Supplementary Methods and Extended Data for

Title: Prodromal dysfunction of α5GABA-A receptor modulated hippocampal ripples in the TgF344-AD Rat Model of Alzheimer's disease

This section includes:

Methods
Supplementary data
Figs. S1 to S19
Table S1 to S16

SUPPLEMENTAL FIGURES FOR METHODS SECTION

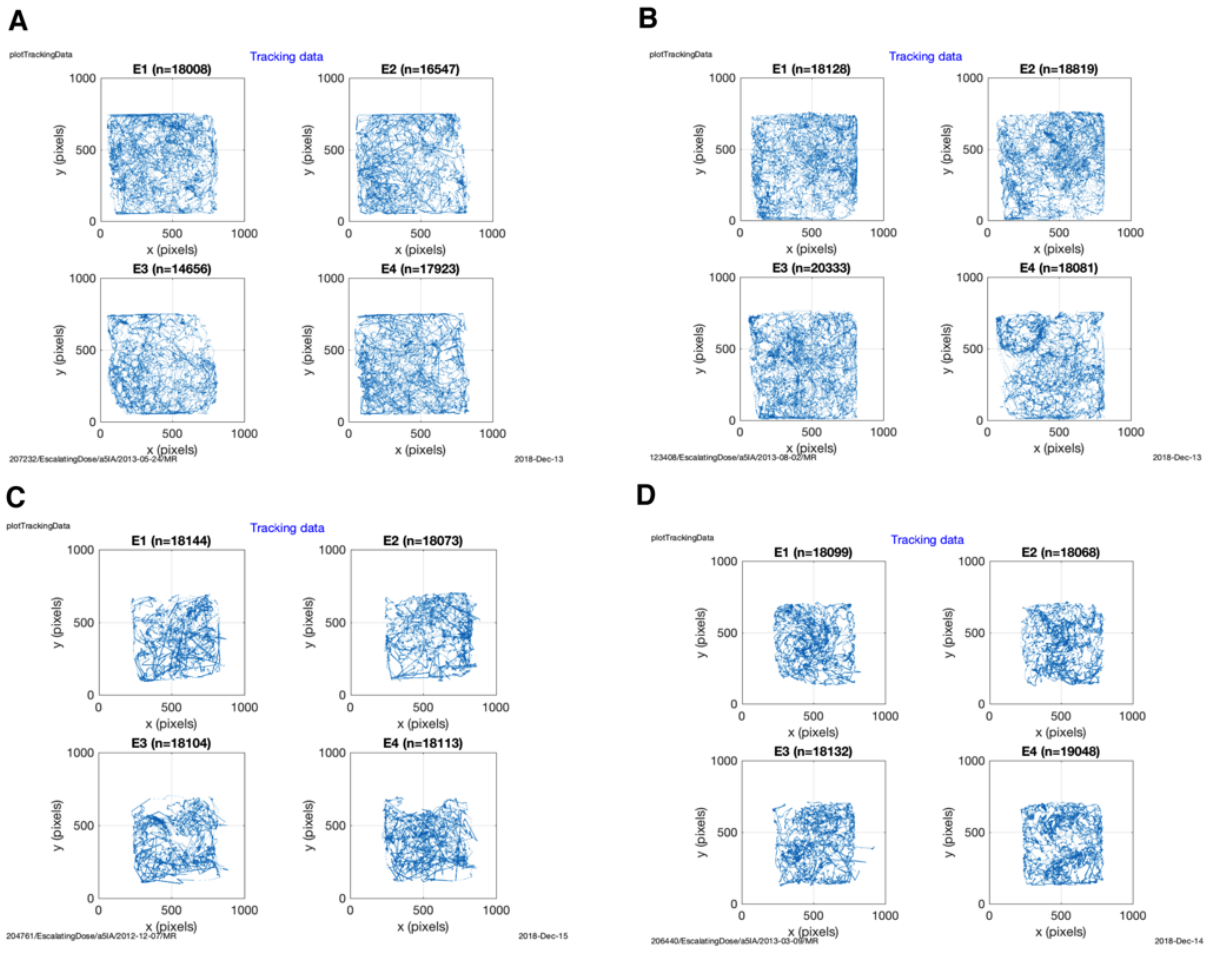

Fig S1: Trajectories of Long Evans Adult Male Rats During Escalating Dose Model.

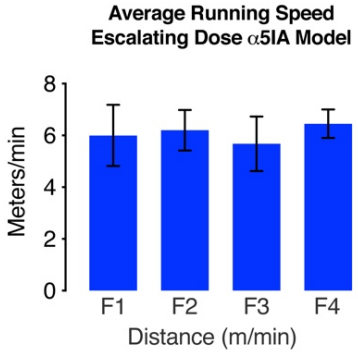

Fig S2: Histogram showing average running speeds of for all rats during escalating dose experiment. Freidman's ANOVA revealed no significant differences in running speed across test sessions.

Table S1: Average running speed of each rat during each session of escalating dose experiment.

Rat Number	F1	F2	F3	F4
Rat 123408	3.7	5	3.4	5.9
Rat 204761	5.3	5.8	5.3	5.9
Rat 206440	5.7	5.5	5.5	5.9

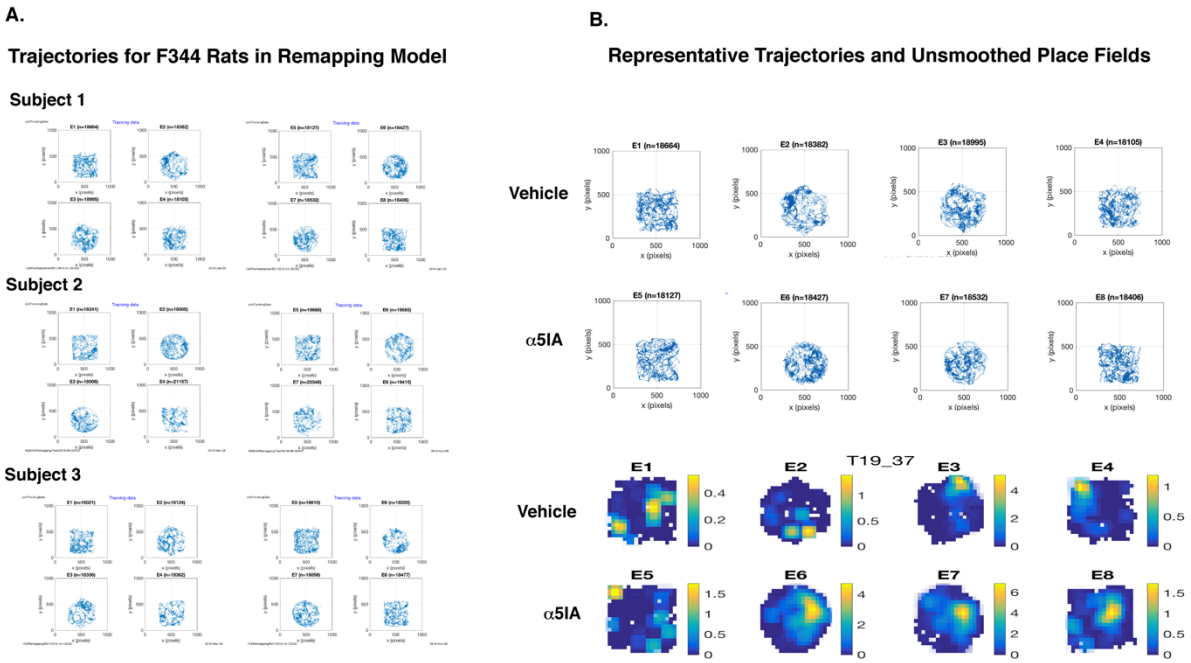

Fig S3. Trajectories **A)** Representative trajectories of F344 male rats during place cell place field remapping experiment. **B)** Representative path trajectories (top) and unsmoothed place fields (bottom) from a remapping experiment in a WT F344 rat (subject 1) showing increased firing rates (rate remapping) due to novelty under vehicle control conditions and following oral administration of α5IA (1.0mg/kg). Figure also shows that the overall mean firing rate is also higher following α5IA administration in both environments as compared with vehicle.

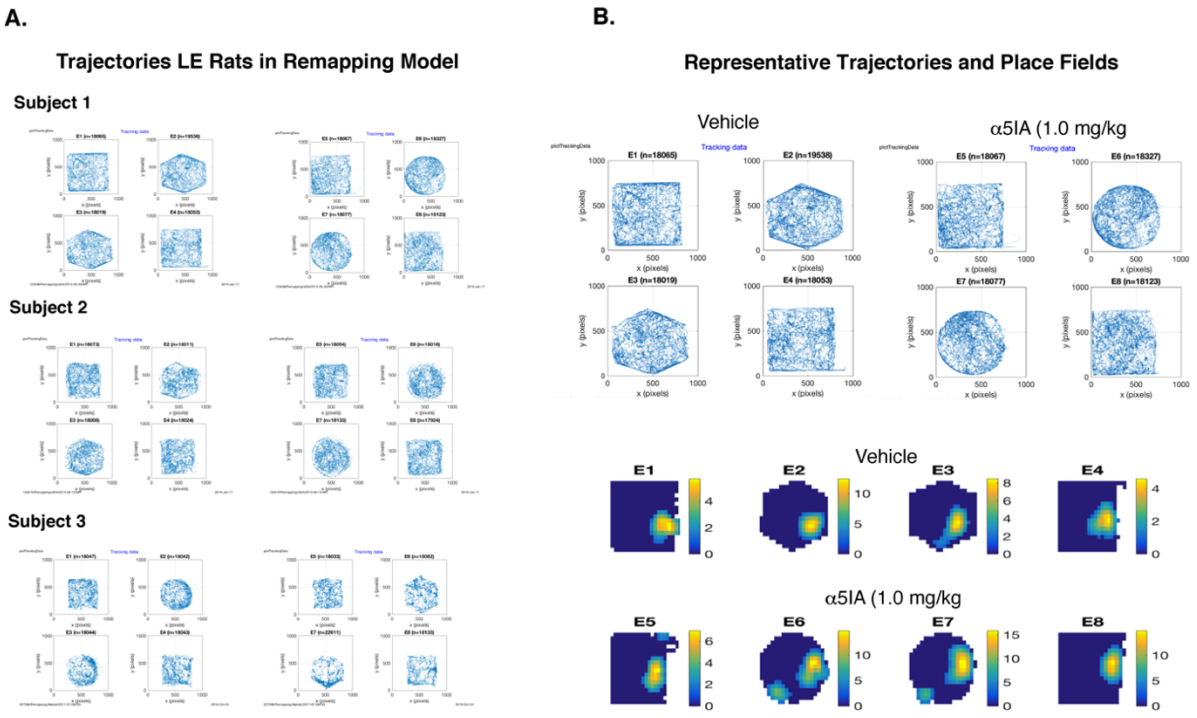

Fig. FigS4: Trajectories A) Trajectories from LE male rats during place cell remapping model. **B)** Representative trajectories (top) and unsmoothed place fields (bottom) from Subject 1 showing characteristic increases in firing rates due to novelty and oral α5IA (1.0mg/kg) administration.

