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Amyloid-β oligomers in the Nucleus Accumbens Decrease Motivation *via* Insertion of Calcium-Permeable AMPA Receptors

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Abstract

It is essential to identify the neuronal mechanisms of Alzheimer's Disease (AD)-associated neuropsychiatric symptoms, e.g., apathy, before improving the life quality of AD patients. Here, we focused on the nucleus accumbens (NAc), a critical brain region processing motivation, also known to display AD-associated pathological changes in human cases. We found that the synaptic calcium permeable (CP)-AMPA receptors (AMPARs), which are normally absent in the NAc, can be revealed by acute exposure to Aβ oligomers (AβOs), and play a critical role in the emergence of synaptic loss and motivation deficits. Blockade of NAc CP-AMPARs can effectively prevent AβO-induced downsizing and pruning of spines and silencing of excitatory synaptic transmission. We conclude that AβO-triggered synaptic insertion of CP-AMPARs is a key mechanism mediating synaptic degeneration in AD, and preserving synaptic integrity may prevent or delay the onset of AD-associated psychiatric symptoms.

Brief

Amyloid-β oligomers in the nucleus accumbens lead to excitatory synapse loss and reduced motivation due to activation of calcium-permeable AMPA receptors.

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Author contributions

Experimental design: CG, MZ, TM, YM. Data collection: CG, DW, YZ, RM, BM, YM. Data analysis: CG, DW, YZ, YM. Paper writing: CG, DW, TM, YM.

Conflict of interest

The authors declare no competing interests.

Keywords

Amyloid-β oligomers; motivation; nucleus accumbens; medium-sized spiny neuron; medial prefrontal cortex; synaptic plasticity; calcium-permeable AMPA receptors; thin spine; silent synapse

Introduction

Among a broad spectrum of Alzheimer's Disease (AD)-associated symptoms, progressive decline of cognition has been extensively investigated[1]. However, limited symptomatic relief in AD patients by available medications demonstrates lack of understanding of the neuronal and circuit substrates of AD, especially the AD-associated neuropsychiatric symptoms (NPSs)[2]. A meta-analysis of 12 NPSs reported in the *NeuroPsychiatric Inventory* identified apathy as the most common NPS in AD patients and the primary cause of caregiver distress[3]. Specifically, apathy, characterized by lack of motivation, is commonly diagnosed in pre-dementia stages, deteriorates as the disease progresses, and predicts phenoconversion from normal cognition to mild cognitive impairment (MCI), and from MCI to dementia[4–7]. Thus, apathy has been widely accepted as a predictor of human AD progression[8]. Unfortunately, little is known about the neuronal mechanisms of AD-associated apathy.

A substantial body of evidence, from both clinical and laboratory studies, demonstrates that the cortical and hippocampal regions are primarily affected in AD[9, 10]. Subcortical structures, including striatum, have been gradually uncovered with significant atrophy in AD by human imaging studies[11]. Among multiple sub-regions of striatum, the nucleus accumbens (NAc), as the major component of the ventral striatum and enriched with excitatory innervation from, among others, the cortex and hippocampus, displayed significantly higher densities of neurofibrillary degeneration[12, 13]. In fact, atrophy of the NAc was found in both MCI and AD human cases[11]. Smaller volume of the NAc, considered as a predictor of AD onset within 2 years, was proportionally associated with increased risk of transition from MCI to AD[14]. Extracellular amyloid Beta (Aβ) plaques, well-investigated molecular events in AD subjects[15, 16], have been detected in the NAc but absent from age-matched non-AD controls[17, 18]. Furthermore, cortical activation, specifically the glutamatergic input from the medial prefrontal cortex (mPFC), is required for the functional activation of corticostriatal synapses onto medium-sized spiny neurons (MSNs), the major neuronal population of the NAc. This cortical excitatory innervation, although essential for normal MSN function, also confers vulnerability of MSNs in the NAc of AD subjects due to AD-associated cortical damage[19–21].

Aβ peptides, particularly the non-aggregated, soluble assemblies of Aβ oligomers (AβOs), are known to be the main source of toxicity[22, 23]. Although extensive investigations have been made on Aβ peptides in relation to cognition impairments in corresponding brain regions such as hippocampus and cerebral cortex[24–26], little is known about the involvement of Aβ peptides in AD-associated NPSs. Synaptic loss in AD, hypothesized to be a consequence of the amyloid cascade[27], could be caused by glutamate excitotoxicity, which happens much earlier than complete neuronal loss[28–31]. While there is a consensus

in the AD field that deranged Ca^{2+} homeostasis is the main cause of synapse and cell loss[32–34], the exact source of excessive intracellular Ca^{2+} flux remains obscure. As one of the major types of ionotropic glutamate receptors, AMPA receptors (AMPARs) are typically GluA2 subunit-containing and Ca^{2+} impermeable (CI). In addition, an atypical form of the AMPA receptor, the GluA2-lacking Ca^{2+} permeable (CP) AMPAR, is widely recognized as an indicator of brain dysfunction when present in adult brains[29]. Here we focused on the role of CP-AMPA receptors, particularly in the mPFC projection to D2-receptor expressing MSNs, in AβO-induced low motivation and excitatory synapse loss in the NAc.

Methods

(more details are available in Supplementary Information)

Animals

All procedures were performed in accordance with the United States Public Health Service Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use committee at Indiana University School of Medicine.

In vivo procedures

Surgical procedures, including guide cannula and catheter implantation, and non-surgical *in vivo* procedures, including self-administration, were done as described in publications from our group[35–37] and others[38–40].

In vitro procedures

Brain slices for whole-cell patch clamp recordings were prepared by standard procedures as detailed in our previous publications[35–37, 41, 42]. These slices were also used for Western blot analysis, immunofluorescent staining, and DiI spine staining.

Data Analysis

Detailed statistics information for Figs.1–5 is available in Tables.S1–S5.

Results

Intra-NAc delivery of AβOs decreases motivation

The first question we addressed was about the behavioral effects of acute delivery of AβOs into the NAc. 3-month-old C57BL/6J (C57) mice were bilaterally injected with PBS vehicle (Veh), Aβ scrambled (AβSs), Aβ monomers (AβMs) or AβOs (including trimer, tetramer and larger soluble oligomers as shown in Fig. S1A) in the NAc, followed by behavioral training and testing within 3 weeks (Fig.1A,D). Similar acquisition of sucrose oral self-administration (SA) under fixed ratio (FR) 1 and FR5 was observed in Veh, AβSs, AβMs, and AβO mice, indicating the intra-NAc injection of AβSs, AβMs and AβOs has no apparent effect on the operant learning and memory-associated behaviors (Fig.1E). However, we saw significant decreases of the break point (BP) value in AβO mice, relative to Veh, AβS or AβM mice, to take sucrose pellets under PR schedule with no pre-feeding sessions (Fig.1F–H). In the Veh mice, the 1h pre-feeding by sucrose pellet right before the PR test

led to a significant decrease of the BP value compared to that without pre-feeding session, indicating that sucrose taking behaviors include a significant motivational component, which can be devaluated by 1h pre-feeding. This pre-feeding-devaluatable component of BP value was absent in AβO, but not AβS or AβM, mice. Thus, a decreased motivation index was detected in these AβO mice (Fig.1I).

