associated processes including synaptogenesis and CREB activation; these actions are dependent upon the receptors being adequately trafficked to the plasma membrane (73-75).

However, these receptors are also responsible for mediating cell death and inhibiting synaptic growth, and are heavily implicated in AD pathology (76-78). Our measures of PM receptor levels did not differentiate between synaptic versus extrasynaptic receptor location. Previous studies found that DIO caused an early pathological increase in glutamate receptor expression and/or activity (9, 79-82), consistent with our findings and in contrast to later-stage AD, where increased endocytosis and decreased expression of glutamate receptors is seen (83-86). One possibility is that downregulation of glutamate receptors in the AD brain may be a protective adaptation to increased PM glutamate receptor expression such as that seen in our DIO animals and reversed by the A β 33-42 treatment, which would potentially cause overexcitation and perhaps excitotoxicity. However, additional work is needed to test this possibility and further characterize the molecular changes that cause transition from DIO to T2D and into AD.

Some studies using antibodies against specifically dimeric A β have shown reversal of amyloid-induced impairment of hippocampal slice LTP *in vivo*, and prevention of glutamate dysregulation in hippocampal neurons *in vitro* (87, 88). This is consistent with several lines of work that have identified glutamatergic dysregulation as being a major transducer of A β -related cognitive and neural impairment: A β selectively depletes glutamatergic terminals within the hippocampus (89), and glutamate release is increased following A β administration (90). Most studies on this topic have focused on NMDAR, finding that A β can bind to NMDAR directly and that in some cases A β may act to abnormally elevate NMDAR-mediated neuronal firing (91, 92), while elevated levels of NMDAR2B have been noted in transgenic AD mice (53). Increased NMDA-mediated firing may also mediate DIO-induced hippocampal excitotoxicity and cognitive impairment, which are reversed by the NMDAR antagonist, and approved AD treatment, memantine (9). Additionally, pretreatment with NMDAR antagonists prevents cell

death produced by an infusion of A β (93). Our data support identification of glutamatergic dysregulation as a key downstream transducer of DIO-induced cognitive impairment, and identify the novel possibility that an abnormal increase in AMPAR translocation to the plasma membrane may be a central element of this dysregulation: the A β 33-42 gammabody treatment that reversed the DIO-associated impairment in CCF performance also reversed the significant abnormal elevation in PM AMPAR seen in DIO rats.

Several previous reports are consistent with this hypothesis. In one study, a high-sucrose diet used to induce a pre-diabetic state increased the expression of the AMPR subunit GluR1 in the hippocampus (71) while impairing spatial memory; similar data have been obtained in the retina and spinal cord (81, 82). Mice fed a HFD show impaired glutamate breakdown and transmission (45), and direct knockdown of hypothalamic insulin signaling impaired both LTP and AMPAR function (47). Moving from DIO to AD, studies suggest that oligomeric A β can directly impair AMPAR localization to the plasma membrane, with the researchers hypothesizing that this dysregulation may be a mechanism for cognitive and synaptic dysfunction in AD (83-85). Interestingly, in those studies administration of A β was correlated with a reduction in cell-surface expression rather than the marked increase seen here following a HFD. It is possible that this may be a key difference in DIO-related development of AD as opposed to other causes of the dementia, or reflect a difference between our *in vivo* findings and the impact of large doses of exogenous A β . The difference may also reflect different stages in the etiology of cognitive impairment: elevation in PM AMPAR would be consistent with an adaptive response to ongoing loss of glutamatergic release. Lastly, there is evidence to suggest that AMPAR activity can suppress A β production and enhance the cleavage of non-toxic forms of A β (94). Hence, the increase in PM AMPAR seen here might be in part a compensatory

mechanism by which neurons are attempting to counteract elevated levels of toxic A β . The role of hippocampal AMPAR in both DIO- and AD-associated cognitive impairment merits further study as a possible target for therapeutic intervention.