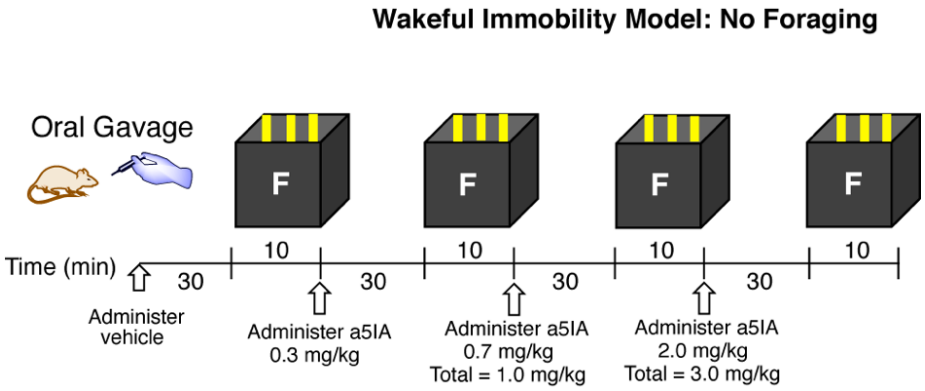

Fig. S5. Detection of CA1 ripples. A) Schematic diagram of LFP recordings for analysis of vehicle and drug effects of ripples. Data was acquired from immobile awake animals placed in a familiar environment. Food was withheld to discourage foraging behavior during the 10 min recording sessions.

Table S2. Ages of Strains of Rats Used in Escalating Dose Experiments for Probe Drug effects on Ripples

Subject/Rat #	Strain	Age at testing
Subject 1/#327396	LE	15
Subject 2/#130	F344	9
Subject 3/#600040	F344	16
Subject 4/#142	F344	18

Subject 5/#113	F344	12
Subject 6/#115	TgF344-AD	12
Subject 7/#128	TgF344-AD	9
Subject 8/#822	TgF344-AD	11
Subject 9/#811	F344	11
Subject 10/#814	F344	11

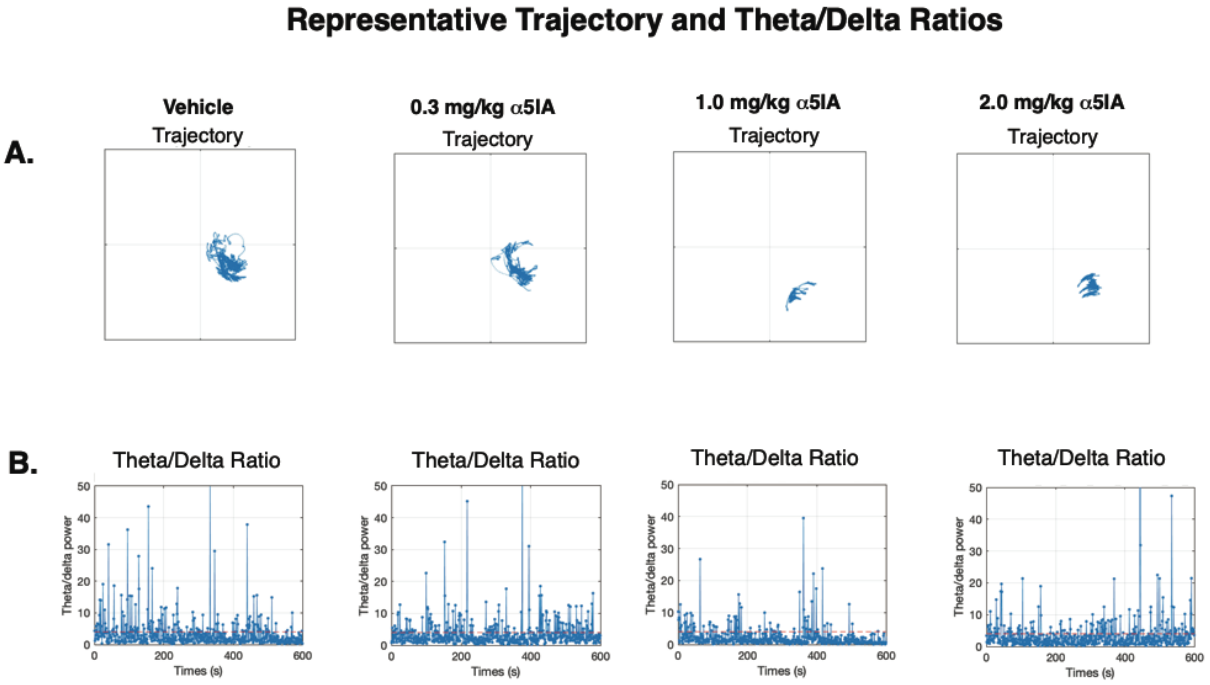

Fig. S6: Representative trajectories and theta/delta power ratio. All data shown are from Rat #2 (9 mo old F344 male). Panel A shows trajectories and panel shows corresponding theta/delta power ratios during serial exposures to the familiar environment in the escalating dose model.

Table S3: Percentage of Time Spent Immobile During Test Sessions

Rat/Drug/Dose/Model	Percent Time Immobile
LEM_Subject-1_Veh_FFFF	49.003
LEM_Subject-1_α5IA_0.3_FFFF	50.332
LEM_Subject-1_α5IA_1.0_FFFF	50.831
LEM_Subject-1_α5IA_3.0_FFFF	43.76
F344 _Subject-2_Veh_FFFF	77.434
F344 _Subject-2_α5IA_0.3_FFFF	74.799
F344 _Subject-2_α5IA_1.0_FFFF	85.578
F344 _Subject-2_α5IA _2.0_ FFFF	77.449
F344_Subject-3_Veh_FFFF	84.242
F344_Subject-3_ α5IA_0.3_FFFF	83.233
F344_Subject-3_ α5IA_1.0_FFFF	94.591
F344_Subject-3_α5IA_2.0_FFFF	89.583

F344 _Subject-4_Veh_FFFF	85.548
F344 _Subject-4_α5IA_0.3_FFFF	84.653
F344 _Subject-4_α5IA_1.0_FFFF	87.725
F344 _Subject-4_α5IA _2.0_ FFFF	83.845

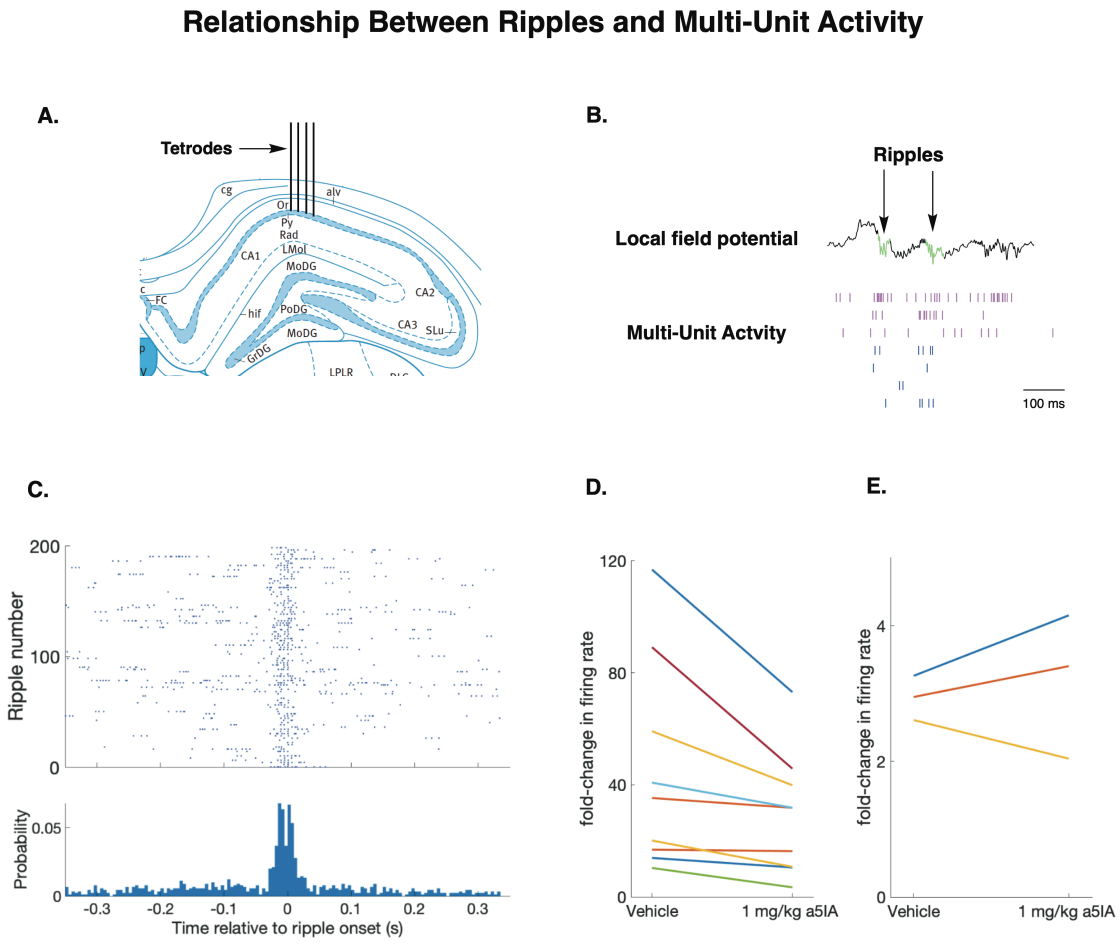

Fig S7: Single unit firing times relative to the onset time of ripples. **A)** Schematic drawing of representative recording sites in the hippocampal CA1 subregion. **B)** Representative multiunit activity during ripples. **C)** Ripple-triggered raster plot (top) and firing probability histogram (lower) for a representative CA1 pyramidical cell. Fold-change in ripple-associated firing rate with and without α5IA for (**D**) pyramid cells and (**E**) interneurons recorded with electrode best located within the pyramidal cell layer.

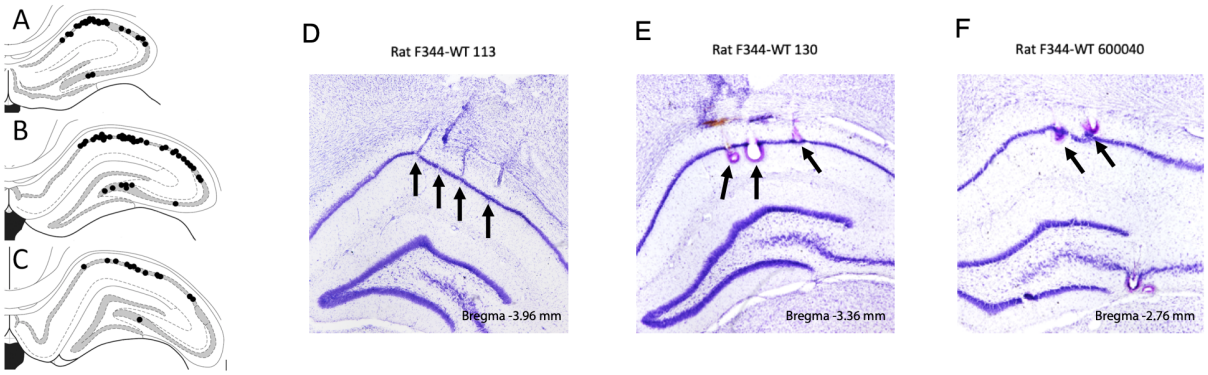

Fig. S8. Histological verification of recording locations. A) Illustration of coronal sections that are in the anterior-posterior plane at -2.5 mm (*A*), -3.4 mm (*B*), and -3.8 mm (*C*) from Bregma for rats used in place field remapping studies. Black dots indicate tetrode locations. Majority of tetrodes were located in CA1 and those that were located elsewhere were excluded from analyses. (D, E, F) Photographic images of coronal sections showing examples of tetrode locations in CA1 subregion for three F344 male adult rats used for ripple analysis.