Further evaluation of positive reinforcer-driven behaviors in AβO mice used cocaine intravenous SA (IVSA) (Fig.1A,J). The dose-response curve of IV cocaine at doses between 0.1–3.0 mg/kg per infusion under FR1 in AβO mice, compared to Veh mice, was shifted downwards and flattened (Fig.1M). The decreased number of active lever presses (ALP), but not inactive lever presses (ILP), was demonstrated in AβO mice at the representative cocaine dose of 0.3 mg/kg per IV infusion (Fig.1K,L). Furthermore, the PR test showed a decreased BP value in AβO *vs.* Veh mice (Fig.1O,P). With regard to the effects of intra-NAc AβO delivery on the despair-like behavioral output driven by an aversive condition, such as in the forced swimming test (FST), we found that AβO mice displayed increased immobility % time (Fig.1B,C). Additional behavioral tests showed that, relative to Veh, AβS or AβM mice, AβO mice showed no differences in the total distance traveled (Fig.S1B,C) and time distribution between central *vs.* peripheral areas (Fig.S1D) in the 5-min open field (OF) test, or the crossover latency in the light-dark transition (LDT) test (Fig.S1E,F). These data reaffirmed our conclusion of significant decreases of motivation to counter negative stimuli and to earn positive rewards in intra-NAc AβO mice. Notably, these mice showed no deficits in operant learning and memory, general locomotion, or anxiety levels, thus other alternative explanations of low motivation could be excluded. Finally, the sucrose SA behavior was also evaluated in mice with intra-DLS delivery of AβOs. We found no difference of sucrose SA acquisition by FR1 and FR5 training, or motivation to take sucrose pellets in the PR test between intra-DLS Veh *vs.* AβO mice (Fig.S2). Thus, low motivation occurred as a NAc-specific consequence of AβOs.

Intra-NAc delivery of AβOs increased synaptic CP-AMPARs, blocking of which restored motivation to take sucrose pellets.

Next, we wondered about the potential synaptic and molecular substrates of AβO effects in the NAc that might be targeted to prevent behavioral deficits, i.e., low motivation. Glutamatergic inputs, particularly those mediated by AMPARs in the NAc, have been identified as the critical substrates of motivational behaviors[39, 43–46]. Thus, the GluA1 and GluA2 AMPAR subunits were quantified in synaptosome extracts from the NAc in mice with intra-NAc delivery of Veh or AβOs (Fig.2A,B). AβO mice showed a significant decrease of AMPAR GluA2 but not GluA1 subunits in the NAc (Fig.2C), indicating a potential synaptic increase of GluA2-lacking CP-AMPARs. This was verified by whole-cell patch clamp recordings from MSNs in the NAc (Fig.2D). The amplitude of electrically evoked AMPAR-mediated EPSCs in AβO, but not Veh, mice displayed significant rectification (i.e., lower rectification index, RI) at positive membrane potentials (Fig.2E) due to intracellular polyamine blockade of CP-AMPARs[47]. Also, the CP-AMPAR antagonist, Naspm, decreased the amplitude of AMPAR-mediated EPSCs in AβO mice (Fig.2F,G). A decreased RI and Naspm-induced decrease of EPSC amplitude could either be explained by increased CP-AMPARs, decreased CI-AMPARs, or combined effects from

both[43, 48]. The RI=1 and no response to Naspm in Veh mice indicated there are no detectable CP-AMPARs in the NAc. Thus, the AβO mice certainly had newly inserted CP-AMPARs in the NAc, while CI-AMPARs could be decreased or unchanged. Further explorations of AMPAR changes were made in our slice AβO studies below. After confirming the increase of CP-AMPARs in AβO mice, we wondered whether the motivation deficits in these AβO mice could be prevented by blocking the CP-AMPARs in the NAc. Considering that cocaine-taking behaviors could involve more than the motivational component[49, 50], and due to the significant stress and relatively high variability of the forced swimming test[51], sucrose SA was selected as the behavioral readout to address this question. Daily intra-NAc injections of Naspm through the pre-implanted guide cannula, at least 1h after the post-training food-pellet feeding session, if there was any, were done during the 1st, 2nd, and the 3rd week, respectively in three batches of C57 mice, after intra-NAc delivery of the AβOs (Fig.2A). We found that daily injections of Naspm in Week 1 (before acquiring sucrose SA, Fig.2H–M), but not Week 2 (during acquisition of sucrose SA, Fig.S3A–D) or Week 3 (when sucrose-taking motivation was tested, Fig.S3E–G), effectively rescued the motivation of AβO mice to orally take the sucrose pellets as indicated by the BP values in the PR test of sucrose SA. The week 1 Naspm-treated AβO mice showed no deficits in their operant learning under FR1 and FR5 schedules and their expression of the sucrose-taking motivation (Fig.2I). Our data indicate that CP-AMPARs in the NAc, specifically right after intra-NAc delivery of AβOs, are necessary for the instigation of motivation deficits.

AβOs affect the pre- and post-synaptic compartments in striatal MSNs

What is the potential origin of excitatory inputs involved in the synaptic effects of AβOs in the NAc? MSNs in the NAc are innervated by multiple sources of excitatory terminals, including those containing vesicular glutamate transporters (VGluT) 1^+ (primarily from mPFC, hippocampus, basolateral amygdala and cerebellar cortex) and those containing VGluT2^+ (primarily from thalamus, brainstem and deep cerebellar nuclei)[52–54]. Thus, the VGluT1/VGluT2 expression in excitatory terminals was used to identify the potential source of inputs involved in AβO-induced synaptic alterations. PSD95, as a post-synaptic membrane-associated scaffolding protein located at the head of MSN dendritic spines[55, 56], was co-stained with VGluT1 or VGluT2. Both 0.5h and 3h AβO pre-incubation (denoted pre-AβO 0.5h and pre-AβO 3h) of acutely cultured-brain slices from naïve 3-month-old C57 mice decreased the volume of PSD95 puncta in the NAc. However, pre-AβO 3h, but not pre-AβO 0.5h, decreased the density of PSD95, reduced both the volume and density of VGluT1 puncta, and decreased the VGluT1^+ excitatory synapses in the NAc (Fig.3 A–G). Similar changes in PSD95^+ puncta were observed after co-staining of VGluT2 and PSD95 in the NAc, i.e., pre-AβO 0.5h decreased their volume but not the density, and pre-AβO 3h decreased both the volume and the density (Fig.S4). However, no changes in both volume and density of VGluT2^+ puncta or the density of VGluT2^+ excitatory synapses were detected in the NAc (Fig.S4). Similar analyses were done in the DLS. We found no change of VGluT1 and VGluT2 signals, although the volume, but not the density, of PSD95^+ puncta in the DLS decreased after pre-AβO 3h (Figs.S5,S6). Thus, although AβOs cause synaptic degeneration in both the NAc and the DLS, synaptic contacts in the NAc, particularly the post-synaptic compartment, are more vulnerable to AβOs; compared to reduced density, the decreased volume of dendritic spines could be an early neurodegenerative event; and

VGluT1^{+} (e.g., the mPFC-NAc projection), relative to VGluT2^{+}, terminals are also more sensitive to AβOs. Our following studies focused on the excitatory synapses in the NAc, particularly the postsynaptic compartment at the mPFC-NAc projection.