The use of antibodies without A β conformational specificity to block deleterious effects of A β has been shown effective in studies both *in vitro* and *in vivo*, albeit with the latter using large volumes of full-sized antibodies in anesthetized animals over a long period (59, 95). Similar approaches have been attempted to sequester and remove A β as a therapeutic intervention in AD with mixed success; such approaches have shown no consistent improvement in cognitive performance even in transgenic mouse models that show improvements in amyloid pathology (96-99). Intrahippocampal delivery, as used here, is clearly not plausible for therapy in humans. However, the efficacy of gammabodies with preferential affinity for oligomeric A β in improving cognitive performance, combined with their small size, suggests a possible approach to improving the efficacy of anti-amyloid immunotherapeutic approaches. Moreover, the conformational selectivity of these domain antibodies may reduce the potential for negative side-effects associated with blockade of monomeric A β . Although in these studies a unilateral treatment was sufficient to fully reverse cognitive impairment, one additional possibility is that bilateral (or i.c.v.) gammabody administration might be required for maximum effect in more pathological models of oligomeric A β accumulation such as AD or long-term T2D.

Taken together, our results extend prior work demonstrating a clinical link between DIO and AD by identifying mechanistic causes for DIO- and A β -induced impairment on hippocampally-mediated tasks, suggesting that glutamatergic dysregulation may be a key component of the increased risk for AD seen as a consequence of DIO. Moreover, we show that DIO induces a pre-AD state in which hippocampal oligomeric A β may produce cognitive

impairment, with blockade of oligomeric A β reversing cognitive impairment and restoring hippocampal plasma membrane glutamate receptors to a control level.

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Figure 1: (A) Rats fed a high fat diet (DIO, n=18) weighed more than chow fed rats (n=16). (B) DIO rats had larger epididymal fat pads than rats fed regular chow (C). DIO rats had higher fasting plasma blood glucose levels than rats fed regular chow. * indicates a significant effect of diet ($p<0.05$).

Figure 2: (A) Leftmost two bars show that DIO animals (n=15) were significantly impaired compared to chow-fed (n=18) animals in their contextual retrieval as measured by freezing time.

* indicates that baseline DIO rats significantly differ from baseline Chow rats ($p<0.05$). The

remaining bars show that memory for a fearful context, measured by freezing when replaced in that context, was not significantly altered by administration of the control gammabody to rats in either diet group. Administration of the A β 33-42 gammabody did not significantly alter performance of chow-fed rats, but improved memory in DIO compared to their baseline-DIO score, causing an improvement in performance to the level of chow-fed animals. N = 6-7 per group. ^ indicates a significant increase in freezing time following administration of A β 33-42 to DIO rats ($p < 0.05$). The effect of treatment on freezing time is more clearly demonstrated in panel B, which depicts the within-animal change in freezing time after treatment and hence corrects for chance variation in the baseline freezing performance of animals randomly assigned to each treatment condition. Only the DIO-gammabody group showed a significant change in performance from baseline after treatment.

Figure 3: Hippocampal plasma membrane (PM) AMPAR levels were altered both by diet and by the interaction of diet and gammabody treatment. Planned group comparisons indicated that DIO/Untreated (n=17) hippocampi had significantly increased levels of PM AMPAR compared to the hippocampi of Chow/Untreated (n=13) rats (* indicates $p < 0.01$). In addition, treatment of DIO animals with the Aβ33-42 gammabody (n=9) significantly reduced the PM AMPAR level (** indicates $p < 0.01$), to the level seen in chow-fed animals.

Figure 4: Neither diet nor gammabody treatment affected total expression levels of hippocampal AMPA receptor.

Figure 5: Hippocampal plasma membrane (PM) NMDAR levels were marginally affected by the interaction of diet and gammabody treatment ($p=0.056$ for interaction). Post hoc analysis showed that among DIO rats, PM NMDAR were higher in hippocampi treated with the Control gammabody ($n=5$) than in hippocampi treated with the Aβ33-42 gammabody ($n=8$) (* indicates $p<0.05$), but that neither group differed significantly from PM NMDAR levels in untreated hippocampi from DIO animals ($n=17$).

Figure 6: Total levels of hippocampal NMDAR were reduced in the DIO group. However, no significant post hoc differences were present between the groups.