Table S4: Aβ42 and Aβ40 Plasma Concentrations in Males and Females.

Aβ42	
Multiple comparisons test	Adjusted P Value
TgF344-AD 3M v. 3F	>0.9999
TgF344-AD 6M v. 6F	>0.9999
TgF344-AD 9M vs. 9F	>0.9999
TgF344-AD 12M v. 12F	>0.9999
Aβ40	
WT F344 3M v. WT3F	>0.9999
WT F344 6M v. WT6F	0.8370
WT F344 12M v. WT12F	0.3586

Table S5: Results of One-Way Wilcoxon Test for Object Validation and Evaluation for Intrinsic Bias

One-Way Wilcoxon Test	**Object 1**	**Object 2**	**Object 3**	**Object 4**	**All Objects**
Theoretical median	50.00	50.00	50.00	50.00	50.00
Actual median	35.80	55.66	39.99	82.04	39.15
Number of values	12	6	4	6	34
Sum of signed ranks (W)	-14.00	9.000	-4.000	15.00	-165.0
Sum of positive ranks	32.00	15.00	3.000	18.00	215.0
Sum of negative ranks	-46.00	-6.000	-7.000	-3.000	-380.0
P value (two tailed)	**0.6221**	**0.4375**	**0.6250**	**0.1562**	**0.1629**
Exact or estimate?	Exact	Exact	Exact	Exact	Exact
P value summary	**ns**	**ns**	**ns**	**ns**	**ns**
Significant (alpha=0.05)?	**No**	**No**	**No**	**No**	**No**

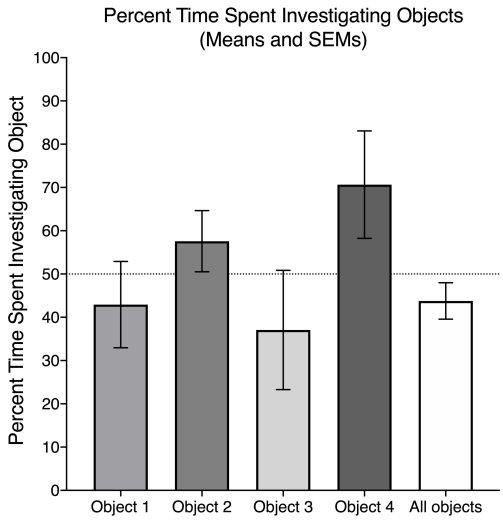

Fig S9: Histogram showing Means and SEMs for percent time spent investigating the different types of objects as compare to chance.

SUPPLEMENTAL RESULTS

Analysis of Mean Firing Rates of LE Rat Place Cells in Escalating Dose Model

The firing rates of LE rat CA1 pyramidal cells were measured while animals explored the familiar environment following administration of vehicle and escalating doses of α5IA. The average percent change in mean firing rate ranged from 23 to 71% with an average change of 38% following administration of the 1.0 mg/kg dose of α5IA (**Table S5**). The effect of vehicle versus α5IA on the activity of individual cells in the scatter plots and frequency distributions (**Fig S10).**

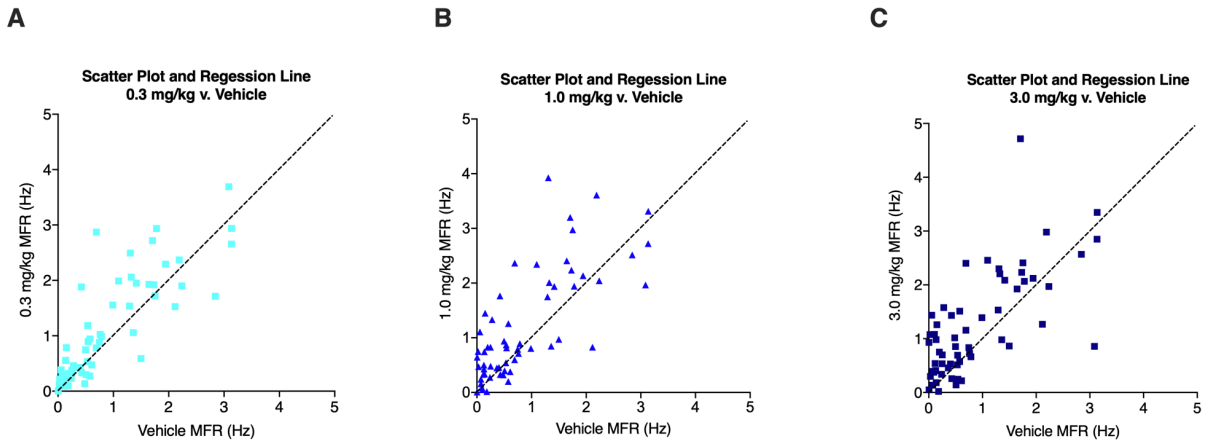

Fig. S10. Scatter Plots for Mean Firing Rates in Escalating Dose Model. A) Scatter plot for vehicle fit curve line versus 0.3 mg/kg dose of α5IA. **B)** Scatter plot for vehicle versus 1.0 mg/kg. **C)** Scatter plot for vehicle versus 3.0 mg/kg.

Table S6: Mean Firing Rates of Individual LE Rats Escalating Dose Model

Rat #	Vehicle	0.3 mg/kg	1.0 mg/kg	3.0 mg/kg	% Change (1.0 mg/kg)
204761	1.08665905	1.22439015	1.34093528	1.48529932	23%
206440	0.74717198	0.95881221	1.27890491	1.17652844	71%
207232	0.96529718	1.40989799	1.28803643	1.31341157	33%
123408	0.46385454	0.60343560	0.61672310	0.58985254	33%
Average	0.81574568	1.04913398	1.13114993	1.14127296	38%

Supplemental Results from Remapping Model Analyses

Oral α5IA administration significantly increased the mean, peak and in-field mean firing rates (MFR) of CA1 place cells in this remapping model in the LE Rats (Rats n= 3; Place cells n = 115). Planned comparisons indicated that α5IA administration significantly increased MFRs in the familiar environment ($p < 0.01$) **(Fig. S11-A and S12; Tables S7, S8 and S9).** The increase MFR of CA1 place cells seen in the LE rats is consistent with that observed in the F344 rats **(see Fig. 3 in manuscript).** Planned comparisons also revealed

significant increases in MFR on exposure to environmental novelty in the vehicle conditions indicating an effect of environment on mean firing rates as observed in WT F344 rats **(Fig. S11-A; Table S9)**. These effects of drug and environment were significant after adjusting the alpha for multiple comparisons. The interaction between drug and environment was not significant on this parameter. Peak firing rates (PFR) also increased significantly in the familiar environment following α5IA administration $p<0.01$). **(Fig. S11-B; Table S9)** Planned comparisons also revealed significant increases in PFR during exposure to environmental novelty in the vehicle ($p<0.05$). Although this was not significant after controlling for multiple comparisons it is important to note that this control experiment was not influenced by subsequent experiments in the drug conditions **(Fig. S11-B; Table S9)**. The interaction between drug and environment was not significant on this parameter. Significant in-field MFRs changes are also seen in this model. **(Fig. S11-C; Table S9)** Planned comparisons revealed a significant in-field MFR increase in the familiar environment following α5IA administration. Planned comparisons indicated that in-field MFRs also increased significantly in the novel environment under both the vehicle indicating an effect of environment on this parameter. The effect of novelty on this parameter in the drug condition was significant after controlling for multiple comparisons. The interaction between drug and environment was not significant on this parameter.

Analysis of variance for SIC per spike revealed significant effects. **(Fig. S11-D; Table S9)** Planned comparisons demonstrated significant decreases in SIC in the novel environment in the vehicle and drug treatment conditions. The interaction between drug and environment was not significant on this parameter.

Significant changes in place field area (PFA) were revealed by ANOVA as well. **(Fig. S11-E; Table S9)** PFA increased with environmental novelty from 366cm^2 to 407cm^2 (11%) and 368cm^2 to 412 cm^2 (12%) in the vehicle and drug conditions respectively but, although there was a strong trend ($p = 0.07$) observed in vehicle condition the increase in PFA only reached statistical significance ($p = 0.001$) in the drug condition. The interaction between drug and environment was not significant on this parameter.

Analysis of variance also revealed significant differences in place field spatial correlations in the vehicle condition. **(Fig. S11-F; Table S9)** Pairwise comparisons indicated spatial correlations between first familiar and each of the two novels were significantly lower than the correlations between the two familiars indicating remapping occurred during both exposures to the novel in the vehicle condition. This effect remained significant when controlling for multiple comparisons. Significant differences in spatial correlations were also revealed by ANOVA in the drug condition. Pairwise comparisons indicated spatial correlations between first familiar and each of the two novels were significantly lower than the correlations between the two familiars indicating remapping occurred during both exposures to the novel in the drug condition as well. Comparisons of spatial correlations across treatment conditions revealed no differences in the correlation between the two familiar environments before or after controlling for multiple comparisons indicating no effect on drug on the stability of well-established highly correlated place fields in this model. The interaction between drug and environment was not significant for this parameter.