AβOs affect synaptic glutamate receptor subunits in the NAc

Was there an upregulation of synaptic CP-AMPARs in the NAc when the acutely cultured brain slices were pre-incubated with AβOs? To answer this question, synaptosomes from the NAc were extracted after AβO pre-incubation of brain slices prepared from naïve 3-month-old C57 mice (Fig.3H). Western blot analyses showed that pre-AβO 3h, but not pre-AβO 0.5h, decreased protein levels of GluN1 (Fig.S7A,B), indicating a potential decrease of synaptic NMDARs. The GluA1 subunit of AMPARs in NAc synaptosome extracts was also down-regulated by pre-AβO 3h, but not pre-AβO 0.5h (Fig.3I,J).The GluA2 subunit was significantly decreased after pre-AβO 0.5h, and was further decreased after another 2.5h (i.e., 3h in total) AβO pre-incubation (Fig.3L,M). Decreased protein levels of both GluA1 and GluA2 subunits indicated down-regulation of total AMPARs. Specific decrease of the protein levels of GluA2 but no changes in GluA1 by pre-AβO 0.5h indicates the possibility of increases in GluA2 lacking CP-AMPARs in the NAc, which could be accompanied by a decrease in synaptic GluA2-containing, calcium impermeable (CI)-AMPARs to explain the lack of changes in GluA1 subunit protein levels. Interestingly, our data showed a significant increase in the ratio of GluA1/A2 protein levels in the NAc synaptosome fraction after pre-AβO 0.5h, and was further increased after another 2.5h (i.e., 3h in total) AβO pre-incubation (Fig.3O). Thus, it is likely that the synaptic CP-AMPARs in the NAc progressively increased with the passage of AβO pre-incubation.

Further Western blot analyses were performed to identify the temporal course of AβO effects. Compared to pre-AβO 0.5h, significant decreases of the glutamate receptor subunit GluN1 (Fig.S7C) and GluA2 (Fig.3N), but not GluA1 (Fig.3K), occurred in the slices treated with pre-AβO 3h, and the slices treated with pre-AβO 0.5h, immediately followed by 2.5h artificial cerebrospinal fluid (ACSF) pre-incubation (denoted pre-AβO 0.5h+). Interestingly, there were no differences between pre-AβO 0.5h+ *vs.* pre-AβO 3h in any of the 3 types of glutamate receptor subunits. Thus, we assumed that the further increase of CP-AMPARs after pre-AβO 0.5h was independent from AβO exposure.

AβOs decrease the density of dendritic spines in NAc MSNs, which can be prevented by blocking CP-AMPARs

In order to identify structural changes in the post-synaptic compartment in the NAc after AβO exposure, morphological analyses of the dendritic spines in MSNs of the NAc were performed on acutely cultured-brain slices from naïve 3-month-old C57 mice *via* DiI staining (Fig.4A). Pre-AβO 3h, but not pre-AβO 0.5h, decreased the total spine density (Fig.4B). Interestingly, both pre-AβO 0.5h and pre-AβO 3h decreased the non-thin spine density (Fig.4D) and increased the ratio of thin *vs.* non-thin spines (Fig.4F), which were accompanied by different changes of thin spines (Fig.4E). The increased thin-spine density observed after pre-AβO 0.5h, but not pre-AβO 3h, were assumed to represent an early synaptic event before spine pruning. The increased thin-spine ratio, which was observed after both pre-AβO 0.5h and pre-AβO 3h, was considered as an index of the subsequent

spine pruning. The continuous spine pruning after pre-AβO 3h was confirmed by further spine decreases in slices treated by pre-AβO 3h followed by 3h of ACSF (Fig.4G). These data indicate that the AβO-induced dendritic spine loss might be initiated during a stage when the pre-existing non-thin spines are degraded or replaced by thin spines.

In order to support the involvement of CP-AMPARs in AβO-triggered progressive degeneration of dendritic spines in the NAc, DiI staining-based morphological measurements of dendritic spines were compared between the slices pre-incubated with Veh/AβOs only *vs*. Veh/AβOs and Naspm (Fig.4H). We found no effects of Naspm on the density of total spines, thin spines, and non-thin spines, or the ratio of thin *vs*. non-thin spines in the Veh-pre-incubated slices (denoted pre-Veh; no difference between pre-Veh 0.5h *vs*. 3h, thus data were pooled together in the first pair of columns in Fig.4I–L), indicating that no detectable or functional CP-AMPARs in nonAβO-treated NAc slices, which is consistent with previous data collected from rat NAc[39, 40, 57, 58]. The increased ratio of thin *vs*. non-thin spines by pre-AβO 0.5h was significantly prevented by 0.5h Naspm co-pre-incubation (denoted co-pre-Naspm 0.5h) to the level of Veh groups with no accompanying effects on the density of total spines (the 2nd pair of columns in Fig.4 I–L). Furthermore, 3h Naspm co-pre-incubation (denoted co-pre-Naspm 3h) brought the density of non-thin and total spines, and the ratio of thin *vs*. non-thin spines back to the pre-Veh control level (the 4th pair of columns in Fig.4 I–L). 3h Naspm co-pre-incubation of the slices treated with pre-AβO 0.5h+ can significantly rescue the density of total/thin/non-thin spines and the ratio of thin *vs*. non-thin spines (the 3rd pair of columns in Fig.4H–K). The loss of total spines and the non-thin spines under both of the 3h procedures, i.e., pre-AβO 0.5h+ and pre-AβO 3h, was a synaptic consequence triggered by acute exposure to AβOs and dependent on the activation of CP-AMPARs. AβO-induced CP-AMPARs might be involved in both early downsizing of non-thin spines to thin spines and the prolonged spine pruning process.

Different from the co-pre-incubation of Naspm by adding Naspm during the entire process of pre-incubation in Fig.4H–L, sequential Naspm pre-incubation for 0.5h (denoted sq-pre-Naspm 0.5h) was performed in slices right after pre-AβO 0.5h or 3h (Fig.4M–O). Remarkably, this sq-pre-Naspm reversed the effects of 0.5h, but not 3h, AβO-pre-incubation. Specifically, the decreased density of non-thin spines and increased ratio of thin *vs*. non-thin spines was reversible by Naspm right after pre-AβO 0.5h procedure. However, this sq-pre-Naspm 0.5h after pre-AβO 3h procedure had no effect on spine morphology, suggesting that the longer the incubation in AβOs, the less likely Naspm can block their toxic effects. Thus, AβO-triggered early increases of synaptic CP-AMPARs in the NAc can be targeted to reverse the acute synaptic alterations (presumably the scaling down of non-thin spines to thin spines) and prevent the prolonged synaptic consequences, such as decreased density of total spines and non-thin spines.

