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Supplemental information

Modulation of social investigation by anterior hypothalamic nucleus rhythmic neural activity

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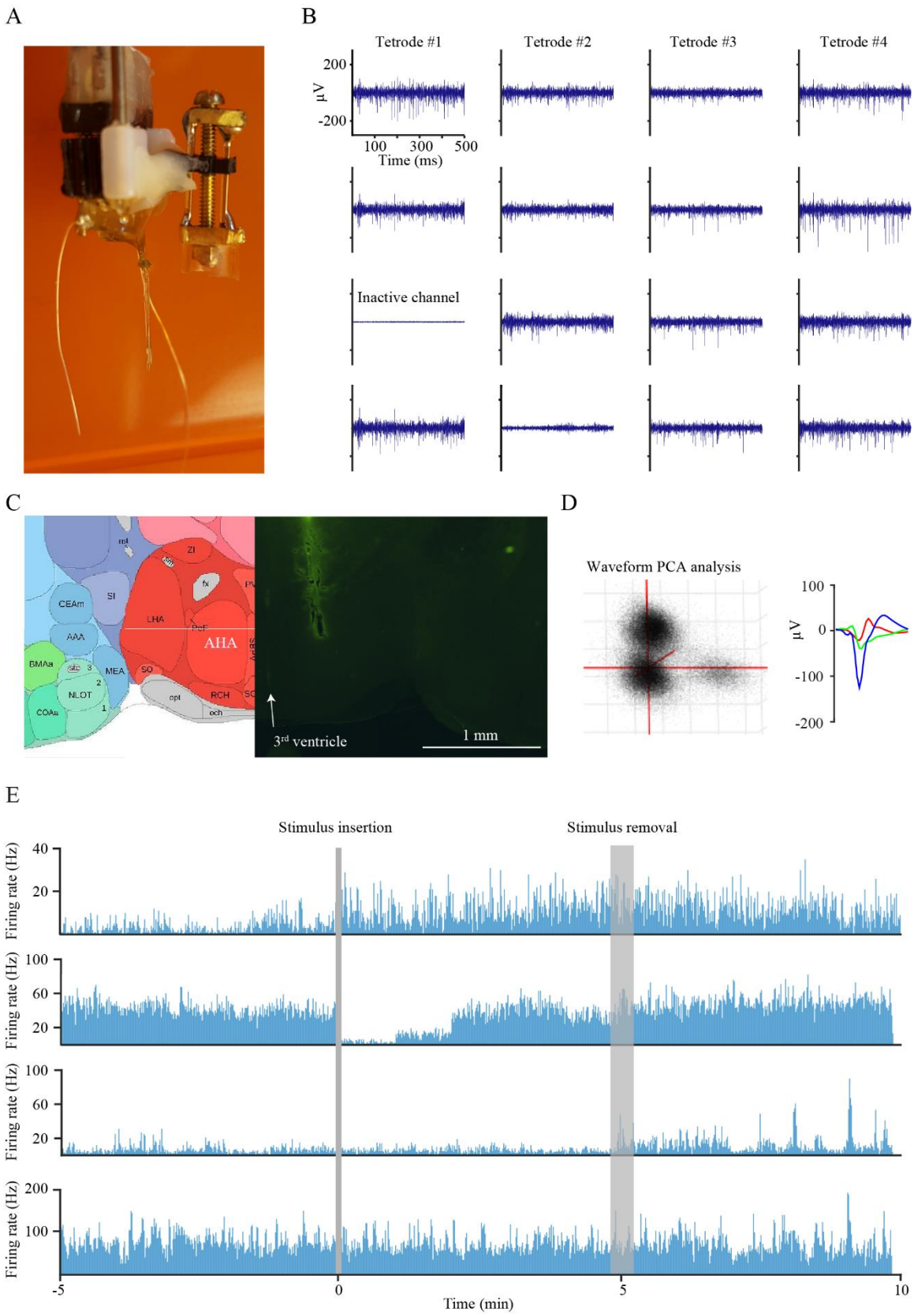

Figure S1. Recording AHN spiking activity (see also Figure 2)

(A) A picture of the custom-made 4 x 4 tetrodes probe.
(B) Representative 0.5-s traces from all 16 probes of one probe implanted into the AHN.
(C) Implantation site of the tetrode probe: brain atlas (left) and representative image (right).
(D) Left: PCA analysis of a single recording session, showing the clusters created by three distinct single-units. Right: superimposed waveforms of the three units.
(E) Firing rate of four distinct multi-units along the time course of the SP session. Gray stripes represent the time of stimulus insertion and removal.