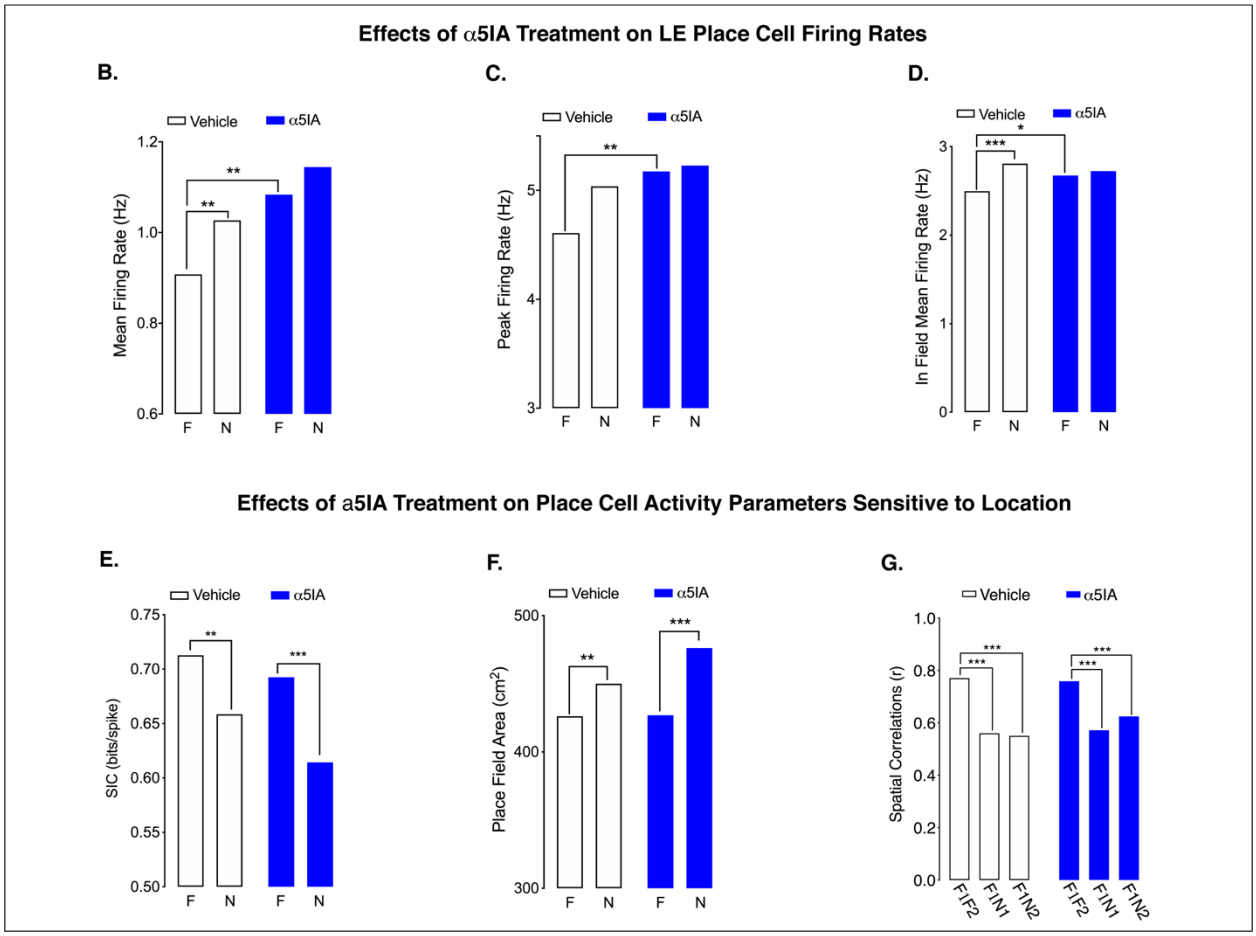

Fig. S11. Results of remapping model experiments designed to assess the within-subject effects of oral α5IA administration on CA1 place cell responses to environmental novelty in adult male LE rats. A) Environment-dependent remapping model. **A)** Histogram shows significant effect of α5IA (1.0 mg/kg) on MFR of CA1 place cells in a familiar environment and significant effects of environmental novelty on MFR. **B)** Histogram showing significant effect of α5IA on PFR of place cells in a familiar environment. **C)** Histogram shows significant effect of α5IA on in-field MFR of CA1 place cells in a familiar environment and significant effects of environmental novelty on in-field MFR. **D)** SIC significantly decreases during exposure to environmental novelty. **E)** Planned comparisons indicate that PFA significantly increases following administration of α5IA but ANOVA reveals no interaction between environment and treatment on this parameter. **F)** Environmental novelty significantly reduces place field spatial correlations novelty in both treatment conditions. Rats n = 3. Place cells n = 115. Significant at *p<0.01 unless otherwise indicated.

Table S7: Means and SEMs for Remapping Data from LE Rats

Parameter	Mean	SEM
MFR Familiar Vehicle	0.91	± 0.062
MFR Novel Vehicle	1.03	± 0.073
MFR Familiar α5IA	1.08	± 0.083
MFR Novel α5IA	1.14	± 0.093
PFR Familiar Vehicle	4.61	± 0.344
PFR Novel Vehicle	5.04	± 0.359
PFR Familiar α5IA	5.17	± 0.395
PFR Novel α5IA	5.23	± 0.338

In Field MFR Familiar Vehicle	2.50	± 0.169
In Field MFR Novel Vehicle	2.67	± 0.150
In Field MFR Familiar α5IA	2.74	± 0.184
In Field MFR Novel α5IA	2.80	± 0.166
SIC Familiar Vehicle	0.71	± 0.046
SIC Novel Vehicle	0.69	± 0.044
SIC Familiar α5IA	0.66	± 0.050
SIC Novel α5IA	0.61	± 0.041
Area Familiar Vehicle	426	± 14.81
Area Novel Vehicle	450	± 18.50
Area Familiar α5IA	427	± 15.25
Area Novel α5IA	476	± 18.13
Correlation F1F4 Vehicle	0.77	± 0.013
Correlation F1N1 Vehicle	0.56	± 0.027
Correlation F1N2 Vehicle	0.55	± 0.027
Correlation F1F4 α5IA	0.75	± 0.014
Correlation F1N1 α5IA	0.57	± 0.023
Correlation NF1N2 α5IA	0.62	± 0.023

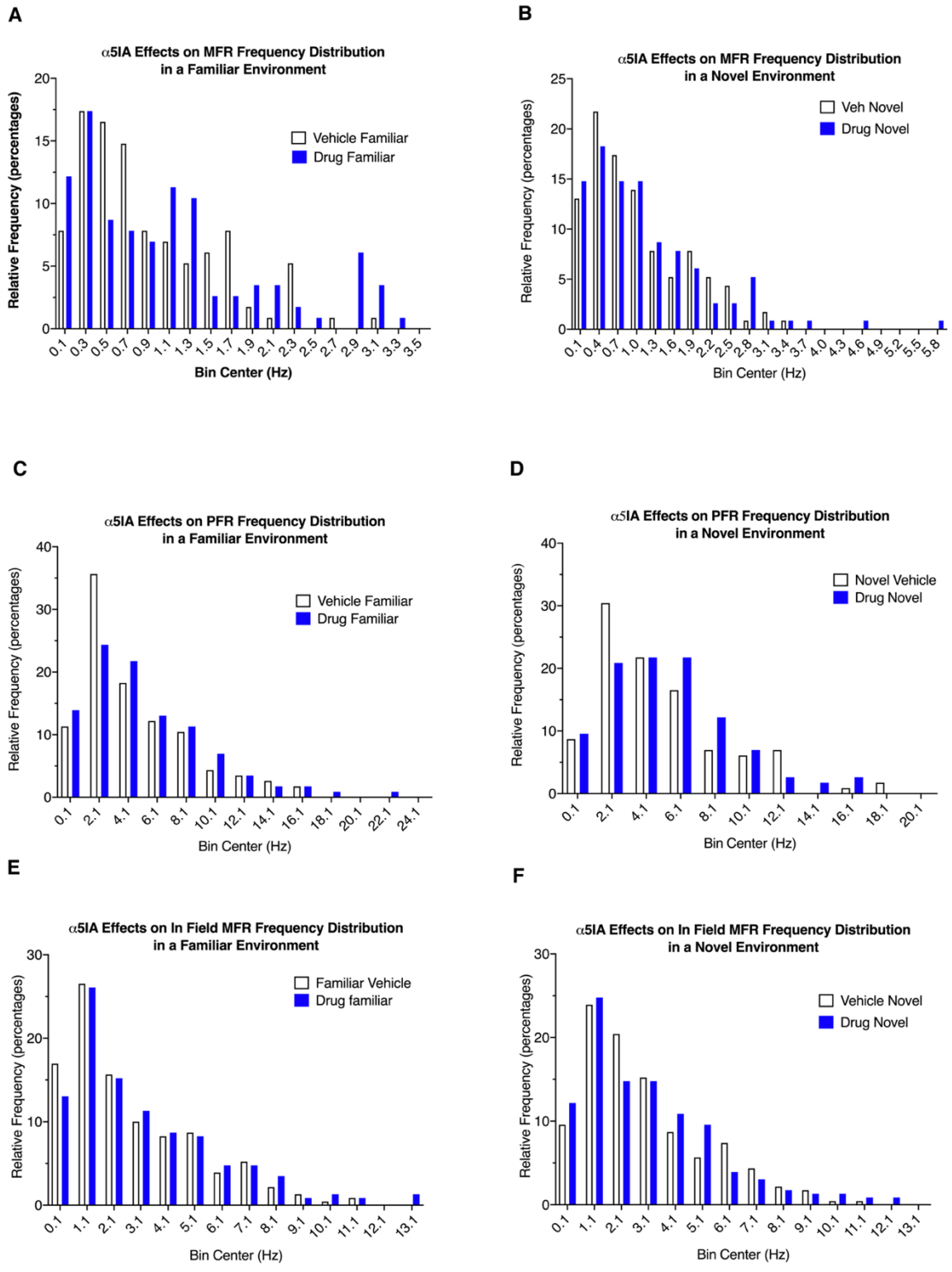

Fig S12. Firing Rate Frequency Distributions Reveal Effects of α5IA on Mean and Peak Firing Rates in the Familiar and Novel Environments in LE rats. A) The mean firing rate frequency distribution in the familiar environment reveals a shift in the distribution with a remarkable increase in the percentage of

cells with mean firing rates ≥ 1.9 Hz following α5IA administration**. B)** Mean firing rate frequency distributions in the novel environment also reveals a shift in the frequency distribution with an increase in the percentage of cells with mean firing rates ≥ 2.8 Hz following α5IA administration**. C)** Peak firing rate frequency distributions in the familiar environment also reveals a shift in the frequency distribution with an increase in the percentage of cells with peak firing rates ≥ 4.1 Hz following α5IA administration**. D)** Peak firing rate frequency distributions in the novel environment also reveals a shift in the frequency distribution with an increase in the percentage of cells with peak firing rates ≥ 6.1 Hz following α5IA administration**. E)** In-field mean firing rate frequency distributions in the familiar environment also reveals a shift in the frequency distribution with an increase in the percentage of cells with peak firing rates ≥ 8.1 Hz following α5IA administration**. F)** In-field mean firing rate frequency distributions in the novel environment also reveals a shift in the frequency distribution with an increase in the percentage of cells with peak firing rates ≥ 10.1 Hz following α5IA.