AβO-enhanced synaptic CP-AMPARs and silent synapses were specific to the excitatory mPFC projection on D2 MSNs in the NAc

Which specific excitatory projection onto the NAc participates in the AβO-induced synaptic recruitment of CP-AMPARs? This question was addressed experimentally by focusing on specific pre-synaptic inputs (mPFC) and post-synaptic neurons (D1 *vs*. D2 dopamine

receptor-expressing MSNs, denoted D1 and D2 MSNs, respectively) in the NAc. In order to identify the D1 *vs.* D2 MSNs involved in the synaptic effects of AβOs, D1 mice and A2a mice (see more details about the mouse lines and identification of D1 and D2 MSNs in Methods and Fig.S8A,B) were used at the age of 3 months. AAV-ChR2-EYFP was injected into the mPFC ~3 weeks before the slice preparation (Fig.5A). Using whole-cell patch clamp recordings and optical stimulation-evoked EPSCs on D1 *vs.* D2 MSNs in the NAc, synaptic CP-AMPARs were evaluated by the rectification index (Fig.5B) and by comparing before and during bath application of Naspm (Fig.5C–G). Relative to pre-Veh 3h treatment, pre-AβO 3h significantly rectified these EPSCs at positive membrane potentials in D2 MSNs. Pre-incubation of AβOs for 0.5h significantly increased the sensitivity of mPFC-NAcD2 synapses to Naspm (i.e., decrease of EPSC amplitude). Noticeably, the extended pre-incubation procedures (i.e., pre- AβO 0.5h+ and pre-AβO 3h) increased the sensitivity of EPSCs to Naspm, indicating that (1) this further % increase of synaptic CP-AMPARs in the mPFC-NAcD2 synapses after pre-AβO 0.5h-triggered enhancement of synaptic CP-AMPARs could be a consequence of potentially decreased total synaptic AMPARs, with or without further recruitment of synaptic CP-AMPARs, (2) this progressively increased ratio of CP-AMPARs among total synaptic AMPARs was independent of AβO-pre-incubation after 0.5 hr, although it was initiated by AβOs, and thus (3) the early recruited CP-AMPARs may be pivotal synaptic events in the further recruitment of CP-AMPARs.

Silent synapses are thought to be glutamatergic synapses containing stable NMDARs, while AMPARs are either absent or highly labile[59]. Because of early decreases of total AMPARs but not NMDARs and increases of CP-AMPARs (Figs.3H–O&S7), we hypothesized that acute AβO exposure-silenced excitatory synaptic transmission in the NAc could be prevented by blocking CP-AMPARs. We did see increased % of silent synapses in the mPFC-NAcD2 projection by pre-AβO 0.5h, pre-AβO 0.5h+, and pre-AβO 3h (Fig.5H–R). Furthermore, we found that pre-AβO 0.5h, pre-AβO 0.5h+, or pre-AβO 3h-increased silent synapses in the mPFC-NAcD2 projection could be prevented by Naspm co-pre-incubation for 0.5h, 3h and 3h, respectively (Fig.5R). However, neither pre-AβO 0.5h nor pre-AβO 3h affected the recruitment of synaptic CP-AMPARs or the percent of silent synapses in the mPFC-NAcD1 MSN projections (Fig. S8C–F). Together with the reported involvement of D2-MSNs in (1) both reward and aversion[60], and (2) greater susceptibility to excitotoxicity from cortical inputs[61, 62], we conclude that mPFC-NAcD2 projection is the synaptic substrate in AβO-induced low motivation.

DISCUSSION

The central goal of the present study was to identify the synaptic and behavioral effects of acute exposure of NAc to Aβ peptides. Thus, Aβ exposure was provided in a spatially and temporally delimited manner. Spatially limited amyloid aggregation demonstrates the effects specifically related to the target brain region, also avoids extraneous influences due to the amyloid aggregation in other brain regions. The central hypothesis was that AβO exposure of the NAc resulted in apathy. We discovered that synaptic CP-AMPARs, normally absent in the NAc, can be unmasked by acute exposure to AβOs, and contribute to the emergence of synaptic loss and motivation deficits. Further, we identified the excitatory mPFC projection onto D2 MSNs in the NAc as the critical input affected by AβO exposure.

Potential of CP-AMPARs as a novel target other than NMDARs:

After years of intensive investigation on NMDAR*s* in synaptic/neuronal excitotoxicity, there are several strong rationales to switch our attention from NMDARs to CP-AMPARs. NMDAR activation is essential for normal neuronal function. Administration of NMDAR blockers as anti-excitotoxicity, neuroprotective agents can block virtually all NMDAR-mediated activity, leading to unacceptable clinical side effects. Only statistically significant but clinically minor positive effects of memantine, a partial antagonist of NMDARs, on cognition have been observed in patients with moderate to severe AD, and no effect on mild to moderate AD[63, 64]. In contrast to NMDARs, which are usually inactive at resting membrane potential due to the channel blockade by Mg^{2+}, CP-AMPARs allow Ca^{2+} and Zn^{2+} entry at any level of receptor activation, and trigger mitochondrial dysfunction and cell death[65–68]. Enhanced CP-AMPARs have been detected in neurodegenerative disease models[69], but decreased, although in a few cases increased, NMDARs have been reported[70–74], suggesting more persistent effects of excitotoxicity from CP-AMPARs. Blockade of CP-AMPARs by CP-AMPAR antagonists has been shown to be neuroprotective in global ischemia[75]. Growing evidence demonstrates that CP-AMPARs, although highly enriched at the early developmental stage, have a low probability of being detected in mature brains unless there are associated pathological events, such as epilepsy, ischemia, traumatic brain injury, drug abuse, *etc*.[39, 76–78]. However, there is evidence to support the role of CP-AMPARs in AD-related neurodegeneration[69]. Postsynaptic density-rich fractions from AD patients' hippocampi showed a significant increase of GluA1 subunits relative to healthy controls, whereas no changes were observed in NMDAR subunits[79]. AβOs induced a rapid enhancement of synaptic CP-AMPAR insertion in hippocampal slices[80]. Thus, CP-AMPARs may be considered as an early marker indicating the onset of pathological progression in AD brains.