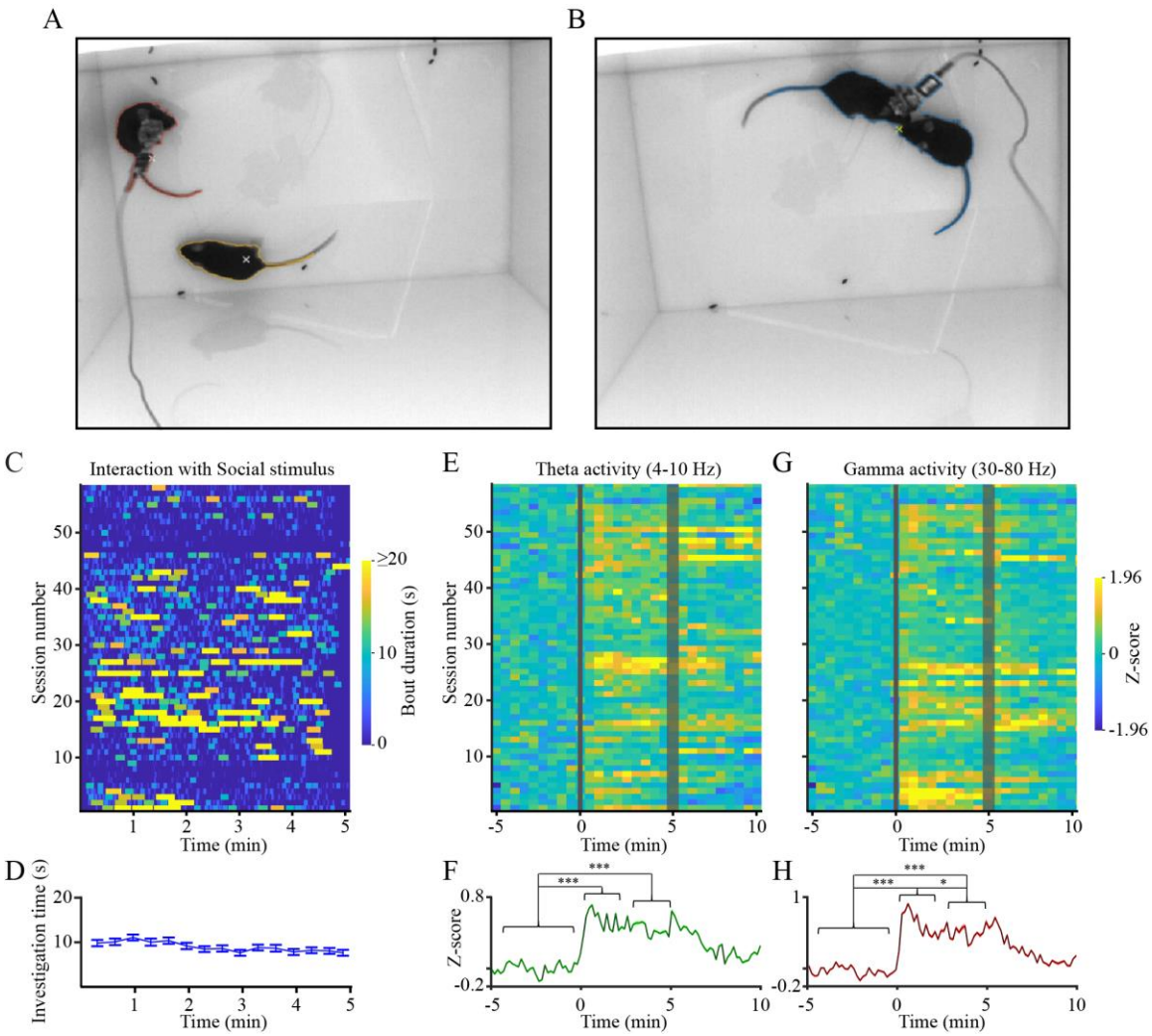

Figure S2. AHN theta and gamma rhythms are enhanced during free social interactions (See also Figure 1)

(A) A picture of the experimental arena of the free social interaction paradigm with the two animals (subject + stimulus), while they are separated.