Table S8. Summary of Significant Effects of Oral α5IA on CA1 Place Cells in LE Rats

Parameter	Drug Familiar	Drug Novel	Response to Novelty	Drug/Env Interaction
Mean FR (MFR)	Yes ↑	No	Yes ↓	No
Peak FR (PFR)	Yes ↑	No	No	Trend
In Field MFR	Yes ↑	No	Yes ↓	No
SIC	No	No	Trend	No
PF Area (PFA)	No	No	No	No
Spatial Correlations (SC)	No	No	Trend for F1:N1 only	No

Significant effects indicated by yes or no at $p < 0.05$. Trends indicated at $p < 0.1$

Table S9. Results of Statistical Analysis of Place Cell and Ripple Response to Oral α5IA in LE Rats

Figure #	Panel	Test statistic (DF) = p value (Dependent Variable)	Significance of Pairwise Comparisons
Fig. 1.	B	*Friedman's ANOVA (F (3, 86) = 7.24; p = 0.003) (Escalating Dose Mean Firing Rate)*	Veh v. 0.3mg/kg; p = 0.03* Veh v. 1.0mg/kg; p = 0.0002*** Veh v. 2.0mg/kg; p = 0.001**
Fig. 2.	B	*ANOVA (F (3,115) = 4.64; p = 0.002) (Mean Firing Rate)*	Fam_{Veh} v. $Nov_{Veh;}$ p = 0.01** Fam_{Veh} v. Fam_{Drg}; p = 0.002**
	C	*Friedman's (χ^2(3, 115) = 21.91; p < 0.001) (Peak Mean Firing Rate)*	Fam_{Veh} v. $Nov_{Veh;}$ p = 0.05† Fam_{Veh} v. Fam_{Drg}; p = 0.002**
	D	*Friedman's (χ^2(3, 115) = 17.6; p < 0.001) (In-Field Mean Firing Rate)*	Fam_{Veh} v. $Nov_{Veh;}$ p < 0.001*** Fam_{Veh} v. Fam_{Drg}; p = 0.013*
	E	*Friedman's (χ^2(3, 115) = 25.9; p < 0.001) (Spatial Information Content)*	Fam_{Veh} v. $Nov_{Veh;}$ p < 0.006** Fam_{Drg} v. $Nov_{Drg;}$ p < 0.001*** Fam_{Veh} v. Fam_{Drg}; p = 0.959 Nov_{Veh} v. Nov_{Drg}; p = 0.645
	F	*Friedman's (χ^2 (3, 115) = 20.3; p < 0.001)*	Fam_{Veh} v. $Nov_{Veh;}$ p < 0.005**

		(Place Field Area)	Fam_{Drg} v. $Nov_{Drg;}$ p < 0.001*** Fam_{Veh} v. Fam_{Drg}; p = 0.867 Nov_{Veh} v. $Novrg_{D}$; p = 0.767
	G	*Friedman's (χ^2(2, 77) = 63.3.0; p < 0.001)* *(Spatial Correlations)*	Fam_{Veh} v. $Nov_{Veh1;}$ p < 0.001*** Fam_{Veh} v. $Nov_{Veh2;}$ p < 0.001*** Fam_{Drg} v. $Nov_{Drg1;}$ p < 0.001*** Fam_{Drg} v. $Nov_{Drg1;}$ p < 0.001*** Fam_{Veh} v. Fam_{Drg}; p = 0.86
Fig. S3.	B	*ANOVA (F (3, 86) = 7.24; p = 0.003)* *(Escalating Dose Mean Firing Rate)*	Veh v. 1.0mg/kg; p = 0.003** Veh v. 2.0mg/kg; p = 0.02* 1.0mg v. kg/3.0mg/kg; p > 0.999
Fig. 4.	E	*Friedman's (χ^2 (4, 125) = 361; p < 0.001)* *(Mean Power Spectral Density)*	PSD_{Veh} v. $PSD_{0.3mg/kg}$; p < 0.001*** PSD_{Veh} v. $PSD_{1.0mg/kg}$; p < 0.001*** PSD_{Veh} v. $PSD_{3.0mg/kg}$; p < 0.001***
	F	*Friedman's (χ^2 (1, 3) = 12; p = 0.007)* *(Peak Power Spectral Density)5*	PSD_{Veh} v. $PSD_{0.3mg/kg}$; p = 0.02† PSD_{Veh} v. $PSD_{1.0mg/kg}$; p = 0.007** PSD_{Veh} v. $PSD_{3.0mg/kg}$; p = 0.273
	H	*Wilcoxon* (χ^2 (1, 125) = 9.16; p < 0.001) *(Mean Power Spectral Density no Theta)*	PSD_{Veh} v. PSD_{D}; p < 0.001***
	K	*Friedman's (χ^2 (4, 125) = 312; p < 0.001)* *(Mean Power Spectral Density)*	PSD_{Veh} v. $PSD_{0.3mg/kg}$; p > 0.05 PSD_{Veh} v. $PSD_{1.0mg/kg}$; p < 0.001*** PSD_{Veh} v. $PSD_{2.0mg/kg}$; p < 0.001***
	L	*Friedman's (χ^2 (1, 3) = 13; p = 0.004)* *(Peak Power Spectral Density)*	PSD_{Veh} v. $PSD_{0.3mg/kg}$; p = 0.462 PSD_{Veh} v. $PSD_{1.0mg/kg}$; p = 0.01** PSD_{Veh} v. $PSD_{2.0mg/kg}$; p = 0.004**
	N	*Wilcoxon (χ^2 (1, 125) = 22; p < 0.001)* *(Mean Power Spectral Density no Theta)*	PSD_{Veh} v. PSD_{D}; p < 0.001***
	O	*Friedman's (χ^2 (3, 125) = 43; p < 0.001)* *(Mean Power Spectral Density)*	PSD_{Veh} v. $PSD_{0.3mg/kg}$; p = 0.462 PSD_{Veh} v. $PSD_{1.0mg/kg}$; p < 0.001*** PSD_{Veh} v. $PSD_{2.0mg/kg}$; p < 0.001***
	Q	*Friedman's* (χ^2 (1, 3) = 11; p = 0.006) *(Peak Power Spectral Density)*	PSD_{Veh} v. $PSD_{0.3mg/kg}$; p = 0.055† PSD_{Veh} v. $PSD_{1.0mg/kg}$; p = 0.17 PSD_{Veh} v. $PSD_{2.0mg/kg}$; p = 0.006**
	R	*Wilcoxon* (χ (1, 125) = 18; p < 0.001) *(Mean Power Spectral Density no Theta)*	PSD_{Veh} v. PSD_{D}; p < 0.001***
Fig. 5.	B	*Kruskal-Wallis* (H (3, 405) = 15; p < 0.0001) *(Ripple Peak Amplitude)*	Amp_{Veh} v. $Amp_{0.3mg/kg}$; p < 0.001*** Amp_{Veh} v. $Amp_{1.0mg/kg}$; p < 0.001*** Amp_{Veh} v. $Amp_{2.0mg/kg}$; p < 0.001***

	C	*ANOVA F (3, 86) = 6.13; p = 0.018)* *(Ripple Counts)*	$Number_{Veh}$ v. $Number_{0.3mg/kg}$; p > 0.724 $Number_{Veh}$ v. $Number_{1.0mg/kg}$; p = 0.022*
	D	*Kruskal-Wallis* (H *(3, 2066) = 11.9; p = 0.008)* *(Ripple Duration)*	Dur_{Veh} v. $Dur_{0.3mg/kg}$; p = 0.943 Dur_{Veh} v. $Dur_{1.0mg/kg}$; p = 0.003* Dur_{Veh} v. $Dur_{2.0mg/kg}$; p > 0.180
	E	*Kruskal-Wallis (χ^2 (3, 2066); p = 0.124)* *(Ripple Peak Frequency)*	

Significant effects indicated by: * at p < 0.05; ** at p < 0.01; and, *** at p < 0.001.
Trends not significant after controlling for multiple comparisons indicated by †.

Supplement Data for Place Cell Firing Analysis in F344 Adult Male Rats

We successfully recorded data from n = 152 place cells in three F344 adult male rats. All data were analyzed using Friedman's non-parametric repeated measures ANOVA with alpha level adjusted for the number of multiple pairwise comparisons unless otherwise indicated.

Friedman's ANOVA for mean firing rate was significant (F (3, 152) = 51.7; p < 0.001). Planned comparisons indicated that α5IA administration significantly increased MFRs by 18.5% in the familiar environment (p < 0.001) **(see Fig. 3A in manuscript and Table S10).** Planned comparisons also revealed significant increases in MFR on exposure to environmental novelty in both the vehicle (p<0.001) and drug (p=0.007) conditions indicating an effect of environment on mean firing rates. These effects of drug and environment were all significant after adjusting the alpha for multiple comparisons. The interaction between drug and environment was not significant on this parameter.

Peak firing rates (PFR) also increased significantly following α5IA administration (χ^2(3, 152) = 43.3; p<0.001). A planned comparison revealed a small but significant (p=0.01) 7% peak firing rate increase in the familiar environment following α5IA administration. **(see Fig. 3B in manuscript and Table S10)** Planned comparisons also revealed significant increases in PFR during exposure to environmental novelty in both the vehicle (p<0.001) and drug (p=0.01) conditions indicating an effect of environment on peak firing rates. The effect of environmental novelty on PFR in the drug condition remained significant after controlling for multiple comparisons. The interaction between drug and environment was not significant on this parameter.

Significant (χ^2(3, 152) = 38.7; p<0.001) in-field MFRs changes are also seen in this model. **(see Fig. 3C in manuscript and Table S10)** Planned comparisons revealed a significant (p = 0.004) 11% in-field MFR increase in the familiar environment following α5IA administration. Planned comparisons indicated that in-field MFRs also increased significantly in the novel environment under both the vehicle (p<0.001) and drug (p=0.01) conditions indicating an effect of environment on this parameter. The effect of novelty on this parameter in the drug condition was not significant after controlling for multiple comparisons. The interaction between drug and environment was not significant on this parameter.

Analysis of variance in CA1 place cell spatial information content (SIC) per spike in this model also revealed significant (χ^2(3, 152) = 46.0; p < 0.001) effects. **(see Fig. 3D in manuscript and Table S10)** Planned comparisons demonstrated a significant (p<0.01) decreases in SIC in the novel environment in the vehicle

and drug treatment conditions of 18% and 10% respectively. The interaction between drug and environment was not significant on this parameter.

Significant (χ^2 (3, 152) = 12.4; p<0.006) changes in place field area (PFA) were revealed by ANOVA as well. **(see Fig. 3E in manuscript and Table S10)** PFA increased with environmental novelty from 366cm^2 to 407cm^2 (11%) and 368cm^2 to 412 cm^2 (12%) in the vehicle and drug conditions respectively but, although there was a strong trend (p = 0.07) observed in vehicle condition the increase in PFA only reached statistical significance (p = 0.001) in the drug condition. The interaction between drug and environment was not significant on this parameter.

Analysis of variance also revealed significant differences in place field spatial correlations in the vehicle condition (χ^2(2, 44) = 28.7; p < 0.001). **(see Fig. 3F in manuscript and Table S10)** Pairwise comparisons indicated spatial correlations between first familiar and each of the two novels (F1N1: r = 0.5, p < 0.001; F1N2: r = 0.48, p < 0.001) were significantly lower than the correlations between the two familiars (F1F2: r = 0.66) indicating remapping occurred during both exposures to the novel in the vehicle condition. This effect remained significant when controlling for multiple comparisons. Significant (χ^2(4, 44) = 19.4; p < 0.001) differences in spatial correlations were also revealed by ANOVA in the drug condition. Pairwise comparisons indicated spatial correlations between first familiar and each of the two novels (F1N1: r = 0.59, p < 0.002; F1N2: r = 0.56, p < 0.001) were significantly lower than the correlations between the two familiars (F1F2: r = 0.69) indicating remapping occurred during both exposures to the novel in the drug condition as well. Comparisons of spatial correlations across treatment conditions revealed no differences in the correlation between the two familiar environments before (p=0.20) or after controlling for multiple comparisons (p; r_{veh} = 0.66$\pm$0.10; r_{drug} = 0.69$\pm$0.11) indicating no effect on drug on the stability of well-established highly correlated place fields in this model (*37*). The interaction between drug and environment was not significant for this parameter.