Reversible and non-reversible synaptic alterations:

We found that the AβO pre-incubation-induced synaptic alterations were fully prevented by adding Naspm during AβO pre-incubation. However, if Naspm is added right after AβO pre-incubation, the synaptic changes induced by 0.5 hr, but not 3 hr, can be prevented. Together with findings of no changes in the density of pre- and post-synaptic elements but potential transformation of non-thin spines into thin-spines and increased silent synapses by 0.5 hr AβO pre-incubation, we postulate that the CP-AMPAR enriched, rather than CP-AMPAR lacking, spines could indicate a reversible stage of synaptic degeneration before the elimination of synaptic components. Blocking CP-AMPARs from the beginning or early exposure to AβOs could be essential to rescue the pre-AβO 3h triggered synaptic degeneration. Density decreases of pre- and post-synaptic elements is the synaptic alteration specifically observed after 3h pre-incubation. Once this morphological degeneration of synaptic contacts occurred, blockade of CP-AMPARs could not fully rescue the synaptic morphology. This may explain our *in vivo* data showing that prevention of AβO-induced low motivation of sucrose-taking behaviors was achieved only if Naspm was delivered into the NAc during the first week, right after the intra-NAc delivery of AβOs, but not in the 2^{nd} or 3^{rd} week. Since Aβ deposition in the brain has been reported years earlier than the onset of AD-associated symptoms, our results indicate that CP-AMPARs should be targeted at the early stage when the Aβ deposition occurs.

No contradiction between decreased synaptic transmission/contacts *vs*. increased CP-AMPARs:

Ca^{2+} influx *via* enhanced synaptic CP-AMPARs may result in excitotoxic effects, and high channel conductance of CP-AMPARs may compensate for the loss of synaptic function, but the price of this compensation is to cause more synaptic loss. These dual roles of increased CP-AMPARs may lead to a self-enhanced excitotoxicity, a process possibly independent from AβOs and facilitating the synaptic degeneration in an accelerated manner. If this hypothetical CP-AMPAR-mediated self-enhanced excitotoxicity (CPAMSEE) is, at least partially, the primary pathological event in AD, one of the ways to delay AD progression is to target CP-AMPARs, but not the upstream pathological events if CPAMSEE is already underway. AβOs, presumably the neurotoxic species in AD brains[81–87], are able to trigger the CPAMSEE. Thus, our study provides a novel avenue (i.e., targeting CP-AMPARs) for AD clinical intervention, especially when failure of anti-Aβ antibodies in AD treatment has been reported[88]. It is worth emphasizing that the upregulation of CP-AMPARs does not always lead to excitotoxicity. For example, the elevated CP-AMPARs in NAc after a prolonged withdrawal period from cocaine exposure is proposed to strengthen excitatory synaptic transmission[39, 58, 89]. The synaptic consequences of CP-AMPAR insertion, including either weakening (i.e., excitotoxicity) or strengthening synaptic transmission, could involve pre- and post-synaptic mechanisms. Decreased pre-synaptic glutamate release could reduce the risk of excitotoxicity by preventing the over-activation of CP-AMPARs, which could explain the lack of synaptic degeneration after cocaine exposure. In contrast, continuous accumulation of CP-AMPARs, due to a positive feedback loop, may lead to CPAMSEE in AD brains. Further investigations need to be performed to understand the full complexity of synaptic effects of CP-AMPARs.

A number of limitations have to be acknowledged. **First**, although the temporally and spatially controlled Aβ exposure used in the present study made it possible to uncover a number of synaptic, molecular, and behavioral alterations in the NAc after acute administration, more studies need to be done before understanding the protracted pathological consequences of Aβ peptides in AD patients. Genetically engineered AD mice[90, 91] could be used to explore the role of chronic exposure to accumulated Aβ in AD-associated phenotypes. Although the current studies focused on AβOs, the potential effects of Aβ plaques with a cumulus of soluble AβOs should not be overlooked, as they may directly alter synaptic transmission or mimic the effects of AβOs[92]. **Second**, the microtubule-associated protein Tau undergoes aberrant hyperphosphorylation in AD-associated pathology, leading to neurodegeneration[93]. Thus, additional experiments need to be performed to tease apart the role of endogenous Tau in AβO-triggered, CP-AMPAR-mediated synaptic maladaptation. **Third**, although the AD-associated apathy is rarely relieved by DA-targeting medication[7], decreased levels of DAergic neurotransmission have been linked to the pathophysiology of AD[94]. As the NAc receives converging glutamatergic afferents from hippocampus, mPFC, etc., as well as DAergic afferents from the ventral tegmental area, the importance of DA modulation of excitatory neurotransmission through axoaxonic, axodendritic and axosomatic connections should not be neglected in AD-associated synaptic alterations.

In spite of these limitations, our study identified CP-AMPARs as a critical synaptic substrate underlying the initial effects of AβOs in the NAc, which may cause motivation decrease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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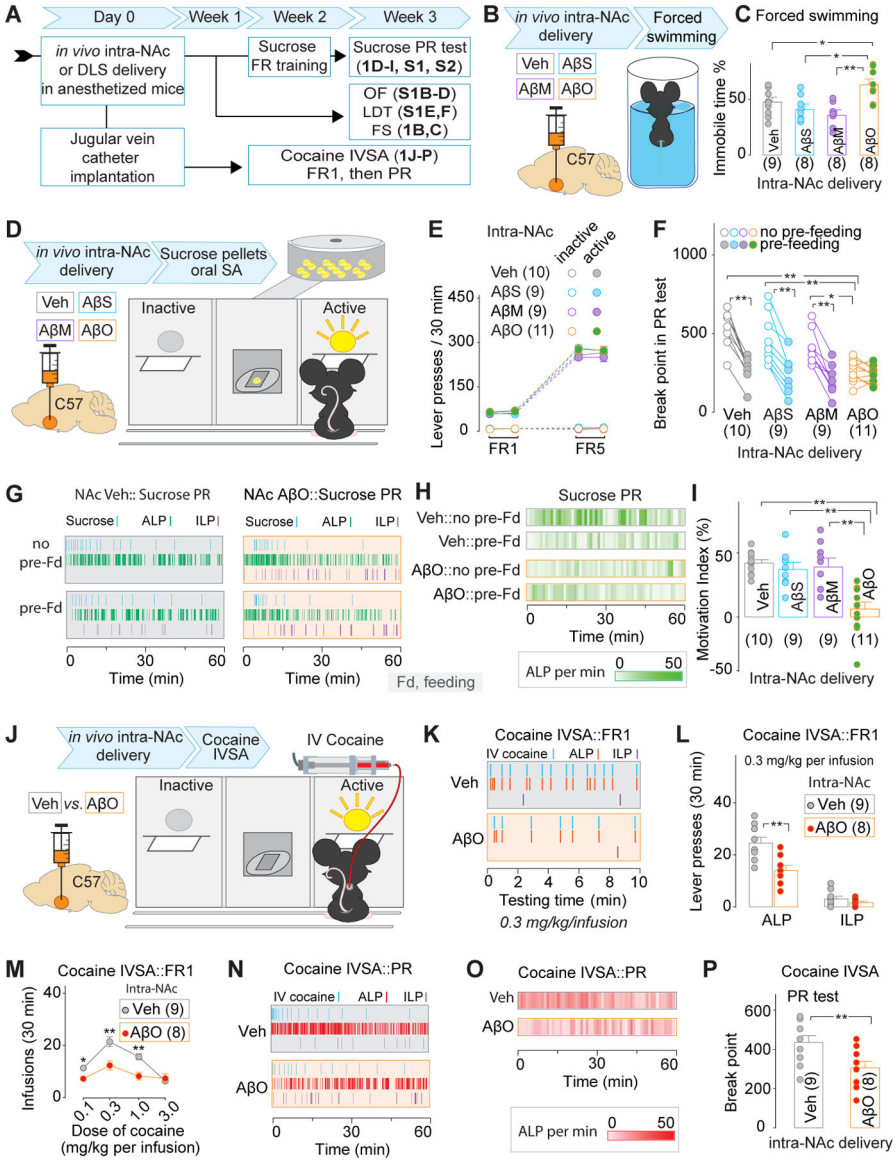

Figure 1. Behavioral effects of intra-NAc delivery of AβOs

A, Experimental timeline for data collection in Figs. 1, S1, and S2.