(B) As in A, for the two animals while they are in contact.

(C) Behavioral raster-plots of interaction (contact) bouts between the two animals along 56 sessions of free social interactions.

(D) Mean interaction time (±SEM, 20-s bins) during the same sessions shown in C, along the time course of the free interaction session.

(E) Heat-map of Z-score analysis of theta power of LFP signals recorded during the same sessions shown in C-D. Gray stripes represent the time of insertion (thin stripe) and removal (thick stripe) of the stimuli from the chambers.

(F) Mean (±SEM) Z-score analysis of theta power of LFP signals recorded during the same sessions shown in D-E. Significant main effect for Time was found in RM ANOVA ($p<0.001$).

(G) As in E, for gamma power.

(H) As in F, for Z-score of gamma power. Significant main effect for Time was found in RM ANOVA ($p<0.001$).

*$p<0.05$, ***$p<0.001$, paired t-test with Bonferroni correction for multiple comparisons following main effect of time in ANOVA

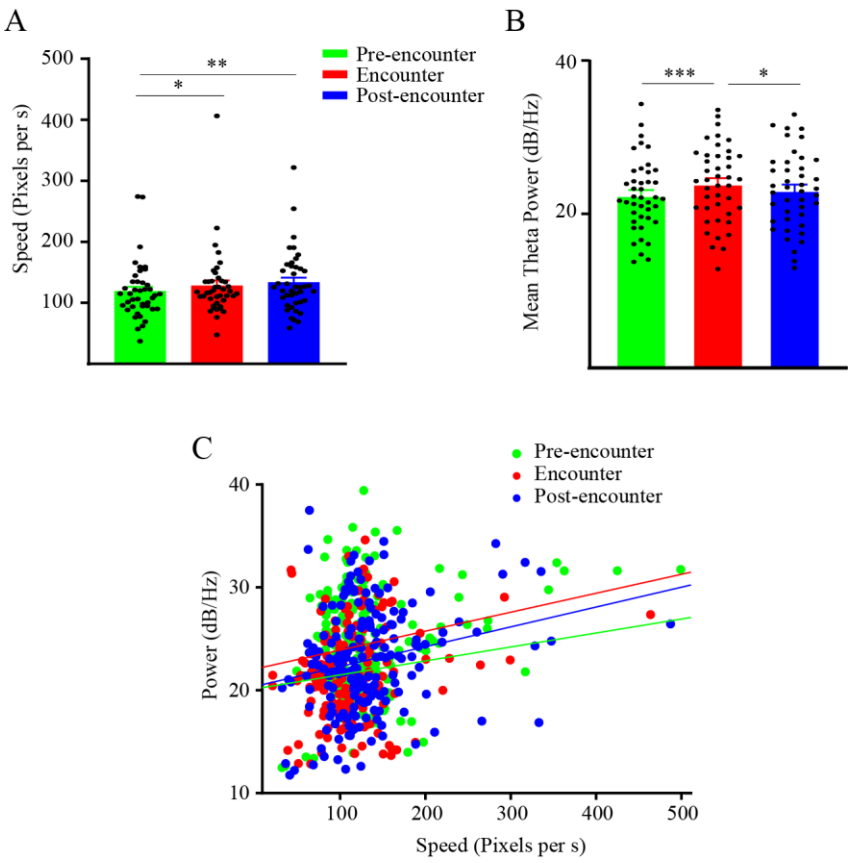

Figure S3. Relationship between subject's speed and LFP theta rhythmicity in the AHN (see also Figures 1 & 2)

(A) Mean (±SEM) speed during each stage of the SP test. Note the lower speed level during the Pre-encounter period, as compared to both the Encounter and Post-encounter periods, which did not differ in speed from each other. Significant main effect for Time was found in Friedman's test ($p<0.001$).

(B) As in A, for mean theta power. Note the significant reduction in theth power during Post-encounter, as compared to the Encounter period, despite the lack of change in mean speed shown in A. Significant main effect for Time was found in RM ANOVA ($p<0.001$).

(C) Analysis of covariance of LFP theta power as a function of the speed for the various stages of the SP test. Each point represents a 1-min time window in a session color-coded according to the session stage. Significant main effects in 2 way mixed-model ANOVA were found for Time ($p=0.01$) and Speed ($p<0.001$), but not in interaction ($p=0.864$).

