Supplementary Figures

for

## Near infrared light decreases synaptic vulnerability to amyloid beta oligomers

Michele M. Comerota, Balaji Krishnan, Giulio Taglialatela



Supplementary Figure 1. ELISA and flow cytometry  $A\beta$  oligomer binding curves Two methods were imploded in the determination of changes in the  $A\beta$  oligomer binding between NIR light treated and sham control treated wild type mice; ELISA and flow cytometry analysis. The flow cytometry analysis as described in the Methods section was used in determining the percentage of synaptosomes in our synaptosomal prep that would bind a fluorescently tagged  $A\beta$  oligomer. The ELISA method was similar, however, the  $A\beta$  oligomers were prepared without a fluorescent tag, so the analysis determined the total amount of  $A\beta$ oligomers bound in our sample. The flow cytometry method was chosen as the main focus in our current study, because of the added ability of the method to selectively analyze the synaptosomes in our prep, excluding nonspecific binding of the tagged  $A\beta$  oligomers to nonsynaptosomal particles. As shown in this figure, both methods illustrated a reduction of binding in the NIR light treated mice compared to the control sham group. Further both methods demonstrated a saturation of  $A\beta$  oligomer binding to isolated synaptosomes, thus further confirming overall validity of the ex vivo binding procedure used here.



## Supplementary Figure 2. Input/output curves for the four treatment groups.

The fEPSP amplitude (mV) obtained at increasing stimulus intensities (mA) show no significant differences in the basal synaptic strength following NIR light treatment and/or exposure to A $\beta$  oligomers compared to sham (no treatment). n=6-8 slices from 3-6 mice; Statistical analysis was carried out using two-way ANOVA with Bonferroni post-hoc analysis, F<sub>9,3</sub>=0.4209, P=0.9954, ns. Error bars represent standard error of mean.