B, **C** Experimental procedure (**B**) and summarized data (**C**) showing that intra-NAc delivery of AβOs increased the % time of immobility in the forced swimming test.

D-I Experimental procedure (**D**) and summarized data (**E**) showing similar acquisition of sucrose SA between NAc-Veh and NAc-AβO mice under FR1 and FR5 schedules. Representative time course of lever presses and delivery of sucrose pellets (**G**) and the heat map of ALP frequency (**H**) during the 1st hour of PR test, and summarized data showing a significant decrease of BP value in NAc-Veh, but not NAc-AβO mice in the PR test with 1h pre-feeding session, compared to that with no pre-feeding session (**F**), leading to decreased motivation indices (**I**).

J-P, Experimental procedure (**J**), representative time course of lever presses and IV delivery of cocaine (**K**), and summarized data (the number of IV cocaine infusions at multiple doses

in **M**, and lever presses when the mice were treated with 0.3 mg/kg per IV infusion in **L**) showing decreased cocaine taking behaviors in NAc-AβO, compared to those in NAc-Veh, mice. Representative time course of lever presses and IV delivery of cocaine (**N**) and the heat map of ALP frequency (**O**) during the 1st hour of PR test, and summarized data (**P**) showing significant decrease of BP value in NAc-AβO, compared to NAc-Veh, mice in the PR test.

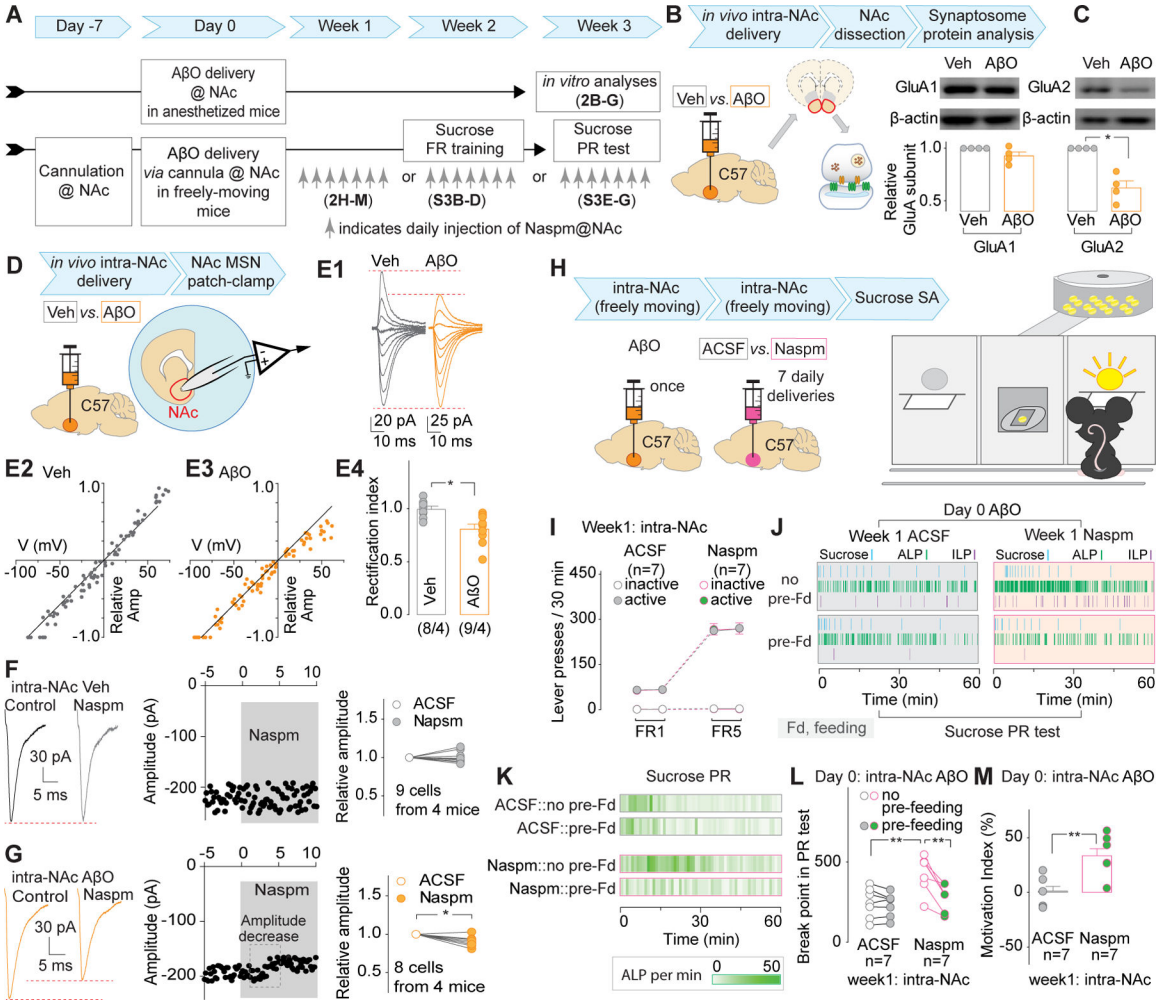

Figure 2. Involvement of NAc CP-AMPARs in sucrose self-administration in mice with intra-NAc delivery of AβOs

A. Experimental timeline of data collection in Figs. 2 and S3.

B, **C**, Experimental procedure (**B**) and representative Western blotting bands and summarized results (**C**) showing that intra-NAc delivery of AβOs decreased the protein level of GluA2 but not that of GluA1 in synaptosome extracts from NAc.

D-G, Experimental procedure (**D**), representative AMPAR-mediated EPSCs at −70 to +70 mV (**E1**), EPSC I-V relationships (**E2**, **E3**), and summarized results (**E4**) showing a decreased rectification index of MSNs in the NAc from NAc-AβO mice. Representative EPSCs (left), time course (middle), and summarized results (right) showing that CP-AMPAR antagonist Naspm decreased AMPAR-mediated EPSCs in MSNs in the NAc from NAc-AβO (**G**), but not NAc-Veh mice (**F**).