*$p<0.05$, **$p<0.01$, ***$p<0.001$, Wilcoxon or paired t-test with Bonferroni correction for multiple comparisons following main effects.

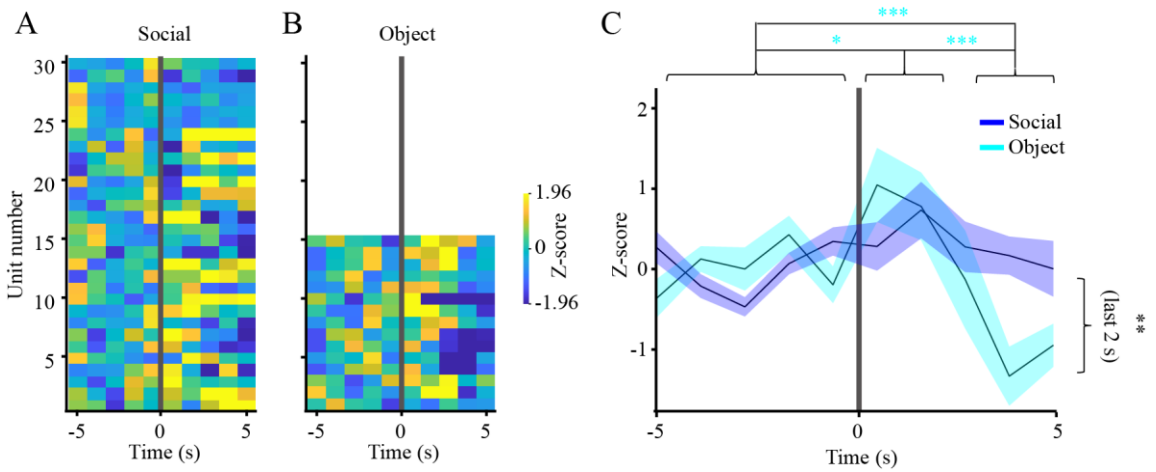

Figure S4. Spiking activity is specifically enhanced during long investigation bouts towards social, but not object stimuli (see also Figure 3)

(A) Heat maps of mean Z-score of MUA activity recorder from 5-s before to 5-s after the beginning of long (>6 s) social investigation bouts during SP sessions. Each line represents the mean value averaged across all bouts for a single session.

(B) As in A, for object investigation bouts during sessions that included long investigation bouts towards the object.

(C) Mean (±SEM) Z-score analysis for all the sessions shown in A-B, averaged separately for social (blue) and object (light blue) stimuli. Significant main effects in 2-way repeated measures ANOVA were found for Time ($p<0.001$), and Time×Stimulus interaction ($p<0.001$). Note the significant reduction in MUA activity observed between social and object investigation bouts during the last two seconds.

*$p<0.05$, ***$p<0.001$, paired t-test for with Bonferroni correction for multiple comparisons following main effect in ANOVA.

