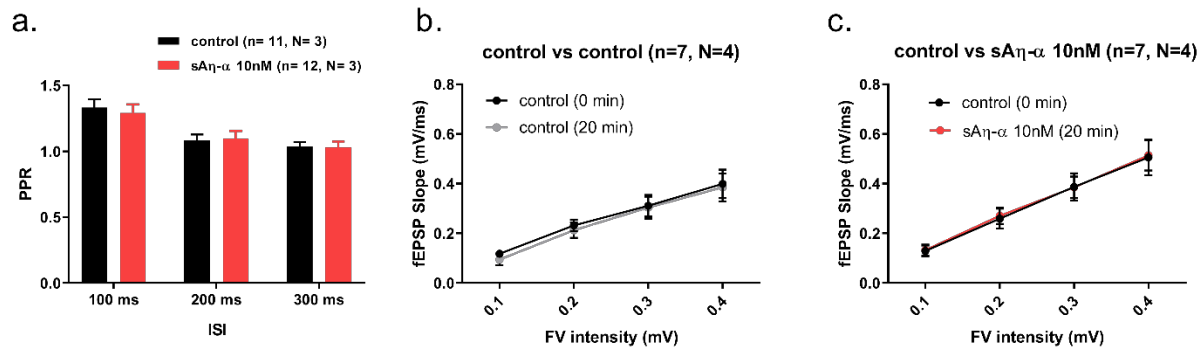
Aη−α and Aη−β peptides impair LTP *ex vivo* within the low nanomolar range and impact neuronal activity *in vivo*.

Maria Mensch et al.

SUPPLEMENTARY MATERIAL

Supplementary Figure S1.
Supplementary Tables S1-5.



Supplementary Figure S1. Acute application of sAη-α (10nM) does not perturb short-term pre-synaptic plasticity nor basal excitatory synaptic transmission.

a) PPRs (fEPSP2 slope/fEPSP1 slope) were similar at all ISIs examined (100ms, 200ms, 300ms) in control condition (aCSF only) and in presence of 10nM sAη-α throughout recording. ISI: inter-stimulus interval.

b) Synapse input–output curves (fEPSP slopes versus increasing afferent FV amplitudes) were similar at time 0 and then after 20 minutes in control condition (aCSF only).

c) Synapse input– output curves (fEPSP slopes versus increasing afferent FV amplitudes) were similar at time 0 in control condition (aCSF only) and then after 20 minutes of incubation with sAη-α (10nM).

(n=slices, N=mice). Detailed statistics are shown in Supplementary Table S1.