H-M, Experimental procedure (**H**) and summarized data (**I**) showing intra-NAc injection of Naspm during the first week after intra-NAc delivery of AβOs did not affect the acquisition of sucrose SA under FR1 and FR5 schedules. Representative time course of lever presses and delivery of sucrose pellets (**J**) and the heat map of ALP frequency (**K**) during the 1st hour of PR test, and summarized data (**L**) showing that daily microinjection of Naspm into the NAc during the 1st week after intra-NAc delivery of AβOs significantly increased the

BP value in the PR test with no pre-feeding session, compared to that in NAc-Veh mice. The AβO-decreased motivation indices were restored by daily intra-NAc delivery of Naspm during the 1st week after intra-NAc delivery of AβOs (**M**).

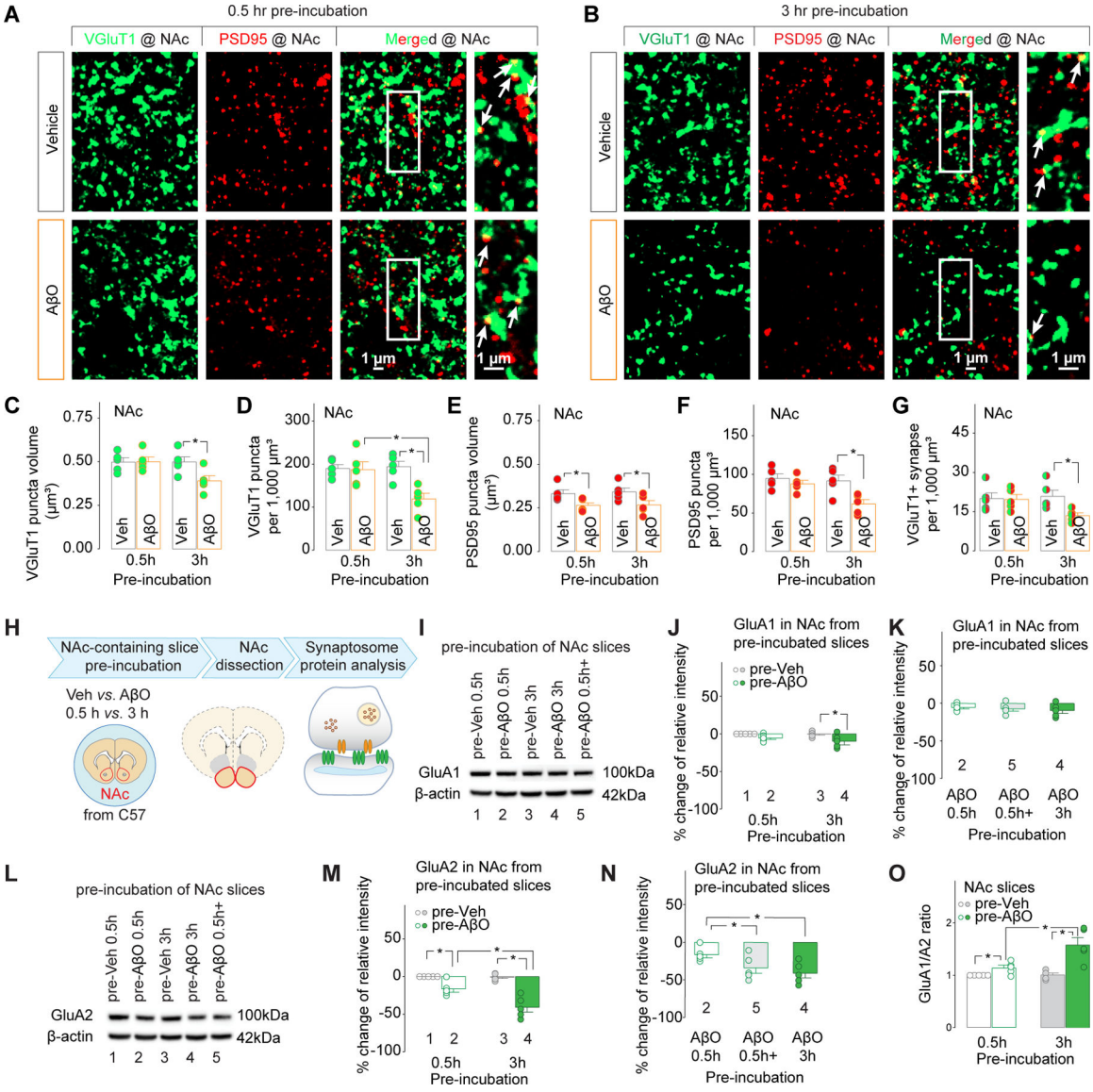

Figure 3. Effects of pre-incubation of striatal slices with AβOs on the morphology of VGluT1$^+$ excitatory synapses (A-G) and the protein levels of AMPAR subunits (H-N) in the NAc.

A-G, morphology of VGluT1$^+$ excitatory synapses in the NAc

A, B, Representative maximum projection confocal images of the NAc showing VGluT1, PSD95 and co-localization of VGluT1 and PSD95 (indicated by white arrows in the rightmost column) after 0.5h (**A**) and 3h (**B**) pre-incubation by Vehicle *vs.* AβOs.

C-G, Quantification of VGluT1$^+$ excitatory synapses in the NAc. 3h, but not 0.5h pre-incubation with AβOs decreased the volume (**C**) and the density (**D**) of VGluT1 puncta, the density of PSD95 puncta (**F**), and the density of VGluT1$^+$ synapses (**G**). Both 0.5h and 3h pre-incubation with AβOs decreased the volume of PSD95 puncta (**E**).

H-N, protein levels of AMPAR subunits (H-N) in the NAc.

H, Experimental procedure.

O, The ratio of GluA1/GluA2 protein levels was significantly increased by 0.5h pre-AβO incubation, and further increased by 3h pre-AβO incubation.

I-N, Representative Western blotting bands (**I**, **L**) and summarized data showing the effects of pre-AβO 0.5h, pre-AβO 0.5h+, pre-AβO 3h on GluA1 (**J**, **K**) and GluA2 (**M**, **N**) in synaptosome extracts of NAc from naive C57 mice. The number in each column in **J** & **K** and **M** & **N** corresponds to the data showing the representative band indicated by the same number in **I** and **L**, respectively.