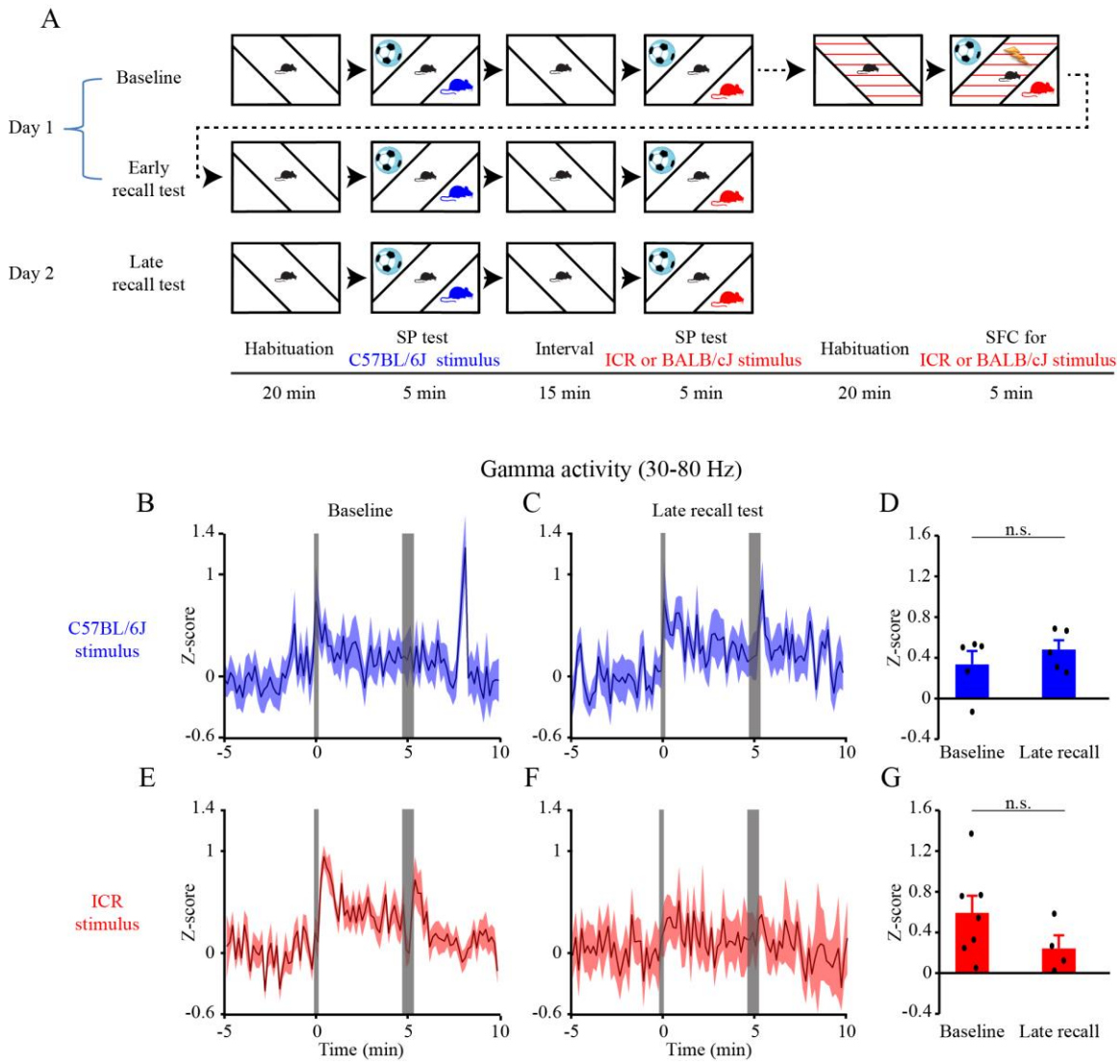

Figure S5. Gamma power of LFP signals in the AHN do not differ between affiliative and aversive social interactions (see also Figure 4)

(A) A schematic depiction of the social fear conditioning (SFC) paradigm. Note that C57BL/6J stimuli are colored in blue while ICR stimuli are colored in red.

(B) Mean (±SEM) Z-score analysis of gamma power along the time course of the Baseline SP test using C57BL/6J mice.

(C) As in B, for the late recall test using the same stimulus.

(D) Mean (±SEM) Z-score analysis of the change in gamma power during the Encounter stage of the SP test using C57BL/6J stimuli, calculated separately for Baseline (left) and Late recall (right) experiments. No significant effect was found using 2-way mixed-model ANOVA.

(E) As in B, for ICR stimuli.

(F) As in C, for ICR stimuli.

(G) As in D, for ICR stimuli.