Table S1 - Statistics of Figure 1 and S1 and C57Bl/6 ex vivo LTP data

Mouse model and genetic background	Experiment		# mice	# slice	Measurement	Treatment	Average	S.E.M.	Units	Statistical Test	p value	F/t/z/R ETC value	Comparison	post-hoc test	p value	q(DF) value	Figure
RjOrl:SWISS	LTP - 100 nM sAη–β		5	13	last 15 minutes	_	148,50	5,33	%	Student t-test	**p=0,0003**	t=4,406 df=20	-	-	-	-	**1b**
			3	9		sAη-β	115,10	4,79	%								
	LTP - 10 nM sAη–α and sAη-β		10	17	last 15 minutes	_	168,40	5,77	%	One-way ANOVA	**p=0,0039**	F(2,31)=6,670	control vs sAη-α	Dunnett's test	**0,0061**	q(31)=3,203	**1d**
			5	8		sAη-α	138,30	7,87	%				control vs sAη-β		**0,0205**	q(31)=2,721	
			3	9		sAη-β	143,80	5,66	%								
	LTP - 5 nM sAη–α and sAPP-β		3	7	last 15 minutes	_	145,10	3,45	%	One-way ANOVA	**p=0,0072**	F(2,15)=6,987	control vs sAη-α	Dunnett's test	**0,0074**	q(15)=3,412	**1f**
			3	7		sAη-α	124,50	4,23	%				control vs sAη-β		**0,0235**	q(15)=2,836	
			3	4		sAη-β	125,00	7,40	%								
	LTP - 1 nM sAη–α and sAPP-β		6	12	last 15 minutes	_	142,30	4,24	%	One-way ANOVA	p=0,2006	f(2,32)=1,657	control vs sAη-α	Dunnett's test	0,2935	q(32)=1,389	**1h**
			6	12		sAη-α	132,60	5,11	%				control vs sAη-β		0,171	q(32)=1,706	
			6	11		sAη-β	130,10	5,66	%								
C57Bl/6	LTP - 100 nM sAη–α		5	17	last 15 minutes	_	158,00	6,84	%	Mann Whitney Test	**p=0,0109**	U=25	-	-	-	-	**-**
			2	8		sAη-α	125,90	8,28	%								
RjOrl:SWISS	PPR 10nM sAη–α	**100 ms**	3	11	EPSP slope	_	1,33	0,06	%	Two-way ANOVA	interaction p=0,8842 time **p<0,0001** treatment p=0,7824	interaction F (2, 63) = 0,1233 time F (2, 63) = 15,53 treatment F (1, 63) = 0,0769	Sidak's	Control vs sAη-α	p=0,9908	t(84)= 0,3998	**S1a**
			3	12		sAη-α	1,29	0,06	%								
		200 ms	3	11		_	1,09	0,04	%					Control vs sAη-α	p=0,9999	t(84)= 0,1266	
			3	12		sAη-α	1,10	0,06	%								
		300 ms	3	11		_	1,04	0,03	%					Control vs sAη-α	p>0,9999	t(84)= 0,08895	
			3	12		sAη-α	1,03	0,05	%								
RjOrl:SWISS	I/O	**0.1 mV**	4	7	EPSP slope	_	0,117634262	0,0140231	%	Two-way ANOVA	interaction p=0,9974 **time p<0,0001** retesting p=0,5680	F (3, 48) = 0,01519 F (3, 48) = 19,02 F (1, 48) = 0,3306	Sidak's	Control vs.Control	p=0,9896	t(48)=0,4139	**S1b**
			4	7		_	0,09444753	0,0225927	%								
		0.2 mV	4	7		_	0,231957891	0,023384	%					Control vs.Control	p=0,9945	t(48)=0,3496	
			4	7		_	0,212371057	0,0309529	%								
		0.3 mV	4	7		_	0,311017213	0,0435328	%					Control vs.Control	p=0,9999	t(48)=0,1302	
			4	7		_	0,303724585	0,0443224	%								
		0.4 mV	4	7		_	0,399198355	0,057836	%					Control vs.Control	p=0,9984	t(48)=0,2562	
			4	7		_	0,384845293	0,0559999	%								
	I/O 10nM sAη–α	**0.1 mV**	3	8	EPSP slope	_	0,129088906	0,0221622	%	Two-way ANOVA	interaction p=0,9991 **time p<0,0001** treatment p=0,8757	F (3, 56) = 0,007462 F (3, 56) = 24,28 F (1, 56) = 0,02471	Sidak's	Control vs. sAη-α	p>0,9999	t(56)=0,05298	**S1c**
			3	8		sAη-α	0,132580932	0,0222377	%								
		0.2 mV	3	8		_	0,259067485	0,0403819	%					Control vs. sAη-α	p=0,9997	t(56)=0,1717	
			3	8		sAη-α	0,270384224	0,0329775	%								
		0.3 mV	3	8		_	0,386970615	0,0543952	%					Control vs. sAη-α	p>0,9999	t(56)=0,02858	
			3	8		sAη-α	0,385086387	0,0433801	%								
		0.4 mV	3	8		_	0,505709119	0,0714783	%					Control vs. sAη-α	p>0,9999	t(56)=0,1183	
			3	8		sAη-α	0,513507936	0,0610509	%								