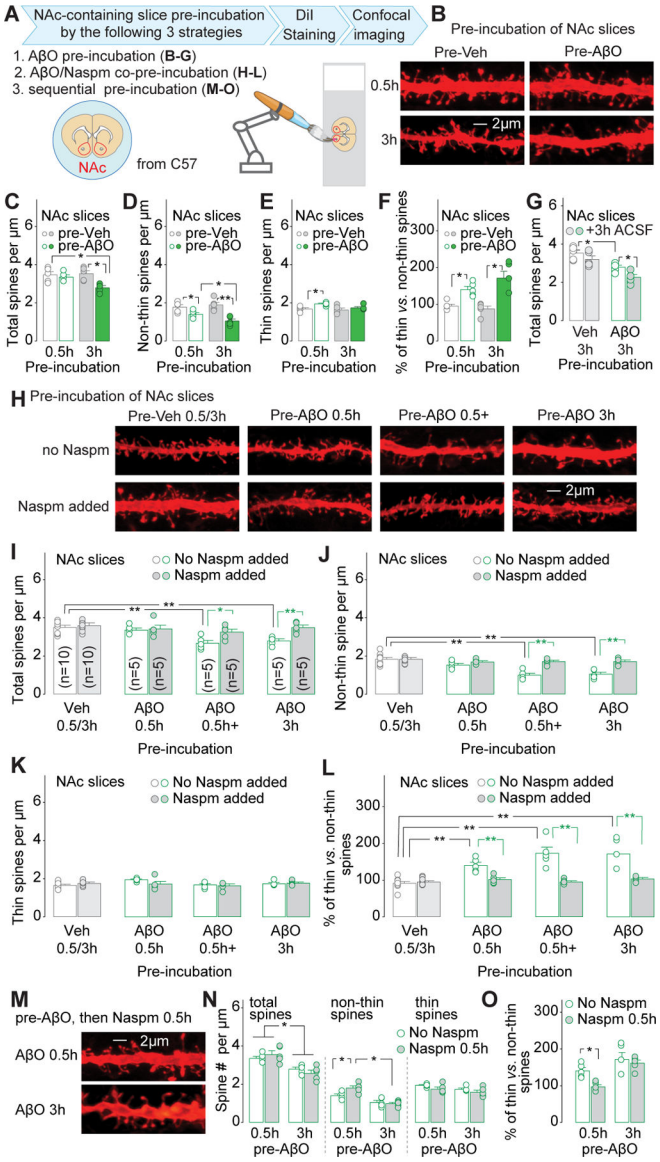

Figure 4. Effects of *in vitro* AβO pre-incubation of striatal slices on dendritic spine morphology in the NAc, which can be prevented by Naspm.

A, Experimental procedure

B-F, Representative maximum projection confocal images of the dendritic spines (**B**) and summarized data showing the effects of pre-incubation of brain slices with vehicle *vs.* AβOs for 0.5 or 3h on densities of total spines (**C**), non-thin spines(**D**) and thin spines (**E**), and the ratios of thin *vs.* non-thin spines (**F**).

G, Additional 3h-ACSF pre-incubation right after pre-AβO 3h decreased total spine density.

H-L, Representative maximum projection confocal images of the dendritic spines (**H**) and summarized data showing the effects of co-pre-incubation of Naspm with AβO pre-incubation on densities of total spines (**I**), non-thin spines(**J**) and thin spines (**K**), and the ratios of thin *vs.* non-thin spines (**L**).

M-O, Representative maximum projection confocal images of the dendritic spines (**M**) and summarized data showing the effects of 0.5h- and 3h-AβO pre-incubation, followed by

0.5h Naspm sequential pre-incubation, on densities of total spines, non-thin spines and thin spines (**N**), and the ratios of thin *vs.* non-thin spines (**O**).

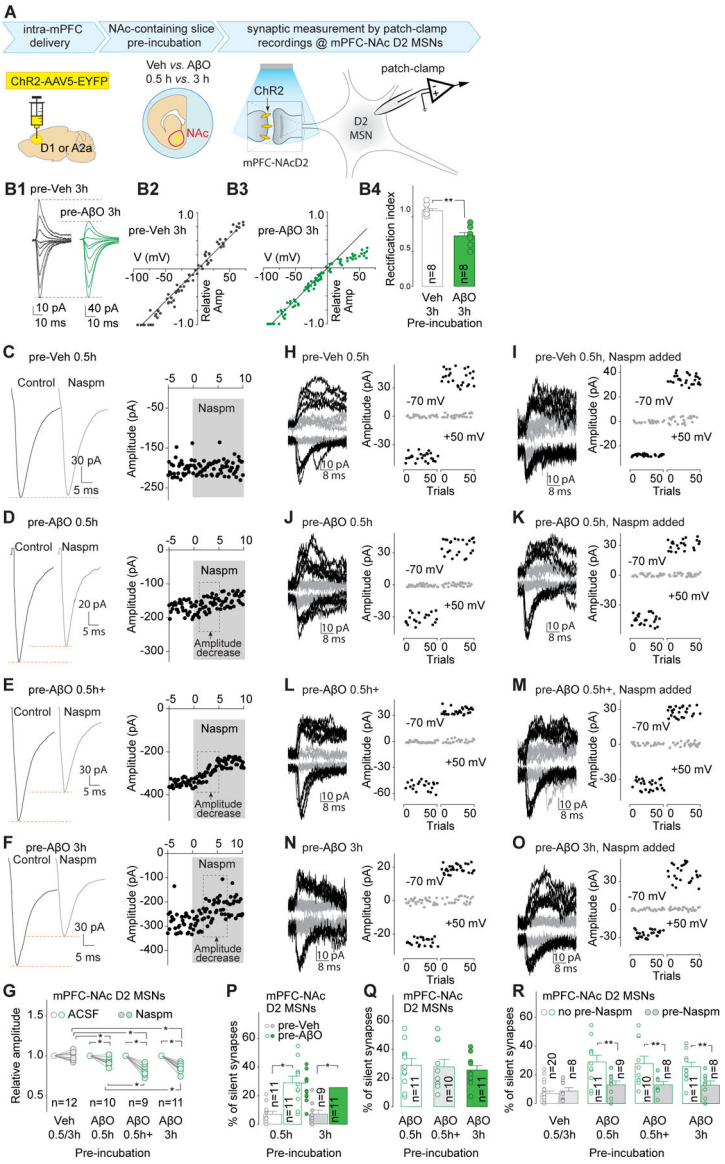

Figure 5. Effects of *in vitro* AβO pre-incubation of NAc slices on synaptic transmission in the mPFC projections to D2 MSNs.

A, Experimental procedure.

B, Representative EPSCs at −70 to +70 mV (**B1**), EPSC I-V relationships (**B2**, **B3**), and summarized results (**B4**) showing pre-AβO-3h decreased the rectification index in mPFC-NAcD2 projections.

C-G, Representative traces (**C-F**, left), time course (**C-F**, right), and summarized data (**G**) of optical stimulation-evoked EPSCs at −70 mV in the mPFC-NAcD2 projections showing that Naspm decreased the amplitude of EPSCs in the slices pre-incubated with 0.5h AβOs, 0.5h AβOs followed by 2.5h ACSF (i.e., 0.5h+ AβO) and 3h AβOs.

H-R, Representative traces of optical minimal stimulation-evoked EPSCs at −70 or +50 mV (**H-O**, left) over 100 trials (time course in the right panels in **H-O**) at the mPFC-NAcD2 projections and summarized data showing increased % of silent synapses by pre-AβO 0.5,

0.5+ and 3h relative to that in pre-Veh 0.5h / 3h groups (**P**, **Q**). Co-pre-incubation of Naspm prevented the increase of silent synapses by pre-AβO 0.5, 0.5+ and 3h (**R**).