Table S10. Means and SEMs for Remapping Data from WT F344s

Parameter	Mean (Hz)	SEM (Hz)
MFR Familiar Vehicle	0.74	$\pm$ 0.047
MFR Novel Vehicle	0.90	$\pm$ 0.057
MFR Familiar α5IA	0.88	$\pm$ 0.053
MFR Novel α5IA	0.98	$\pm$ 0.059
PFR Familiar Vehicle	3.80	$\pm$ 0.213
PFR Novel Vehicle	4.44	$\pm$ 0.240
PFR Familiar α5IA	5.07	$\pm$ 0.210
PFR Novel α5IA	4.86	$\pm$ 0.222
In Field MFR Familiar Vehicle	2.38	$\pm$ 0.130
In Field MFR Novel Vehicle	2.76	$\pm$ 0.144
In Field MFR Familiar α5IA	2.64	$\pm$ 0.137
In Field MFR Novel α5IA	2.93	$\pm$ 0.140
SIC Familiar Vehicle	0.73	$\pm$ 0.042
SIC Novel Vehicle	0.59	$\pm$ 0.034
SIC Familiar α5IA	0.70	$\pm$ 0.036
SIC Novel α5IA	0.60	$\pm$ 0.033
Area Familiar Vehicle	366	$\pm$ 13.48
Area Novel Vehicle	408	$\pm$ 20.25
Area Familiar α5IA	369	$\pm$ 13.56
Area Novel α5IA	413	$\pm$ 16.36
Correlation F1F4 Vehicle	0.66	$\pm$ 0.016

Correlation F1N1 Vehicle	0.50	± 0.025
Correlation F1N2 Vehicle	0.48	± 0.028
Correlation F1F4 α5IA	0.69	± 0.016
Correlation F1N1 α5IA	0.60	± 0.022
Correlation NF1N2 α5IA	0.56	± 0.027

Supplemental Analysis for Cell by Cell Effects of Oral α5IA on Activity of Individual LE CA1 Pyramidal Cell and Interneurons

Results of the cell by cell analysis for the percentages of pyramidal neurons increasing versus decreasing MFRs was performed using data from the 4 LE rats in included in this study. This analysis revealed that significantly (χ^2 = 8.1; DF = 2; p<0.0174; 45% (60/134) more of these pyramidal cells increased MFR by ≥ 20% in a familiar environment after α5IA administration; a finding consistent with the data from the escalating dose-response experiment **(Table S11 and Fig. S13).**

Chi square analysis of interneuron activity indicates a significantly (χ^2 = 22.5; DF = 1; p < 0.0001) greater portion of cells 56% (19/34) increased their MFR by ≥ 20% following administration of α5IA, while only 18% (6/34) showed a decrease of this magnitude. The average MFR of these interneurons across are all 4 environments following vehicle and α5IA administration were 12.2 and 16.2 Hz respectively. The results of a pair subject T-test was also significant (t (33) = 2.08; p = 0.045) demonstrating that α5IA administration modulates the activity of interneurons as well as pyramidal cells in the CA1 subregion.

Place cells were also characterized based on the percentage of cells that rate remapped following vehicle versus α5IA administration. The number of place cells that rate remapped in the vehicle and drug conditions were 31% (42/134) and 25% (33/134) respectively. The observed percentage of cells showing rate remapping in this model which used different shaped environments in the same room is consistent with previous reports (*S4*). Of the place cells that showed a ≥ 20% increase in overall mean firing rate following drug administration, 25% (15/60) also showed rate remapping based on a ≥ 50% change in their in-field mean firing rates. A slightly smaller percentage 21% (9/42) of the place cells that showed a decrease in overall mean firing rate with following α5IA administration also showed rate remapping in the drug condition. A similar percentage 21% (7/33) of the place cells that did not show a change of this magnitude in overall mean firing rate due to drug still showed rate remapping suggesting rate remapping does not require a drug-induced change in overall mean firing rate. A similar number of these place cells (vehicle = 11; drug = 10) showed global remapping based on a change in their spatial correlations as well as a change in their firing rates in the vehicle control and drug conditions respectively.

Table S11. Cell by Cell Mean Firing Rate Responses of Pyramidal Cells and Interneurons to 1.0 mg/kg α5IA

Neuron type	D +	D -	D0	Local Network Changes (% cells with new activity patterns)
Pyramidal Cell (Pyr)	60 Pyr (+)	41 Pyr (-)	33 Pyr (0)	(|60(+) + 41(-)|) = 75% (101/134) of pyramidal cells
Interneurons (Int)	19 Int (+)	6 Int (-)	9 Int (0)	(|19(+) + 6(-)|) = 73% (25/34)of interneurons
Total Neurons (Units)	|79 Tot (+)|	|47 Tot (-)|	42 Tot (0)	(|79(+) + 47(-)|) = 75% (126/168) of total units

Pyramidal cells and interneurons showing net changes in overall mean firing rate in response to 1.0mg/kg α5IA. A neuron is judged to be excited by drug D+ if MFR(drug)-MFR(veh)/MFR(veh)>,=1.2 MFR(veh); inhibited by drug D- if MFR(drug)-MFR(veh)/MFR(veh)<,=0.8 MFR(veh); and, not responsive to drug D0 if MFR(drug)-MFR(veh)/MFR(veh)<,=1.2 MFR(veh) or >,=0.8 MFR(veh).

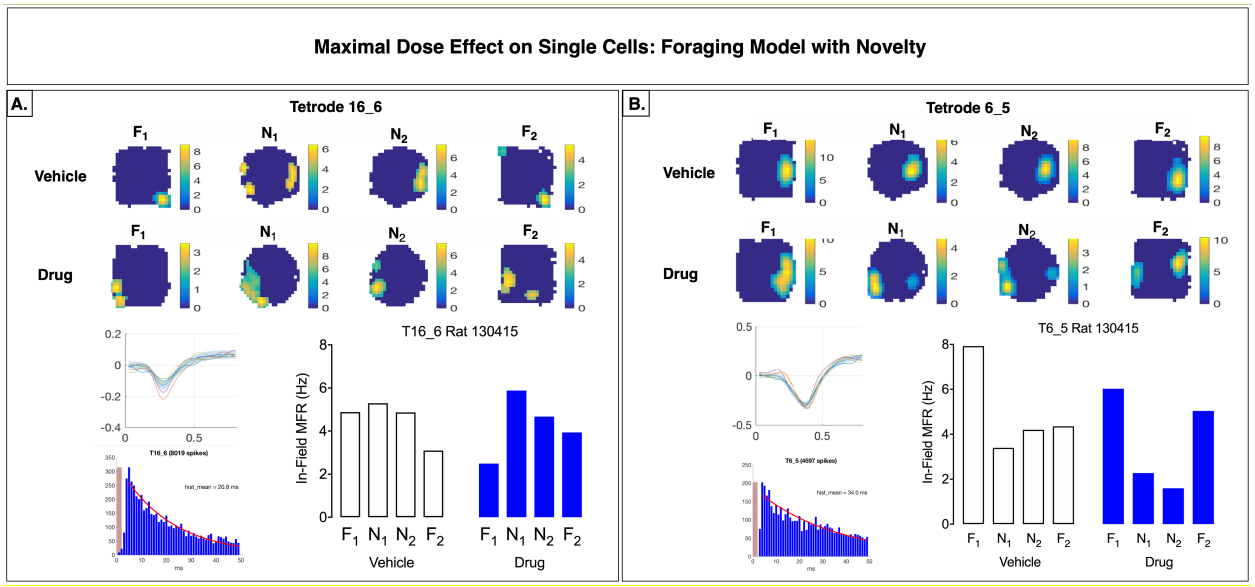

Fig. S13. Representative place fields showing changes in spatial correlations before and after administration of α5IA in LE Rats. **A)** CA1 neuron showing overt global remapping of place field heat maps across familiar (F) and novel (N) environments, following oral administration of vehicle (Top: F1-N1-N2-F2), without overt global remapping in the presence of α5IA (Bottom: F1-N1-N2-F2), but cell shows in field firing rate changes with both vehicle and drug (A, bottom middle panel). **B)** A different CA1 neuron showing stable place field heat maps across familiar (F) and novel (N) environments, following oral administration of vehicle (Top: F1-N1-N2-F2), demonstrates global remapping (Bottom: F1-N1-N2-F2) with a change in both location and firing rate (bottom middle panel) following administration of α5IA. The environments for CA1 neuron A and B were: F, square shape, black wood walls and epoxy wood floor, yellow vertical stripes; N1, hexagonal shape, black wood walls, epoxy wood floor, yellow vertical stripes; N2, cylindrical shape, silver metallic walls, black epoxy coated wood floor, yellow vertical stripes.

Supplemental Results for Dose-Dependent Effects of α5IA on Ripples in F344 and TgF344-AD Rats

To control for systematic error, we performed additional experiments using vehicle only in the wakeful immobility model in two F344 male rats and one TgF344-AD rat age 11 mo. These experiments revealed that repeated exposure to a familiar environment, was consistently associated with a decrease in ripple band power and peak ripple amplitudes in both strains under vehicle control even when the number of ripple events increased **(Fig S14 and S14; Tables S12 and S13).**

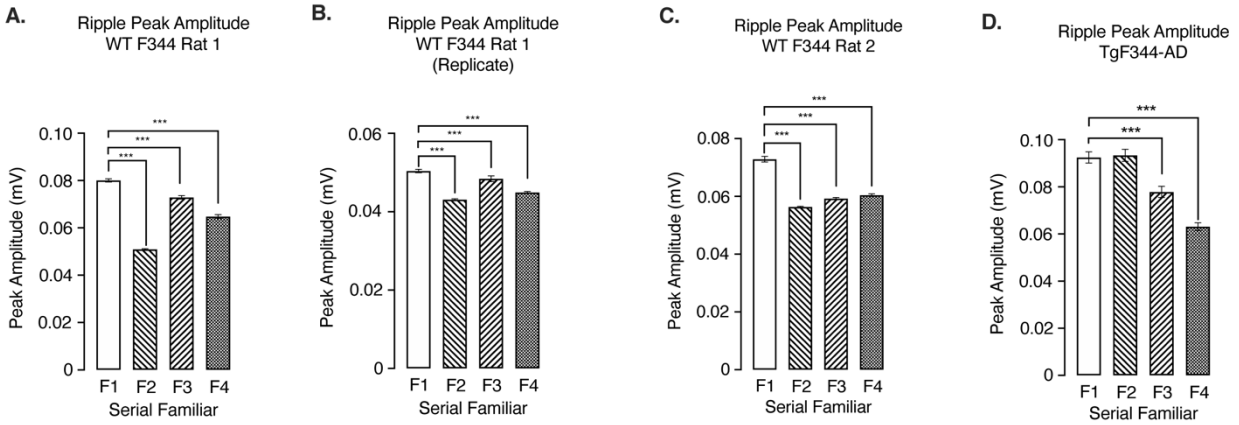

Fig S14: Wakeful immobility model vehicle control experiments. A Repeated exposure to a familiar environment is associated with significant decreases in peak ripple amplitude for subject 9. **B)** Results of replicate experiment in this same animal. **C)** Peak ripple amplitudes for subject 10. **D)** Peak ripple amplitudes for TgF344-AD (TgF344-AD subject 8). Significant effects indicated by: *** at $p < 0.001$.