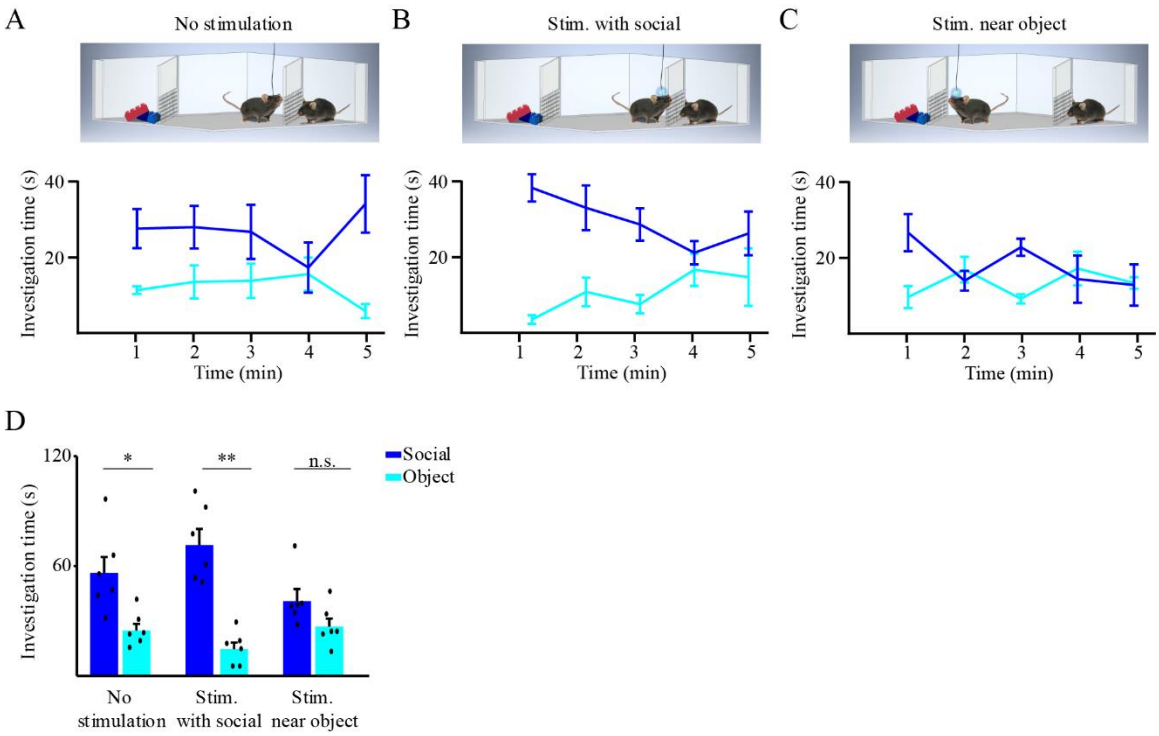

Figure S6. Optogenetic activation of AHN neurons modulates investigation of Optogenetic activation-associated stimulus, even when only the animals that passed all conditions are considered (see also Figure 6)

(A) Mean (±SEM) investigation time across the SP session (1-min bin) for each stimulus, plotted for the no optogenetic stimulation (control) condition (schematically depicted above). Schematic depiction of the condition is presented above.

(B) As in A, for optogenetic stimulation given during social investigation bouts.

(C) As in A, when stimulation was given when the subject was near the object (away for the social stimulus). Note the loss of social preference specifically in this condition.

(D) Mean (±SEM) investigation time throughout the session for the three conditions shown in A-C. Significant main effect in 2-way repeated measures ANOVA were found for Stimulus ($p<0.01$), Condition ($p<0.05$), and Stimulus × Condition interaction ($p<0.01$).

*$p<0.05$, **$p<0.01$, paired t-test following main effect in ANOVA.

Table S2. Kolmogorov-Smirnov Test for Normal Distribution, related to STAR Methods.

Figure		original data			Transformed data		
		Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
1F -Investigation Time During Encounter	**Social**	0.085	42	0.2	0.123	42	0.116
	Object	0.172	42	0.003	0.086	42	0.2
1J - Gamma in SP	**Baseline**	0.099	42	0.2	0.094	41	0.2
	First 2 min	0.171	42	0.003	0.126	41	0.101
	Last 2 min	0.109	42	0.2	0.086	41	0.2
2C - Spikes in SP	**Baseline**	0.063	34	0.2	0.056	34	0.2
	First 2 sec	0.082	34	0.2	0.109	34	0.2
	Last 2 sec	0.205	34	0.001	0.083	34	0.2
2D- Spikes in Free Interaction	**Baseline**	0.089	89	0.081	0.084	89	0.157
	First 2 sec	0.14	89	0	0.043	89	0.2
	Last 2 sec	0.125	89	0.002	0.066	89	0.2
3I- Spiking Activity during Bouts <u>Social</u>	**Baseline**	0.186	30	0.01	0.152	34	0.044
	First 2 sec	0.156	30	0.06	0.088	34	0.2
	Last 2 sec	0.177	30	0.018	0.083	34	0.2
3I- Spiking Activity during Bouts <u>Object</u>	**Baseline**	0.163	15	0.2	0.147	15	0.2
	First 2 sec	0.194	15	0.133	0.119	15	0.2
	Last 2 sec	0.201	15	0.104	0.118	15	0.2
Sup. 3H- Gamma in Free Interaction	**Baseline**	0.054	58	0.2	0.053	58	0.2
	First 2 min	0.138	58	0.008	0.09	58	0.2
	Last 2 min	0.149	58	0.003	0.113	58	0.061