Expanded View Figures

A List of α3-NKA peptides identified from neurons exposed for 10 min to fibrillar α-syn, cross-linked with DTSSP, pulled-down, reduced and alkylated, digested by trypsin, and analyzed by MS/MS through an α3-NKA-targeted identification with SEQUEST.

B MS/MS spectrum of α3-NKA peptide TVNDLEDSYGGQW TYEQR.

C MS/MS spectrum of α3-NKA peptide VVEFTCHTAFFVSIVVVQQ WADLICK with one N-terminal acetylation (+42 Da), two carbamidomethyl cysteines (Cys908 and Cys927, +57 Da), and one DTSSP cross-linked residue (S915 or K928, +145 Da).

Figure EV1. Assessment of the interaction between α3-NKA and α-syn cross-linking/MS.
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B MS/MS spectrum of α3-NKA peptide TVNDLEDSYGGQW TYEQR [884-901].
C MS/MS spectrum of α3-NKA peptide VVEFTCHTAFFVSIVVVQQ WADLICK [903-928] with one N-terminal acetylation (+42 Da), two carbamidomethyl cysteines (Cys908 and Cys927, +57 Da), and one DTSSP cross-linked residue (S915 or K928, +145 Da).
Figure EV2. \(\alpha\)-NKA diffusion in the presence of monomeric \(\alpha\)-syn and A\(\beta\) oligomers.

A, B Monomeric \(\alpha\)-syn-exposed (50 nM, 60 min, green) neurons showed no slowdown in diffusion coefficient of pHluorin-\(\alpha\)-NKA (A) (number of QDs: control, 674; monomer, 865, Kolmogorov–Smirnov test, \(* * P < 0.01\)). (B) No slowdown in the diffusion coefficient of \(\alpha\)-NKA following exposure (200 nM, 60 min) to A\(\beta\) oligomers (number of QDs: control, 674; A\(\beta\) oligomers, 450, Kolmogorov–Smirnov test, ns = non-significant).
**Figure EV3. In vivo synaptic clustering of fibrillar α-syn and association with α3-NKA following intra-striatal injection.**

A–D Distribution of fibrillar α-syn-ATTO-550 (red) 24 h after injection. (A) Limited spread (outlined by a dashed line) showing ATTO-550 signal. (B) Clusters of fibrillar α-syn (arrow). Note that the clusters are excluded from striato-pallidonigral axon bundles (*). Some of the fibrillar α-syn clusters co-localized with homer (Ca, Cc green) or gephyrin (Cb, Cc, blue) (plot: median, quartile, and min. to max. distribution). (Da-Db) Association of fibrillar α-syn clusters (red, Db) and α3-NKA (green, Da, Db) can be seen (arrow). Unless indicated, scale bars: 5 μm.
Figure EV4. α3-NKA-dependent Na+ deregulation by oligomeric but not monomeric α-syn.

A, B Neurons exposed to monomeric α-syn recover to basal level (A) and exhibit no change in Na+ pumping rate (B).

C, D Na+ imaging in the presence of ouabain (1 μM, 3–5 min before 0 mM K+ application) on control or oligomeric α-syn (25 nM) exposed cells. Both oligomeric α-syn-exposed (column 2) and ouabain-treated (column 3) neurons show reduction in the recovery (C) and Na+ pumping rate (D). Note no additive effect of ouabain on oligomeric α-syn-exposed (column 4) compared to control (column 3) neurons.

Data information: Plots represent median, quartile, and 0–100% distribution; Mann–Whitney U-test: *P < 0.05, **P < 0.01, ***P < 0.001; two (A, B) or three (C, D) independent experiments have been performed.

Figure EV5. Increase in glutamate-induced Ca2+ influx following α-syn exposure.

A, B DIV 16–19 striatal neurons were exposed (1 h) to oligomeric (25 nM, blue) or fibrillar (0.03 nM, orange) unlabeled α-syn. Unexposed control is shown in black. Glutamate-evoked (arrow, 100 μM, A) Ca2+ rise was measured following Fluo-4-AM dye labeling. Note a larger Ca2+ influx in cells exposed to α-syn assemblies. Averaged normalized (dF/F0) fluorescence intensity for all cells is shown (B). Box plot in (B) shows the distribution of change in fluorescence intensity for all cells (box plot: median, quartile, and 10–90% distribution; Mann–Whitney U-test: ***P < 0.001; number of cells (n) is shown in parentheses in B; five independent experiments).