MEAN (n=6)	Sham (No treatment)		Sham (Aβ treatment)		NIR (No treatment)		NIR (Aβ treatment)	
mV	Pre	Post	Pre	Post	Pre	Post	Pre	Post
10	0.012	0.034	0.018	0.060	0.020	0.056	0.014	0.081
20	0.026	0.033	0.045	0.046	0.069	0.034	0.089	0.082
30	0.061	0.077	0.083	0.083	0.107	0.140	0.129	0.167
40	0.098	0.107	0.156	0.139	0.182	0.175	0.229	0.302
50	0.117	0.140	0.178	0.163	0.260	0.258	0.303	0.308
60	0.160	0.174	0.228	0.189	0.311	0.292	0.313	0.382
70	0.190	0.215	0.248	0.226	0.376	0.360	0.404	0.455
80	0.212	0.248	0.278	0.262	0.420	0.390	0.406	0.483
90	0.231	0.262	0.307	0.273	0.464	0.424	0.512	0.550
100	0.252	0.255	0.332	0.299	0.500	0.431	0.487	0.559
SEM (n=6)	Sham (Ur	ntreated)	Sham (Abe	ta Treated)	NIR (Un	treated)	NIR (Abeta	a Treated)
<mark>SEM (n=6)</mark> mV	Sham (Ur Pre	ntreated) Post	Sham (Abe Pre	ta Treated) Post	NIR (Un Pre	treated) Post	NIR (Abeta Pre	a Treated) Post
SEM (n=6) mV	Sham (Ur Pre	ntreated) Post	Sham (Abe Pre	ta Treated) Post	NIR (Unt Pre	treated) Post	NIR (Abeta Pre	a Treated) Post
SEM (n=6) mV 10	Sham (Ur Pre 0.006	ntreated) Post 0.009	Sham (Abe Pre 0.006	ta Treated) Post 0.018	NIR (Un Pre 0.006	treated) Post 0.014	NIR (Abeta Pre 0.005	a Treated) Post 0.030
SEM (n=6) mV 10 20	Sham (Ur Pre 0.006 0.006	ntreated) Post 0.009 0.011	Sham (Abe Pre 0.006 0.012	ta Treated) Post 0.018 0.014	NIR (Un Pre 0.006 0.034	treated) Post 0.014 0.014	NIR (Abeta Pre 0.005 0.031	a Treated) Post 0.030 0.035
SEM (n=6) mV 10 20 30	Sham (Ur Pre 0.006 0.006 0.017	ntreated) Post 0.009 0.011 0.017	Sham (Abe Pre 0.006 0.012 0.018	ta Treated) Post 0.018 0.014 0.022	NIR (Un <sup>1</sup> Pre 0.006 0.034 0.052	treated) Post 0.014 0.014 0.041	NIR (Abeta Pre 0.005 0.031 0.054	a Treated) Post 0.030 0.035 0.048
SEM (n=6) mV 10 20 30 40	Sham (Ur Pre 0.006 0.006 0.017 0.017	ntreated) Post 0.009 0.011 0.017 0.026	Sham (Abe Pre 0.006 0.012 0.018 0.026	ta Treated) Post 0.018 0.014 0.022 0.023	NIR (Un <sup>1</sup> Pre 0.006 0.034 0.052 0.083	treated) Post 0.014 0.014 0.041 0.075	NIR (Abeta Pre 0.005 0.031 0.054 0.074	a Treated) Post 0.030 0.035 0.048 0.074
SEM (n=6) mV 10 20 30 40 50	Sham (Ur Pre 0.006 0.006 0.017 0.017 0.015	ntreated) Post 0.009 0.011 0.017 0.026 0.023	Sham (Aber Pre 0.006 0.012 0.018 0.026 0.030	ta Treated) Post 0.018 0.022 0.023 0.026	NIR (Un <sup>1</sup> Pre 0.006 0.034 0.052 0.083 0.110	treated) Post 0.014 0.014 0.041 0.075 0.106	NIR (Abeta Pre 0.005 0.031 0.054 0.074 0.102	a Treated) Post 0.030 0.035 0.048 0.074 0.085
SEM (n=6) mV 10 20 30 40 50 60	Sham (Ur Pre 0.006 0.006 0.017 0.017 0.015 0.026	ntreated) Post 0.009 0.011 0.017 0.026 0.023 0.036	Sham (Aber Pre 0.006 0.012 0.018 0.026 0.030 0.037	ta Treated) Post 0.018 0.022 0.023 0.026 0.026	NIR (Un Pre 0.006 0.034 0.052 0.083 0.110 0.131	treated) Post 0.014 0.014 0.041 0.075 0.106 0.112	NIR (Abeta Pre 0.005 0.031 0.054 0.074 0.102 0.086	a Treated) Post 0.030 0.035 0.048 0.074 0.085 0.097
SEM (n=6) mV 10 20 30 40 50 60 70	Sham (Ur Pre 0.006 0.017 0.017 0.015 0.026 0.033	ntreated) Post 0.009 0.011 0.017 0.026 0.023 0.036 0.047	Sham (Aber Pre 0.006 0.012 0.018 0.026 0.030 0.037 0.036	ta Treated) Post 0.018 0.014 0.022 0.023 0.026 0.026 0.031	NIR (Un Pre 0.006 0.034 0.052 0.083 0.110 0.131 0.155	treated) Post 0.014 0.014 0.041 0.075 0.106 0.112 0.132	NIR (Abeta Pre 0.005 0.031 0.054 0.074 0.102 0.086 0.135	a Treated) Post 0.030 0.035 0.048 0.074 0.085 0.097 0.136
SEM (n=6) mV 10 20 30 40 50 60 70 80	Sham (Ur Pre 0.006 0.007 0.017 0.015 0.026 0.033 0.040	ntreated) Post 0.009 0.011 0.017 0.026 0.023 0.036 0.047 0.055	Sham (Aber Pre 0.006 0.012 0.018 0.026 0.030 0.037 0.036 0.044	ta Treated) Post 0.018 0.014 0.022 0.023 0.026 0.026 0.031 0.028	NIR (Un Pre 0.006 0.034 0.052 0.083 0.110 0.131 0.155 0.183	treated) Post 0.014 0.014 0.041 0.075 0.106 0.112 0.132 0.139	NIR (Abeta Pre 0.005 0.031 0.054 0.074 0.102 0.086 0.135 0.125	a Treated) Post 0.030 0.035 0.048 0.074 0.085 0.097 0.136 0.134
SEM (n=6) mV 10 20 30 40 50 60 70 80 90	Sham (Ur Pre 0.006 0.007 0.017 0.015 0.026 0.033 0.040 0.040	ntreated) Post 0.009 0.011 0.017 0.026 0.023 0.036 0.047 0.055 0.061	Sham (Aber Pre 0.006 0.012 0.018 0.026 0.030 0.037 0.036 0.034 0.044	ta Treated) Post 0.018 0.014 0.022 0.023 0.026 0.026 0.031 0.028 0.038	NIR (Un <sup>1</sup> Pre 0.006 0.034 0.052 0.083 0.110 0.131 0.155 0.183 0.187	treated) Post 0.014 0.014 0.041 0.075 0.106 0.112 0.132 0.139 0.151	NIR (Abeta Pre 0.005 0.031 0.054 0.074 0.074 0.102 0.086 0.135 0.125 0.142	a Treated) Post 0.030 0.035 0.048 0.074 0.085 0.097 0.136 0.134 0.160

## Supplementary Table 1. Table of input/output averages for the four treatment groups.

The averages of the amplitudes (mV) measured in the four treatment groups after increasing stimulus intensities. There was no change in the pre-HFS and post-HFS amplitudes for all four treatment groups. n=6-8 slices from 3-6 mice; Statistical analysis was carried out using two-way ANOVA with Bonferroni post-hoc analysis (Pre-HFS –  $F_{9,3}$ =0.34799, P=0.9991, ns; Post-HFS - two-way ANOVA,  $F_{9,3}$ =0.4209, P=0.9954, ns; Pre- vs Post-  $F_{9,7}$ =0.3395, P=1, ns).



## Supplemental Figure 3. Flow cytometry analysis of condensed NIR light treatment regimen Aβ oligomer binding curve.

Pooled synaptosomes from cortex of WT mice receiving a condensed NIR light treatment schedule (20 treatments over 5 days) (black square) had a similar reduction in Aβ binding compared to sham treated mice (white circle) that was demonstrated in WT mice receiving 20 treatments over 4 weeks. Because the synaptosomes of the mice receiving a condensed schedule treatment regimen displayed similar reductions in binding, this schedule was used before performing electrophysiology experiments.



Uncropped Western blot analysis of Tg2576 mice receiving NIR light treatment or sham treatment (Figure 6).

(a) Representative Western blot probed with 6E10 antibody. (b) The membrane was reprobed using the antibody  $\beta$ -tubulin to serve as a total loading control for each sample.