Table S12. F344 Vehicle Control Experiments Total Number of Ripples Per Session

Subject (Number)	Vehicle	Vehicle	Vehicle	Vehicle
Subject 1 (F344 Rat #9)	502	579	430	409
Subject 1 (F344 Rat #9)	463	605	435	608
Subject 2 (F344 Rat #10)	320	648	558	508
Subject 3 (Tg344-AD Rat #8)*	579	564	472	508
Average for all F344s	428	611	474	417
Percent change F344s	N/A	150%	118%	124%
Statistical significance	N/A	p = 0.094	p = 0.999	P = 0.999

Total number ripple events per 10-minute recording session normalized by periods of immobility.
* See Table S12

Table S13. F344s and TgF344-AD Vehicle Control Experiments Total Number of Ripples Per Session

Subjects	Vehicle	Vehicle	Vehicle	Vehicle
Average for all subjects	466	599	474	508
Percent change all subjects	N/A	129%	102%	109%
Statistical significance	N/A	p = 0.112	p = 0.999	P = 0.999

Total number ripple events per 10-minute recording session normalized by periods of immobility.

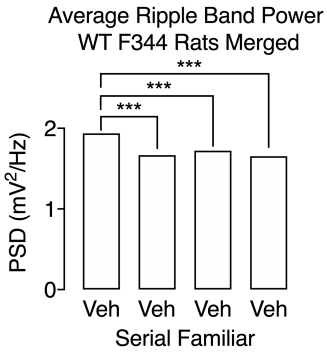

Fig S15: Average Ripple Band Power F344 Vehicle Control Condition. Within subject ANOVA reveals reduction in average ripple band power (140 to 200 Hz) in 11 mo old WT F344 male rats. Significant effects indicated by: *** at $p < 0.001$.

In addition, administration of single bolus doses of 1.0 mg/kg and 2.0 mg/kg of α5IA immediately after vehicle to two of the F344 rats used in the escalating dose experiments and one of the rats used in the vehicle control experiments demonstrated that the increase in ripple band power induced by α5IA administration are seen under these bolus dosing conditions as well. (**Fig. S16**)

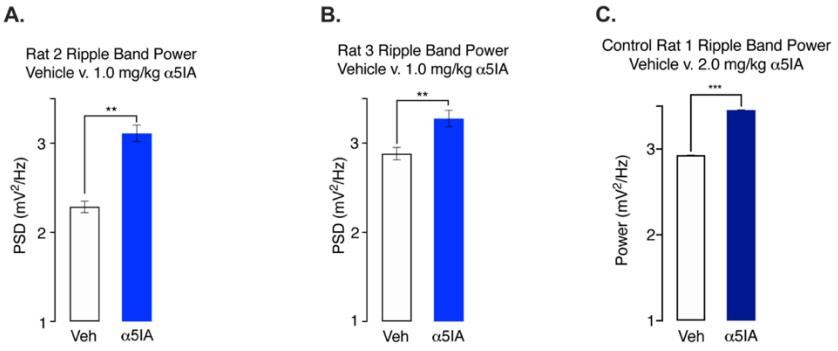

Fig. S16. Increase in Ripple Band Power induced by single bolus dose of α5IA (1.0mg/kg). A) Single bolus dose of α5IA (1.0 mg/kg) in F344 subject 2. **B)** Single bolus dose of α5IA (1.0 mg/kg) in WT F344 subject 3. **C)** Single bolus dose of α5IA (2.0 mg/kg) in WT F344 subject 1. All PSD values are x 10^{-6}. Significant effects indicated by: ** at $p < 0.01$; and, *** at $p < 0.001$.

Supplemental Results for Effects of α5IA on Ripple Band Power in Wildtype Rats

Table S14: 95% Confidence Intervals for α5IA Escalating Dose PSD Power in Ripple Band of WT Rats

Rat #1 (15 mo LE)	**Vehicle**	**0.3 mg/kg**	**1.0 mg/kg**	**2.0 mg/kg**
Lower 95% CI	3.312e-006	3.974e-006	4.605e-006	4.000e-006
Upper 95% CI	3.493e-006	4.217e-006	4.935e-006	4.240e-006
Rat #2 (9 mo WT F344)				
Lower 95% CI	2.513e-006	2.661e-006	3.880e-006	3.732e-006
Upper 95% CI	2.745e-006	2.932e-006	4.322e-006	4.199e-006
Rat #3 (16 mo WT F344)				
Lower 95% CI	2.237e-006	2.366e-006	2.514e-006	2.551-006
Upper 95% CI	2.330e-006	2.480e-006	2.638e-006	2.716e-006
Rat #4 (12 mo WT F344)				
Lower 95% CI	2.668e-006	2.668e-006	3.234e-006	2.723e-006
Upper 95% CI	2.935e-006	2.925e-006	3.670e-006	3.030e-006

1. Effects of α5IA on Total Number of Ripple Events per Session in WT Male Rats

In all four wildtype (WT) animals tested (one LE and three F344) and all three TgF344-AD rats, treatment with oral α5IA increased the total number of ripples per session. Kruskal-Wallis ANOVA with planned comparisons revealed a significant increase in ripples per session of 49% (p = 0.022) following administration of the 1.0 mg/kg oral dose of α5IA in WT rats (**Table S14**). This increase remained significant after controlling for multiple comparisons with an adjusted alpha level of 0.025. No significant effect of α5IA administration on average number of ripples per session is seen in the TgF344-AD rats (**Table S15**).

Table S15. Oral α5IA Treatment Increases Total Number of Ripple events Per Session

		Oral Dose of α5IA		
Subject Number	**Vehicle**	**0.3 mg/kg**	**1.0 mg/kg**	***2.0 mg/kg**
Subject 1 (#LE-327396)	212	336	346	345
Subject 2 (#F344-130)	178	178	245	265
Subject 3 (#F344-600040)	123	191	227	224
Subject 4 (#F344-113)	191	189	236	204
Average for all WT subjects	176	223.5	263.5	259.5
Change from vehicle baseline	N/A	27%	***50%***	47%
Statistical significance	N/A	p = 0.724	***p = 0.022***	N/A†

Total number ripple events per 10-minute recording session normalized by periods of immobility
*Highest dose administered to this LE male animal was 3.0 mg/kg. †Statistical significance not calculated due to dosing differences. Significance indicated at alpha = 0.025.

Table S16. Oral α5IA Treatment Increases Total Number of Ripple events Per Session TgF344-AD Rats

		Oral Dose of α5IA		
Subject Number	**Vehicle**	**0.3 mg/kg**	**1.0 mg/kg**	***2.0 mg/kg**
TgF344-AD Subject 1 (#128)	120	127	142	119
TgF344-AD Subject 2 (#126)	87	107	140	144
TgF344-AD Subject 3 (#115)	223	218	242	248
Average for all TgF344-AD subjects	143	151	175	170

Change from vehicle baseline	N/A	6%	*22%*	19%
Statistical significance	N/A	p = 0.999	p = 0.924	p = 0.999

Total number ripple events per 10-minute recording session normalized by periods of immobility
Significance indicated at alpha = 0.025.

2. Effects of α5IA on Peak Amplitude of Ripple in WT Rats

Non-parametric analysis of variance of peak ripple amplitudes revealed significant drug-induced increases in all four wildtype animals tested (**Fig. S17**). For subject 1 (LE Rat: #327396), Kruskal-Wallis ANOVA test was significant (χ^2 (3, 104) = 24.8; $p < 0.0001$), revealing dose-dependent increase in average peak amplitude of ripple events of 18% ($p < 0.0001$) and 9% ($p < 0.0001$) following administration of the 1.0 and 2.0 mg/kg doses of α5IA respectively. (**Fig. S17-A**). For subject 2 (WT F344 Rat: age 9 mo; #130), Kruskal-Wallis ANOVA revealed significant (χ^2 (3, 133) = 72.1; $p < 0.0001$) dose-dependent increases in average peak amplitude of ripple events of 17% ($p < 0.0001$), 28% ($p < 0.0001$) and 34% ($p < 0.0001$) following administration of the 0.3, 1.0 and 2.0 mg/kg doses of α5IA respectively in this rat (**Fig. S17-B**). The Kruskal-Wallis test of the peak ripple amplitude data from Subject 3 (WT F344 Rat: #600040) also revealed significant (χ^2 (3, 104) = 26.0; $p < 0.0001$) dose-dependent increases in average peak amplitude of 14% ($p < 0.0002$) and 22% ($p < 0.0001$) following administration of the 1.0 and 2.0 mg/kg doses of α5IA respectively (**Fig. S17-C**). Kruskal-Wallis ANOVA of the ripple peak amplitudes from Subject 4 (WT F344 Rat: #113) was significant (χ^2 (3, 160) = 85.2; $p < 0.0001$) as well, revealing dose dependent increases of 15% ($p < 0.0001$), 31% ($p < 0.0001$) and 20% ($p < 0.0001$) following administration of the 0.3, 1.0 and 2.0 mg/kg doses of α5IA respectively in this animal (**Fig. S17-D**). A Kruskal-Wallis test with Dunn's correction for multiple comparisons of the merged peak amplitude data from all three WT F344 rats was also significant (χ^2 (3, 405) = 150; $p < 0.0001$). Dose-dependent increases in average peak amplitude of ripple events of 11% ($p < 0.0007$), 26% ($p < 0.0001$) and 31% ($p < 0.0001$) are seen following administration of the 0.3, 1.0 and 2.0 mg/kg doses of α5IA respectively (**Fig. S17-E**). The Kruskal-Wallis test of the merged peak amplitude data from all four rats was also significant (χ^2 (2, 509) = 41; $p < 0.0001$). Planned comparisons revealed dose-dependent increases in average ripple peak amplitude of 10% ($p < 0.033$) and 24% ($p < 0.0001$) following administration of the 0.3 and 1.0 mg/kg doses respectively (**Fig. S17-F**). However, the effect on peak amplitude observed at 0.3 mg/kg dose was not significant when controlling for multiple comparisons.