Table S2 - Statistics of Figure 2																
Mouse model and genetic background	**Experiment**	**# mice**	**# slice**	**Measurement**	**Treatment**	**Average**	**S.E.M.**	**Units**	**Statistical Test**	**p value**	**F/t/z/R ETC value**	**Comparison**	**post-hoc test**	**p value**	**q(DF) value**	**Figure**
RjOrl:SWISS	**LTP - recAη-α and recAη–β**	5	8	last 15 minutes	_	137,70	7,50	%	One-Way ANOVA	**p=0,0106**	F(2,18)=0,4396	control vs recAη-α	**Dunnett's test**	**0,0104**	q(18)=3,155	**2d**
		2	6		recAη-α	99,04	6,58	%				control vs recAη-β		**0,0311**	q(18)=2,639	
		3	7		recAη-β	106,70	10,71	%								

Table S3 - Statistics of Figure 3																
Mouse model and genetic background	**Experiment**	**# mice**	**# slice**	**Measurement**	**Treatment**	**Average**	**S.E.M.**	**Units**	**Statistical Test**	**p value**	**F/t/z/R ETC value**	**Comparison**	**post-hoc test**	**p value**	**q(DF) value**	**Figure**
RjOrl: SWISS	**LTP - 100 nM sAη-NT and sAη–β–CT**	8	11	last 15 minutes	-	127,30	8,03	%	One_Way ANOVA	**p=0,0288**	f(2,22)=1,093	control vs sAη-β-CT	Dunnett's test	0,9955	q(22)=0,081	**3c**
		3	6		sAη-β-CT	139,20	5,03	%				control vs sAη-NT		**0,0285**	q(22)=2,638	
		3	8		sAη-NT	100,00	7,42	%								
	LTP - 10nM sAη-NT	4	8	last 15 minutes	-	138,10	2,79	%	Unpaired t test	**p=0,0051**	t=3,363; df=13	-	-	-	-	**3e**
		3	7		sAη-NT	124,00	3,18	%								

Table S4 – Statistics of Figure 4

Figure 4A-C

Mann Whitney U-Test

	Baseline	Wash-In	p-value
Aeta-Beta-CT (Figure 4A)	12 [8.5, 17.5]	14 [9.5, 17.5]	0.2
Aeta-Alpha (Figure 4B)	18 [11.1, 21.5]	5 [1.3, 11.3]	1.1e-23
Aeta-Beta (Figure 4C)	13 [9.5, 18.5]	1.8 [0.5, 4]	2e-33

Values as Median [25th percentile, 75th percentile]

Figure 4D

Descriptives

	Change from Baseline
Aeta-Beta-CT	0.5 [-3, 4.5]
Aeta-Alpha	-9.3 [-14, -3.7]
Aeta-Beta	-10.5 [-15, -6]

Values as Median [25th percentile, 75th percentile]

Kruskal-Wallis ANOVA Table

'Source'	'SS'	'df'	'MS'	'Chi-sq'	'Prob>Chi-sq'
'Groups'	4609047.1	2	2304523.6	198.8	6.7e-44
'Error'	7584742.4	524	14474.69		
'Total'	12193789.5	526			

Pairwise results from multiple comparison test (with Tukey-Kramer correction)

Group A	Group B	Lower (95%) Confidence Interval	Estimate	Upper (95%) Confidence Interval	p-value
Aeta-Beta-CT	Aeta-Alpha	140.9	178.1	215.3	9.6e-10
Aeta-Beta-CT	Aeta-Beta	164.3	202.2	240.2	9.6e-10
Aeta-Alpha	Aeta-Beta	-16.1	24.1	64.3	0.3

Table S5 - Statistics of Figure 5											
Rat model	**Experiment**	**# rats**	**Measurement**	**Treatment**	**Average**	**S.E.M.**	**Units**	**Statistical Test**	**p value**	**F/t/z/R ETC value**	**Figure**
Sprague-Dawley	**In vivo LTP**	9	first 10 minutes	sAη-α	159,20	8,00	%	Student t-test	**p=0,013**	t=2,915 df=14	**5b**
		7	first 10 minutes	sAη-β-CT	192,20	5,34	%				
Sprague-Dawley	**In vivo LTP**	9	last 10 minutes	sAη-α	166,00	10,60	%	Student t-test	**p=0,024**	t=2,530 df=14	**5c**
		7	last 10 minutes	sAη-β-CT	212,00	15,50	%				