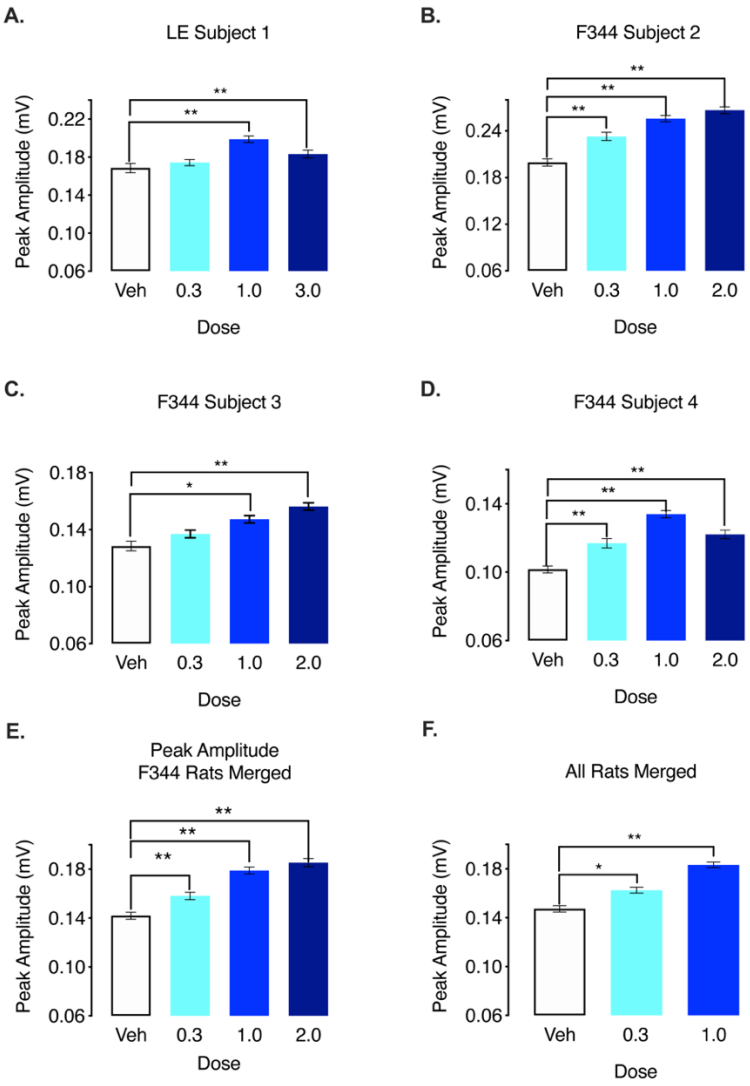

Fig. S17. Oral treatment with α5IA dose-dependently increases peak amplitude of ripples in adult WT male rats. **A)** Histogram of Kruskal-Wallis ANOVA results for LE Subject 1 showing significant dose-dependent increases in average peak amplitude of ripple events following administration of 1.0 and 2.0 mg/kg doses of α5IA. **B)** Histogram for F344 Subject 2 showing significant dose-dependent increases in average peak amplitude of ripple events at all three doses of α5IA tested. **C)** Histogram for F344 Subject 3 showing significant dose-dependent increases in average peak amplitude of ripple events following administration of 1.0 and 2.0 mg/kg doses of α5IA. **D)** Histogram for F344 Subject 4 showing significant dose-dependent increases in average peak amplitude of ripple events following administration of α5IA. **E)** Histogram showing average peak amplitude of ripples from all three F344 rats demonstrates significant dose dependent increases of 15% (0.3 mg/kg), 31% (1.0 mg/kg), and 20% (2.0 mg/kg). **F)** Histogram showing average peak amplitude of ripples from all four rats tested demonstrates significant dose dependent increases of 15% (0.3 mg/kg), 31% (1.0 mg/kg), and 20% (2.0 mg/kg). Significance indicated by * at $p < 0.01$ and ** at $p < 0.001$.

3. Effects of α5IA on Ripple Duration in Wildtype Rats

Kruskal-Wallis ANOVA revealed no consistent significant within subject effects of drug on ripple duration. Subject 1 (LE Rat; age 15 mo: #327396; showed no significant drug induced effects on ripple duration (χ^2 (3, 104) = 1.0; p = 0.78). (**Fig. S18-A**) For Subject 2 (WT F344 Rat; age 9 mo: #130) the Kruskal-Wallis test

was significant (χ^2 (3, 104) = 8.5 p = 0.036), but the pairwise comparisons of each dose with vehicle did not reveal any significant changes in ripple duration. (**Fig. S18-B**) A significant effect of drug on ripple duration is observed following administration of 1.0 mg/kg α5IA in Subject 3 (WT F344 Rat age 16 mo: #600040; (χ^2 (3, 104) = 3.9; p = 0.002). (**Fig. S18-C**) A significant effect of drug on ripple duration is observed in Subject 4 (WT F344 Rat age 12 mo: #113; χ^2 (3, 160) = 4.4; p = 0.04) on a planned comparison at the 0.3 mg/kg dose but this was not after controlling for multiple comparisons (χ^2 (3, 160) = 4.4; p = 0.07). (**Fig. S18-D**). Despite the lack of any consistent within subject changes in ripple duration, the Kruskal-Wallis test of average merged ripple duration data from all three F344 rats revealed a small but significant (χ^2 (3, 405) = 10.4; p = 0.04) 11% decrease following administration of the 1.0 mg/kg dose of α5IA. (**Fig. S18-E**) A significant (χ^2 (3, 509) = 10.4; p = 0.018) decrease in ripple duration of 10% is also seen when the data from all for four animals including Subject 1 (LE Rat age 15 mo: # 327396) are merged prior to analysis (**Fig. S18-F**).

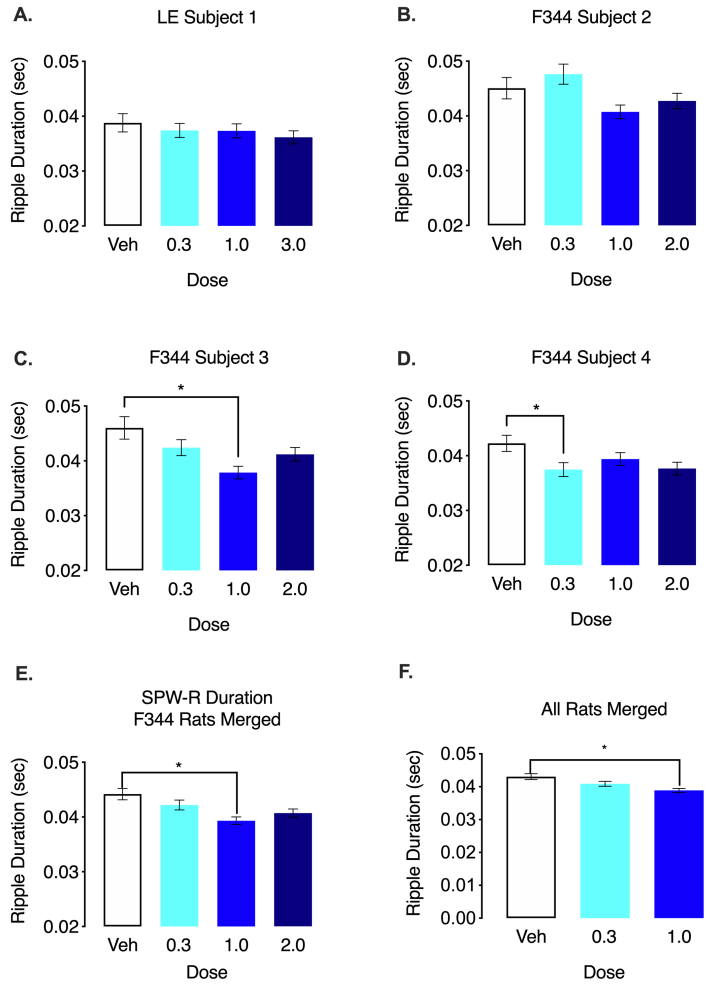

Fig. S18. Oral treatment of adult WT rats with 1.0 mg/kg α5IA decreases the average duration of ripple events. **A)** Subject 1 (LE Rat #600040) shows no significant dose-dependent changes in average ripple duration following administration of α5IA. **B)** Subject 2 (F344 Rat #130) shows no significant changes in average ripple duration. **C)** Subject 3 (F344 Rat #600040) shows a significant decrease in ripple duration following 1.0 mg/kg α5IA. **D)** Subject 4 (F344 Rat #113) shows a significant decrease in average ripple duration following administration of 0.3mg/kg α5IA. **E)** Merged data from all three F344 rats reveals a significant decrease in ripple duration following administration of

1.0 mg/kg dose of α5IA. **F)** A significant 10% decrease in ripple duration is seen following administration of 1.0 mg/kg dose of α5IA in the merged data from all 4 wildtype rats. Significance indicated by * at $p < 0.05$.

4. Effects of α5IA on Peak Frequency of Ripples in Wildtype Rats

No significant dose-dependent changes in peak ripple frequency are observed following treatment of wildtype rats with α5IA (**Data not shown**).

5. *Supplemental Results for Effects of α5IA on Ripple Band Power in TgF344-AD Rats*

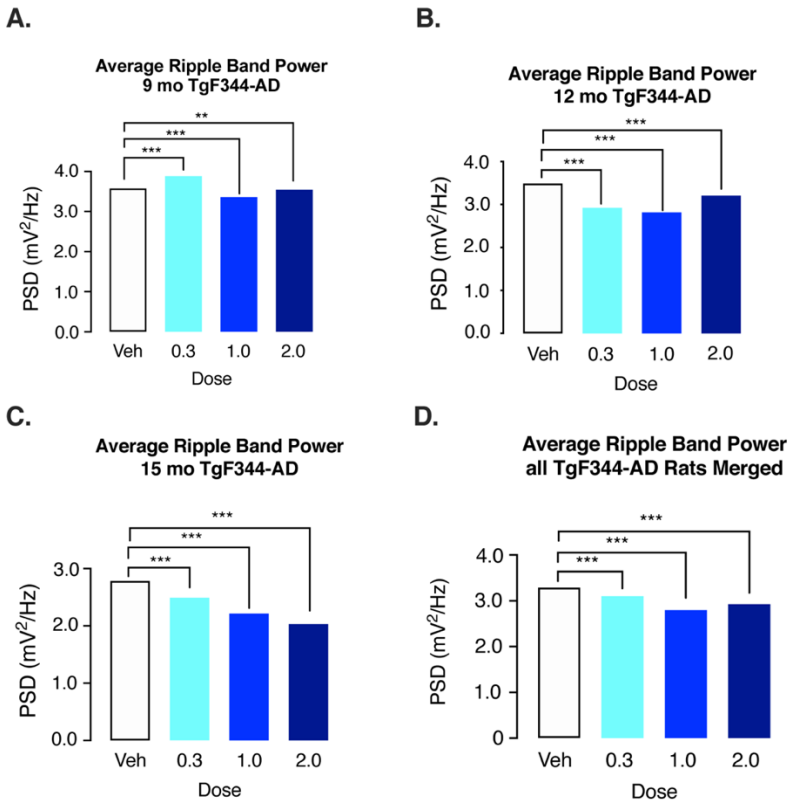

Fig S19: Treatment of adult TgF344-AD rats with α5IA decrease average power in ripple band. **A)** Data from 9 mo TgF344-AD shows increase at 0.3 mg but a decrease in average ripple band power following 1.0 and 2.0 mg/kg α5IA. **B)** Analysis of ripple band power in 12 mo TgF344-AD shows significant decrease in ripple band power following all 3 doses of α5IA. **C)** Analysis of ripple band power in 15 mo TgF344-AD shows significant decrease in ripple band power following all 3 doses of α5IA. **D)** Merged data from all three F344 rats reveals a significant decrease in ripple band power following administration of all three doses of dose of α5IA. Significance indicated by *** at $p < 0.001$.